e-ISSN 2544-1361

European Journal of Clinical and Experimental Medicine

Formerly: Medical Review

Quarterly

Vol. 20, No. 1

Publication date: March 2022

Rzeszów, Poland 2022

Technical development, layout and interior design: Wojciech Pączek
Cover design: Wiesław Grzegorczyk

ICV 2020: 100.00
MEiN: 20.00

Indexing:
Ministry of Science and Higher Education (Poland)
Index Copernicus
The Central EuropeanJournal of SocialSciences and Humanities (CEJSH)
POL-Index
Central Medical Library (Poland)
ARIANTA – Science and branch Polish electronic journals
J-Gate

e-ISSN 2544-1361

EDITORIAL CORRESPONDENCE
European Journal of Clinical and Experimental Medicine Editorial Office
35-959 Rzeszów, ul. Kopisto 2A,
tel. 17 872 11 53, fax 17 872 19 30
http://www.ejcem.ur.edu.pl
e-mail: ejcemur@gmail.com
https://mc04.manuscriptcentral.com/pmur

PUBLISHER: PUBLISHING OFFICE OF THE UNIVERSITY OF RZESZÓW
35-959 Rzeszów, ul. prof. S. Pigonia 6,
tel./fax 17 872 14 26, e-mail: wydaw@ur.edu.pl

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 4

Contents

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.1

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 5–10

ORIGINAL PAPER

Berrin Erok [1], Ali Önder Atca [2], Hakan Önder [1]

Neurological complications encountered in imaging studies in association with COVID-19 – a single center analysis

[1] Department of Radiology, University of Heath Sciences, Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey
[2] Department of Radiology, Altınbas University School of Medicine Bahcelievler Medical Park Hospital, İstanbul, Turkey

ABSTRACT

Introduction and aim. COVID-19 is a viral infectious disease, which was first reported in patients with unusual pneumonia in December 2019. However, as the pandemic progressed, extrapulmonary manifestations including various neurologic complications have been started to be increasingly reported. In this retrospective study, we tried to search the neurological complications seen in our patients with positive rRT-PCR test for COVID-19 and examine the underlying associated risk factors.

Material and methods. We have retrospectively analyzed the neuroimaging studies performed in our patients with positive rRT-PCR test for COVID-19 between April, 2020 and August, 2021. Both computed tomography (CT) scans and magnetic resonance imagings (MRI) of brain, head & neck region and the spine were retrospectively evaluated for the presence of any complications in patients with positive rRT-PCR test for COVID-19.

Results. There were 147 patients having neuroradiological imaging studies performed for various neurological symptoms. Among these patients we detected 10 acute neurologicalcomplications.The most common was acute ischemic stroke in 5 patients and intracranial hemorrhage in 3 patients, two of which were intraventricular hemorrhage. The other complications included a preasumed cytotoxic lesion of corpus callosum in a 18 year old girl and lumbar spondylodiscitis complicated with psoas abcess in a 47 year-old man.

Conclusion. In COVID-19 patients severe neurological complications can occur even as a presenting manifestation. Early cytotoxic endothelial injury can be the underlying cause in these patients and should be further studied in larger series in terms of what the susceptibility factors in these patients.

Keywords. COVID-19, direct viral toxicity, endotheliitis

Introduction

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) associated coronavirus disease 2019 (COVID-19) is a viral infectious disease, which was first reported in patients with unusual pneumonia in December 2019 at Wuhan, China.[1] It was recognized as a pandemic by the World Health Organization (WHO) on 11 March 2020.[2] The main mode of transmission is human-to-human spread via respiratory droplets presenting primarily with pulmonary manifestations. However, as the pandemic progressed, extrapulmonary complications started to be frequently reported. Neurological manifestations, ranging from headache, myalgia, hyposmia/anosmia, hypogeusia/ageusia to severe complications such as impaired consciousness, stroke, encephalitis and encephalopathies have been also reported particularly in severelly ill patients from the disease.[3-6] In a case series of 214 patients, neurologi-

Corresponding author: Berrin Erok, e-mail: drberrinerok@hotmail.com

Received: 1.11.2021 / Revised: 17.11.2021 / Accepted: 18.11.2021 / Published: 30.03.2022

Erok B, Atca AÖ, Önder H. *Neurological complications encountered in imaging studies in association with COVID-19 – a single center analysis.* Eur J Clin Exp Med. 2022;20(1):5–10. doi: 10.15584/ejcem.2022.1.1

cal complications have been observed in 36.4% of the overall cases and the more severe complications were reported in patients with more severe infection. In this case series 5.7% (5 patients) of the neurological complications were acute cerebrovascular diseases (4 patients with ischemic stroke and 1 patient with cerebral hemorrhage).[4] Acute cerebrovascular diseases most of which are acute ischemic stroke were reported up to 6% of hospitalized patients with severe inflammatory state.[7] The hyperinflammatory state and associated edothelial damage has been suggested as an endogenous pathway in the pathophysiology. However, although less frequent, some neurological complications have been reported in young COVID-19 patients without severe disease, even as an presenting manifestation leading to the diagnosis of COVID-19.

Aim

In this retrospective study, we tried to search the neurological complications seen in our patients with positive rRT-PCR test for COVID-19 and examine the underlying associated risk factors.

Material and methods

We have retrospectively analyzed the neuroimaging studies performed in our patients with positive rRT-PCR test for COVID-19 between April, 2020 and August, 2021. Ethics approval has been obtained from Altınbas University School of Medicne Bahcelievler Medical Park Hospital Ethics Committee. Both computed tomography (CT) scans and magnetic resonance imagings (MRI) of brain, head & neck region and the spine were retrospectively evaluated for the presence of any complications in patients with positive rRT-PCR test for COVID-19. The CT images were obtained using 64 channel MDCT scanners (Philips Medical Systems, Brilliance 64, the Netherlands). MR imaging was performed with the Siemens 3T MAGNETOM Skyra MRI scanner. The pulse sequences were coronal FLAIR (TE/TR =125/10000 msec; TI=2800 msec), axial T2 (TE/TR=80/3000 msec), axial T1 (10/2000 msec), diffusion-weighted imaging (DWI) (TE/TR=120/3500 msec) with apparent diffusion coefficient (ADC) maps.

Results

There were 147 patients having neuroradiological imaging studies performed for various neurological symptoms including headache, anosmia, vertigo, impaired consciousness, transient ischemic attacks, acute neurological deficits and peripheral neuropathic symptoms in patients with positive rRT-PCR test for COVID-19. Among these patients we detected 10 acute neurologicalcomplications. The most common was acute ischemic stroke (AIS) (5 patient with M/F; 3/2, mean age; 75) (Fig. 1, 2) followed by intracranial hemorrhage (ICH) (Fig. 3, 4) (3 patients with F/M: 2/1, mean age; 73); all of these 8 cases were older age patients (older than 60 years). All of them had at least one traditional cardiovascular risk factors including hypertention (HT), type 2 diabetes (T2DM), obesity and/or smoking.

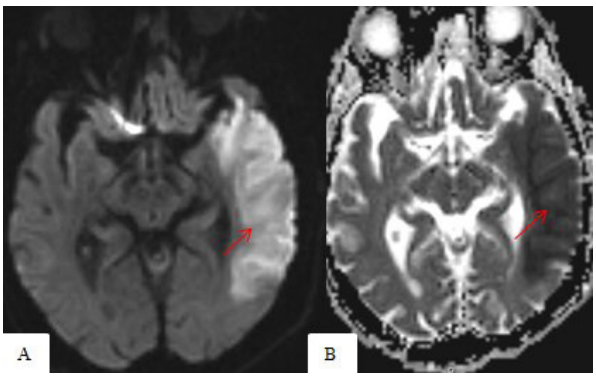

Fig. 1. A 72-year-old male patient with T2DM, HT, obesity and smoking history presented with COVID-19 pneumonia and followed in ICU. A) DWI and B) ADC mapping of the brain show restricted diffusion at the left MCA territory characterized with hyperintensity on DWI (A, arrow) and corresponding low signal intensity on ADC mapping (B, arrow) compatible with AIS

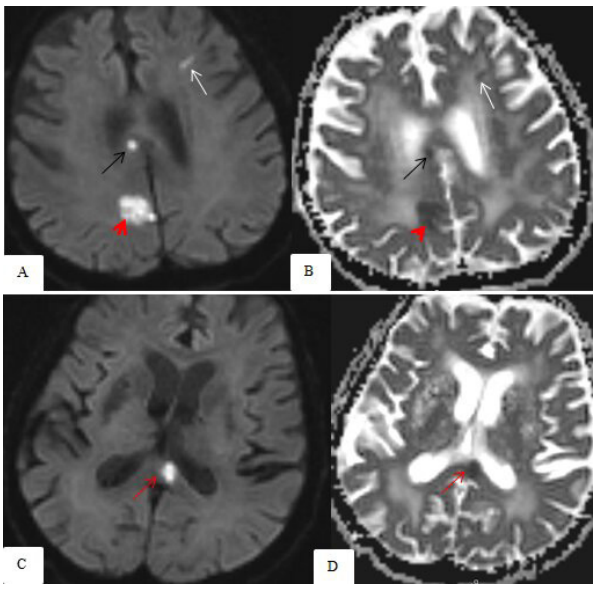

Fig. 2. A 92-year-old female patient with with hypertention who presented with COVID-19 pneumonia and followed in ICU. A) DWI and B) ADC mapping of the brain show multifocal restricted diffusion at the left anterior corona radiata (A,B white arrow), at the right parasagittal frontal (A,B ablack arrow) and parietal lobes (A,B red arrow head), at the splenium of corpus callosum (C,D red arrow)

In all of the ICH patients and 3 of the AIS patients the cerebrovascular events occured in intensive care unit (ICU) when they were under management for severe COVID-19 pneumonia. The other two patients

with AIS had also COVID-19 pneumonia but were being followed in hospital setting, not in ICU. All of these patients were under anticoagulation treatment, except one patient with AIS of left MCA territory, due to his previous history of ICH and one patient with intraventricular hemorrhage (IVH) due to uncontrolled HT during the ICU course (Table 1).

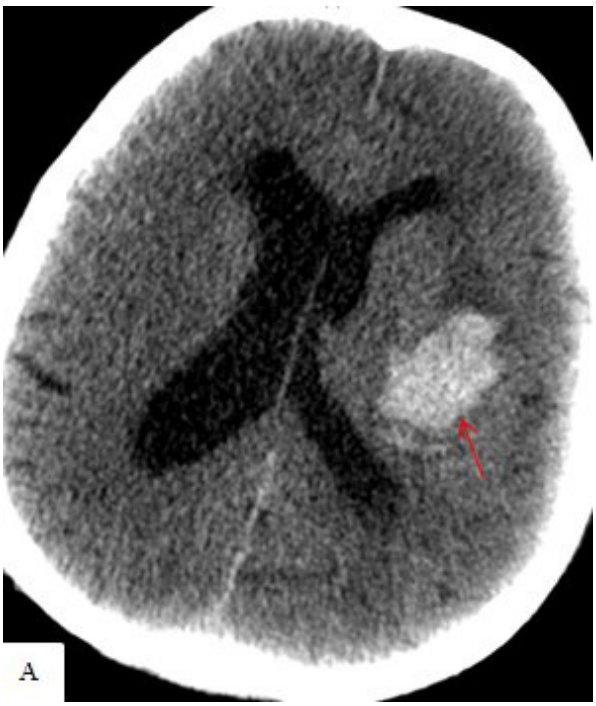

Fig. 3. A 60-year-old male patient presented with COVID-19 pneumonia and followed in ICU with anticoagulation treatment. Parenchymal window axial CT image shows acute left thalamic cerebral hematoma (arrow)

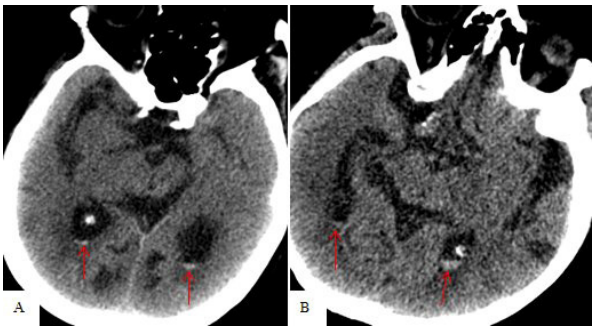

Fig. 4. A 78-year-old female patient under anticoagulation (A) and a 81 year old female patient without prophylactic anticoagulatiob presented with COVID-19 pneumonia and followed in ICU. Axial CT images show small amoutns of IVH (A,B, red arrows) without obvious parenchymal hemorrhage

The other complications included a preasumed cytotoxic lesion of corpus callosum (CLCC) in a 18 year old girl (Fig. 5).

In this young patient with positive rRT-PCR test for COVID-19 presenting with presyncope, a focal restricted diffusion in the splenium of the corpus callosum was present on MRI and CLCC was considered in this previously healthy patient despite the lack of follow-up MRI to show its reversibility.There was no other white matter lesion to suggest demyelinating processes.The last complication was lumbar spondylodiscitis (LSD) complicated with psoas abcess in a 47 year-old man with no relevant medical history except long lasting smoking (Fig. 6).

These two complications occured at the absence of any pulmonary disease and were the presentations of the patients that lead to the diagnosis of COVID-19 (Table 2).

Table 1. Demographic features of the patients

	Age	Sex	Medical history	Presentation with COVID-19 pneumonia	ICU	Location	Anticoagulation
AIS	92	Female	HT	Yes	Yes	Bifrontal, right parietooccipital sulcus, splenium of the corpus callosum	yes
	67	Female	HT, smoking	Yes	No	Right hemipontine	Yes
	75	Male	Obesty,T2DM, HT, smoking	Yes	Yes	Left MCA territory	No (previous ICH)
	72	Male	Smoking	Yes	Yes	Left MCA territory	Yes
	69	Male	HT, T2DM, smoking	Yes	No	right frontal and left parietal	Yes
ICH	81	Female	HT	Yes	Yes	IVH	No (uncontrolled HT)
	78	Female	Obesity, T2DM, HT	Yes	Yes	IVH	Yes
	60	Male	HT, smoking	Yes	Yes	Left thalamic	Yes

AIS; acute ischemic stroke, ICH; intracranial hemorrhage, HT; hypertention, T2DM; type 2 diabetes mellitus, ICU; intensive care unit, MCA; middle cerebral artery, IVH; intraventricular hemorrhage

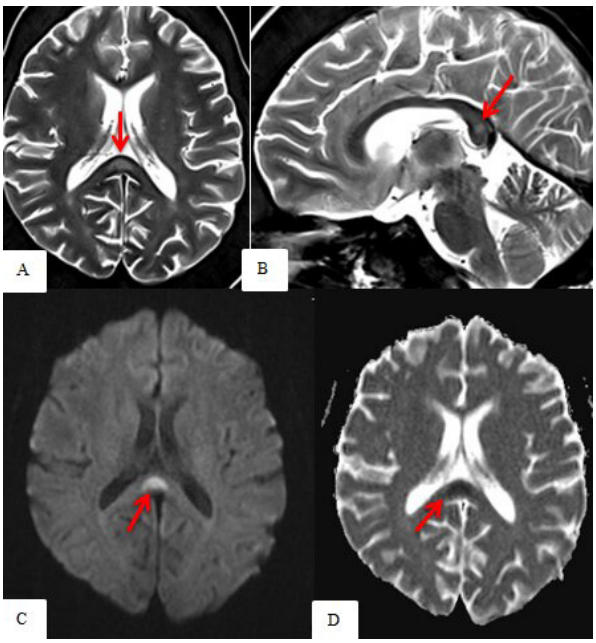

Fig. 5. MRI showing CLCC; A) axial T2w and B) sagittal T2w images showing hyperintense lesion at the splenium of the corpus callosum (A,B; red arrows). C) DWI and D) ADC map show restricted diffusion of the lesion (C,D; red arrows)

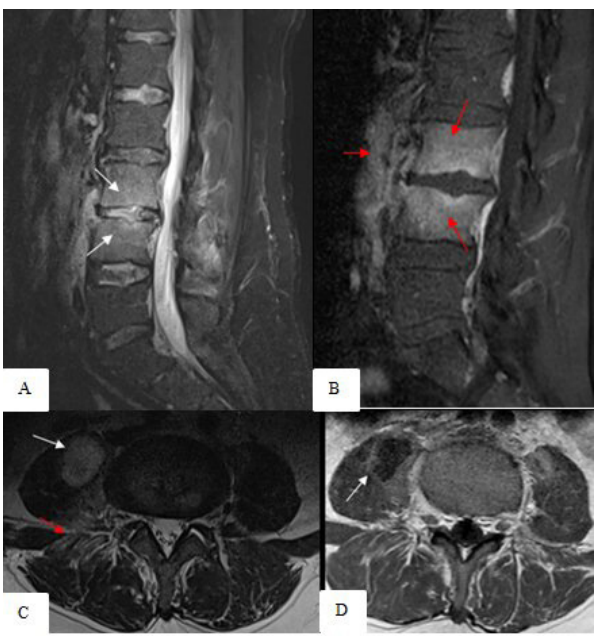

Fig. 6. Lumbar MRI demonstrating abnormally increased signal intensity involving L3-L4 vertebral bodies on sagittal STIR image (A, white arrows) and the contrast enhancement in both the involved vertebral bodies and also in the inflammatory prevertebral soft tissue on sagittal postcontrast T1w image (B, red arrows). Abnormal signal intensity involving the right prevertebral soft tissue (C, red arrow) and the encapsulated hyperintens collection in the right psoas muscle (C, white arrow) are demonstrated on axial T2w image. The enhancing wall with central necrotic cavity on axial poscontrast T1w images (D, white arrow) is shown

Table 2. The complications other than acute cerebrovascular diseases

	Age	Sex	Medical history	COVID-19 pneumonia	Presenting symptom
CLCC	18	Female	None	No	Presyncope
LSD	47	Male	Smoking	No	Severe back pain

CLCC: cytotoxic lesion of corpus callosum, LSD; lumbar spondylodiscitis

Discussion

The mechanisms underlying the neurological complications caused by SARS-CoV-2 have become a research of interest due to various neurological symptoms that have been reported increasingly as the pandemic progresses. Moreover, many of the neurological complications are probably underreported due to the lack of further diagnostic evaluations. In COVID-19, an inflammatory state with increased level of cytokines, including IL-6, is generated and suggested as an endogenous pathway in the pathophysiology of most of the complications including cerebreovascular diseases in severe cases. The hyperinflammatory state has been shown to be associated with subsequent coagulopathy characterized with increased level of procoagulant mediators, which can present with thrombotic events like venous thrombosis, pulmonary thromboembolism, acute myocardial infarction and acute ischemic stroke, resulting in prophylactic anticoagulation in the management of COVID-19 patients, if the coagulation parameters are suitable.[8] In a study conducted in 16 critically ill patients, it was found that in correlation with increased level of IL-6; the fibrinogen, platelet and D-dimer levels were also increased.[9] In this endogenous pathway the hyperinflammatory state is also suggested as a cause of endothelial damage. Early in the pandemic, the endothelial damage was shown in the form of lymphocytic endotheliitis in the lungs of deceased COVID-19 patients. An angiocentric inflammation with pulmonary vessel enlargement having extensive endothelial injury, as opposed to the normally expected lung's vasoconstriction response to pneumonia was well demonstrated.[10,11] Although histopathologic evaluation of CNS is scarce, this endotheliopathy has been also demonstrated in various other organs including heart, kidney, liver and small intestine of three COVID-19 patients in a postmortem examination.[12] Endothelial damage in the microcirculation can cause ischemia as a result of subsequent vasoconstriction and also serves as an additional predisposing factor for prothrombotic events with dysregulation of antithrombotic functions of the endothelium. Moreover, although less frequently reported and most often considered as a result of thereupathic anticoagulation in COVID-19 patients, hemorrhagic manifestations have

been also supposed to be associated with the endothelial damage as a possible cause of rupture of arterial wall.[13] Cezar-Junior et al. demonstrated four cases of subarachnoid hemorrhage (SAH) in patients with COVID-19 and suggested to some extent the exacerbated systemic inflammatory process as a cause.[14] As our knowledge, IVH which is much more common as a secondary complication of intraparenchymal hemorrhage or SAH, has been very rarely reported as a primary in association with COVID-19.[15] Two of our ICH cases were presented with primary IVH, one of which was not under anticoagulation due to uncontrolled HT. Older patients with already damaged endothelium due to one or more traditional cardiovascular co-morbidities are expected to be more prone to these complications, as seen our patients. On the other hand, Harris CL. et al. reported a fatal case of IVH with associated hydrocephalus without obvious parenchymal source of bleeding in a 32 year-old young man who was not on any anticoagulation or antiplatelet medication and had no significant comorbidities.[16] We did not see AIS and ICH in patients without preexisting cardiovascular risk factors at the absence of moderate to severe COVID-19. Likewise, in a study including 219 hospitalized patients with COVID-19 in Wuhan, Chine acute stroke were found more likely in older patients presenting with severe pulmonary infection, having cardiovascular risk factors, as in our patients.[17] In addition, a few studies demonstrated AIS cases at the absence of severe pulmonary/systemic disease and cardiovascular comorbidities.[18,19] An example is a case series by Oxley et al. reporting five cases of large vessel stroke in COVID-19 patients younger than 50 with no previous medical history. [20] In these cases a direct toxic endothelial injury rather than the endogenous pathway of exacerbated systemic hyperinflammatory state seen in severe disease, can be considered. The most proposed mechanism underlying this direct neurotropism of COVID-19 is the expression of angiotensin-converting enzyme 2 (ACE-2) receptors in the endothelium which are used by SARS-CoV-2 for viral entry to the host cells via their large spike glycoproteins present in all Coronaviruses as a family. ACE-2 receptors are widely expreseed in the type 2 alveolar cells, epithelial cells of the gastrointestinal tract, and also in the endothelial cells.[21-23] SARS-CoV-2 would theoretically use ACE2 receptors expressed in cerebrovascular endothelial cells and cause direct virus induced toxicity. Our two atypical cases of CNS complications occured as a presenting manifestation of COVID-19 also suggest the direct viral toxicty as the underlying pathophysiology of early neurological presentations. A few reported cases of CLCC and posterior reversible encephalopathy syndrome in association with COVID-19 and also our preassumed case of CLCC suggest a direct cytotoxic endothelial injury in the cerebral vasculature resulting in damage to vascular autoregulation and cerebral perfusion.[24-28] Furthermore, in our last patient presenting with LSD, there was no previous history of spinal instrumentation or any trauma. Laboratory results excluded tuberculosis and brucellosis. Abdominopelvic imaging excluded the other possible causes of psoas abcess originating from gastrointestinal diseases like appendicitis, diverticulitis or perforation and urinary diseases. We thought that these pyogenic complications could have been associated with hematogenous dissemination from asymptomatic colonization sites via SARS-CoV-2 induced endotheliitis.[29] In this patient, the longlasting smoking history could have served as a contributing factor for the occurance of early endothelial damage. Another support from our experience is that, we encountered and reported in our previous studies that the pulmonary vasoplegia is present not only in severe systemic disease associated with cytokine storm but also in very limited early pneumonia, meaning that vasoplegia can present early in the disease and could have been also present in the systemic vasculature including the cerebral vessels and can responsible for early extrapulmonary and neurological complications.[30]

Conclusion

In COVID-19 patients despite the paucity of data, severe neurological complications can occur and clinicians should have a high clinical suspicion particularly in patients with severe disease. Moreover, it should be kept in mind that neurological complications can also occur even as a presenting manifestation in the absence of any pulmonary disease. Early cytotoxic endothelial injury can be the underlying cause in these patients and should be further studied in larger series in terms of what the susceptibility factors in these patients, like receptor polymorphism. Our study also shows that, in addition to AIS which has been more frequently reported till now, ICH cases and interestingly primary IVH can be included in severe neurological manifestations seen in patients with COVID-19. In addition, as a viral infectious disease SARS-CoV-2 can be also associated with pyogenic complications from asymptomatic bacterial colonization sites possibly via hematogenous dissemination due to viral induced endotheliitis.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, B.E.; Methodology, B.E.; Formal Analysis, B.E. and H.Ö.; Investigation, B;E; and A.Ö.A.; Writing – Original Draft Preparation, B.E.; Writing – Review & Editing, B.E and H.Ö.; Supervision, B.E. and HÖ.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Ethics approval has been obtained from Altınbas University School of Medicne Bahcelievler Medical Park Hospital Ethics Committee.

References

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506.
2. Mahase E. COVID-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. *BMJ*. 2020;368:m1036.
3. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323:1061-1069.
4. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with Coronavirus disease 2019 in Wuhan, China. *JAMA Neurol*. 2020;77:683-690.
5. Ellul M, Benjamin L, Singh B, et al. Neurological associations of COVID-19. *Lancet Neurol.* 2020;19:767-783.
6. Poyiadji N, Shahin G, Noujaim D, Stone M, Patel S, Griffith B: COVID-19-associated acute hemorrhagic necrotizing encephalopathy: imaging features. *Radiology.* 2020;296:119-120.
7. Li Y, Wang M, Zhou Y, Chang J, Xian Y, Mao L. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *SSRN Electron J.* 2020;5(3):279-284.
8. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost.* 2020;18:1023-1026.
9. Connors J, Levy J. Thromboinflammation and hypercoagulability of COVID-19. *J Thromb Haemost.* 2020;8(7):1559-1561.
10. Kwee T. C., Kwee R. Chest CT in COVID-19: What the Radiologist Needs to Know. *RadioGraphics*. 2020;40:1848-1865.
11. Ackermann ME, Verleden S, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in COVID-19. *N Engl J Med.* 2020;383:120-128.
12. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* 2020;395(10234):1417-1418.
13. Sharifi-Razavi A, Karimi N, Rouhani N: COVID-19 and intracerebral haemorrhage: causative or coincidental? *New Microbes New Infect*. 2020;35:100669.
14. Cezar-Junior AB, Faquini IV, Silva JLJ, et al. Subarachnoid hemorrhage and COVID-19: association or coincidence? *Medicine (Baltimore).* 2020;99:e23862.
15. Mousa-Ibrahim F, Berg S, TPDetola OO, Teitcher M, Ruland S. Intracranial hemorrhage in hospitalized SARS--CoV-2 patients: a case series. *J Stroke Cerebrovasc Dis*. 2021;30:105428.
16. Harris CL, Achuthanandan S, Para K, Shetty V. A Fatal Extrapulmonary Manifestation of COVID-19. *Cureus.* 2021;13(3):e14068.
17. Li Y, Li M, Wang M, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vasc Neurol*. 2020;5(3):279-284.
18. Qureshi AI, Baskett WI, Huang W, et al. Acute Ischemic Stroke and COVID-19. *Stroke.* March 2021;52(3):905-912.
19. Cheruiyot I, Sehmi P, Ominde B, et al. Intracranial hemorrhage in coronavirus disease 2019 (COVID-19) patients. *Neurol Sci.* 2021;42(1):25-33.
20. Oxley TJ, Mocco J, Majidi S, et al. Large vessel stroke as a presenting feature of COVID-19 in the young [letter]. *N Engl J Med*. 2020;382(20):e60.
21. Saavedra JM: Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities. *Cell Mol Neurobiol*. 2005;25:485-512.
22. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res.* 2020;176:104742.
23. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B beta-coronaviruses. *Nat Microbiol.* 2020;5(4):562-569.
24. Gaur P, Dixon L, Jones B, Lyall H, Jan W. COVID-19- Associated Cytotoxic Lesions of the Corpus Callosum. *Am J Neuroradiol.* 2020;41:1905-1907.
25. Lin J, Lawson E, Verma S, Peterson R, Sidhu R. Cytotoxic Lesion of the Corpus Callosum in an Adolescent with Multisystem Inflammatory Syndrome and SARS-CoV-2 Infection. *Am J Neuroradiol*. 2020;41:2017-2019.
26. Rasmussen C, Niculescu I, Patel S, Krishnan A. COVID-19 and Involvement of the Corpus Callosum: Potential Effect of the Cytokine Storm? *Am J Neuroradiol.* 2020;41:1625-1628.
27. Lallana S, Chen A, Requena M, et al. Posterior reversible encephalopathy syndrome (PRES) associated with COVID-19. *Journal of Clinical Neuroscience.* 2021;88:108-112.
28. Fountain JH, Lappin SL. Physiology, Renin Angiotensin System. *Stat Pearls Publishing, Treasure Island*. FL; 2020.
29. Erok B, Kıbıcı K. COVID-19 Infection Manifesting with Lumbar Spondylodiscitis Complicating With Psoas Abscess without Pneumonia. *Indian J Neurosurg*. 2021. doi: 10.1055/s-0041-1741405
30. Erok B, Atca AO, Kıbıcı K. Chest CT imaging features of progressive phase COVID-19 pneumonia and evolution of findings. *Acta Medica Mediterr.* 2021,37:1.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.2

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 11–17

ORIGINAL PAPER

Paulina Opalińska-Zielonka [1,2], Katarzyna Wiącek [1,2], Paweł Marczak [1,2], Krystyna Piasecka [1,2], Bartosz Korczowski [1,2]

Clinical profile and management of patients with pediatric inflammatory multisystem syndrome – temporally associated with SARS-CoV-2 – single-center experience

[1] Department of Pediatrics Regional Hospital No.2 in Rzeszow, Rzeszow, Poland
[2] Institute of Medical Sciences, Medical College, University of Rzeszow, Rzeszow, Poland

ABSTRACT

Introduction and aim. Pediatric Inflammatory Multisystem Syndrome (PIMS-TS) is a new condition that has emerged in children during the COVID-19 pandemic. Many clinical signs and symptoms resemble those found in Kawasaki disease (KD).

Material and methods. The following data were considered: clinical presentation, comorbidities, laboratory findings, abnormalities in additional tests, exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the child and his family members, applied treatment and return to full health.

Results. In the presented study nineteen children were analyzed. Fever was a universal finding in our group and it's mean duration was 7 days (range 5-9). Other common symptoms included abdominal pain and severe weakness (in 89.5%), rash and conjunctivitis (in 84.2%), vomiting (in 73.7%) and mucous membrane involvement (in 63.2%). In nearly half of cases, echocardiography revealed fluid in the pericardial sac and left ventricular systolic dysfunction (in 52.6% and 47.4% respectively). 21.1% of patients had coronary artery abnormalities. 26,3% of the children required treatment with dopamine and/or milrinone. In 15.7% ICU admissions and assisted ventilation was necessary. No deaths were recorded.

Conclusion. One should bear in mind that PIMS-TS can mimic KD, appendicitis and meningitis, which may pose a diagnostic challenge.

Keywords. COVID-19, KawaCOVID, Kawasaki disease PIMS-TS, MIS-C, SISCoV

The list of abbreviations: **PIMS-TS/MIS-C** – Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2, **KD** – Kawasaki Disease, **CI** – confidence interval, **ICU** – Intensive Care Unit, **IVIG** – intravenous immunoglobulin, **PCT** – procalcitonin, **CRP** – C-reactive protein, **WHO** – World Health Organization, **CDC** – Centers for Disease Control and Prevention

Introduction

Pediatric inflammatory multisystem syndrome temporarily associated with COVID-19 (PIMS-TS), also known as a multisystem inflammatory syndrome in children (MIS-C), systemic inflammatory syndrome in COVID-19 (SISCoV), pediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (KawaCOVID), is a new condition that has emerged in children during

Corresponding author: Paulina Opalińska-Zielonka, e-mail: paulina.opal@gmail.com

Received: 10.10.2021 / Revised: 9.12.2021 / Accepted: 11.12.2021 / Published: 30.03.2022

Opalińska-Zielonka P, Wiącek K, Marczak P, Piasecka K, Korczowski B. *Clinical profile and management of patients with pediatric inflammatory multisystem syndrome – temporally associated with SARS-CoV-2 – single-center experience.* Eur J Clin Exp Med. 2022;20(1):11–17. doi: 10.15584/ejcem.2022.1.2

the COVID-19 pandemic.[1-3] In early 2020, it was expected that children will be the least affected population in terms of morbidity and mortality associated with COVID-19.[4] However, since April 2020 researchers mainly from Europe and the United States have reported many cases of severely unwell children presenting with Kawasaki disease-like features. Its regional incidence correlated with the frequency of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. Although, the incidence peaks were shifted by 2-6 weeks.[5,6] Most children met the genetic or serological criteria for SARS-CoV-2 infection.[7,8]

It is suggested that PIMS-TS results from dysregulation of the immune response to the SARS-CoV-2 infection. Many clinical signs and symptoms resemble those found in Kawasaki Disease (KD). These include systemic inflammation, persistent fever, rash, mucositis, conjunctivitis and cardiac involvement.[9] Despite those similarities, recent studies suggest that distinct inflammatory pathways are involved in the pathogenesis of KD and PIMS-TS.[10-14] In addition, patients with PIMS-TS are older, more often complain of gastrointestinal symptoms and present with features of heart failure, in comparison to children with KD. They also more frequently require Intensive Care Unit (ICU) admission, as well as cardiovascular and respiratory support.[15,16] The incidence of both entities differ in various regions around the world – most cases of KD are diagnosed in children of Asian origin, whereas PIMS-TS affects primarily patients of African, Afro-Caribbean, and Hispanic descent.[8,17]

Aim

The aim of the article was to present the clinical characteristics and management of children diagnosed with PIMS-TS. In addition, we wanted to find out the differences between the previously known and having similar features, KD and draw attention to possible difficulties in diagnosing this disease, the cases of which will continue to appear in connection with the ongoing global COVID-19 pandemic.

Material and methods

Our work is a retrospective case series study carried out in the Department of Pediatrics of the State Hospital in Rzeszów, Poland. Nineteen cases of children diagnosed with PIMS-TS were analyzed between November 15, 2020, and January 2, 2021. The diagnosis was made according to the case definitions presented in WHO and CDC publications: persistent fever, elevated inflammatory markers, features of multi-organ dysfunction, epidemiologic link to SARS-CoV-2 infection, and exclusion of other viral and bacterial pathogens as the cause of inflammation.[18,19]

The following data were collected: clinical presentation, comorbidities, laboratory findings (parameters of inflammation, blood count with smear, parameters of cardiomyocyte damage, kidney damage, coagulation, nutrition, transaminase, electrolytes), abnormalities in imaging examinations (chest X-ray, ultrasonography, echocardiography), exposure to SARS-CoV-2 (presence of antibodies against the disease in a child, confirmed/probable infection in the last weeks in one of the child's family members), applied treatment (immunoglobulin infusions, administration of glucocorticosteroids, positive inotropic agents and antithrombotic therapy), the need to stay in the ICU and return to full health.

Results are presented as counts and percentages for categorical data and medians and interquartile ranges (IQRs) for continuous data.

Ethical approval was obtained from the Bioethics Committee at University of Rzeszow (resolution 9/01/2021).

Results

The median age of the patients was 8.3 years (5.3–9.6 IQR). All cases occurred in the period from November 15, 2020, to January 2, 2021. All children were Caucasian. 42.1% of cases were female. In 3 children (15.7%) the following diseases coexisted: bronchial asthma, overweight or obesity. No other comorbidities were reported.

The entire group of 19 children was tested for active infection with the SARS-CoV-2 virus (a genetic test for RNA detection from a nasopharyngeal swab or an antigen test) and all were negative. The level of SARS-COV-2 IgG antibodies were determined in 18 patients. Their positive result was obtained in 17 out of 18 (94.4%) cases. The mother of the patient whose SARS-CoV-2 antibody level was not marked had COVID-19 infection confirmed by genetic testing 4 weeks earlier.

Out of 18 interviewed families, 13 (72.2%) reported symptoms of respiratory tract infection in a close family member of the child 4-6 weeks before hospitalization. However, only 5 out of 19 (26.3%) patients reported such symptoms at similar time.

Clinical characteristics

The mean duration of the fever was 7 days (range 5-9). Other common symptoms included abdominal pain and severe weakness (in 89.5% of cases), rash and conjunctivitis (in 84.2%), vomiting (in 73.7%) and mucous membrane involvement (in 63.2%). Hypotension, diarrhea, hyperesthesia and headache were observed in half of the children. The summary of the clinical presentations is shown in Table 1.

Our study group included a few patients with a clinical picture of the disease suggesting a different diagnosis. Persistent headache, impaired consciousness and hyperestesia were predominant in one patient. Due to

symptoms suggestive of meningitis, this child underwent a head Computed Tomography scan and lumbar puncture which showed no abnormalities. The second child presented with clinical and sonographic symptoms suggestive of appendicitis. Appendectomy was performed but no significant changes were found on histopathology. Another patient with pyuria and kidney oedema on ultrasound was initially diagnosed with pyelonephritis. Subsequently, when despite the antibiotic therapy new symptoms developed, the PIMS-TS diagnosis was established.

Table 1. Clinical presentation

	Number of patients/19 (%)
Fever	19/19 (100)
Cervical lymphadenopathy	6/19 (31.6)
Gastrointestinal symptoms: - abdominal pain - vomiting - diarrhoea	 17/19 (89.5) 14/19 (73.7) 10/19 (52.6)
Dermatological and mucosal symptoms: - rash - swollen hands/feet - conjunctivitis - red lips/strawberry tongue - peeling of the skin	 16/19 (84.2) 8/19 (42.1) 16/19 (84.2) 12/19 (63.2) 5/19 (26.3)
Cardiovascular symptoms: - hypotension - arrhythmia/bradycardia	 10/19 (52.6) 3/18 (15.8)
Respiratory symptoms - dyspnea - cough	 7/19 (36.8) 2/19 (10.5)
Neurological symptoms: - hyperesthesia - headache - confusion - photophobia	 9/19 (47.4) 9/19 (47.4) 3/19 (15.8) 5/19 (26.3)
Physical weakness	17/19 (89.5)
Petechiae	2/19 (10.5)
Swollen eyelids	4/19 (21.2)
Myalgia	4/19 (21.2)

Laboratory investigations

The highest level of inflammatory parameters was recorded on average on the 6.0 day (range 4-9) of the fever. Increased levels of CRP, D-dimers and NT-proBNP were found in all children, elevated PCT in 17 (89.5%) patients. A notable number of patients had elevated fibrinogen, ferritin and cardiac troponin levels. Abnormalities in platelet count (thrombocytopenia on admission and thrombocytosis observed later during hospitalization), anaemia, lymphopenia, neutrophilia, hypoproteinemia and hypoalbuminemia were also noticed. A detailed list of all laboratory investigations is included in Table 2.

Table 2. Laboratory findings – most abnormal level during hospitalization

	Reference value	**Abnormal n/ total (%)**	**Median (IQR)**
Leukocytes max	4.5–13.5 [10^3/µl]	7/19 (36.8)	11.8 (10.4 – 15.7)
Leukocytes min	4.5 – 13.5 [10^3/µl]	4/19 (21.1)	6.5 (5.2 – 8.3)
Granulocytes max	1 – 8.0 [10^3/µl]	11/19 (57.9)	8.4 (4.7 – 12.2)
Lymphocytes min	1.5 – 5.0 [10^3/µl]	12/19 (63.2)	1 (0.5 – 1.6)
Thrombocytes min	180 – 450 [10^3/µl]	13/19 (68.4)	144.5 (112.5 – 188)
Thrombocytes max	180 – 450 [10^3/µl]	8/19 (42.1)	398 (314 – 722)
Haemoglobin min	11.5 – 14.5 [g/dl]	16/19 (84.2)	9.7 (8.9 – 10.7)
CRP	<10 [mg/l]	19/19 (100)	183.1 (105.1 – 240.7)
PCT	<0.5 [ng/ml]	17/19 (89.5)	3.1 (1 – 11.4)
Ferritin	22 – 322 [ug/l]	10/18 (55.6)	407.1 (183.9 – 543)
Nt-proBNP	<125 [pg/ml]	19/19 (100)	5 878 (2 316.5 – 16 762.5)
Troponin T	<2,5 [ng/l]	11/19 (57.9)	288.4 (182.9 – 433.7)
D-dimers	<500 [ng/ml]	19/19 (100)	6 839 (3 115.5 – 11 369)
Fibrinogen	2 – 4 [g/l]	14/17(82.4)	5 (4 – 7.1)
Serum sodium min.	136 – 145 [mmol/l]	15/19 (78.9)	133.0 (131.5 – 134)
Serum calcium min.	9.12 – 10.2 [mg/dl]	16/19 (84.2)	8.1 (7.4 – 8.8)
Albumin min.	3.5 – 5.6 [g/dl]	12/19 (63.2)	3.1 (2.6 – 3.7)
Protein	6.8 – 8.0 [g/dl]	17/19 (89.5)	5.7 (5 – 6.1)
INR	0.85 – 1.2	11/16 (68.8)	1.3 (1.2 – 1.4)
APTT	24 – 36 [s]	9/15 (60)	37.2 (32.8 – 38.4)

Depending on the marking: max – the highest recorded value, min – the lowest recorded value and with no designation the maximum value, INR – international normalized ratio, APTT – activated partial thromboplastin time, IQR – interquartile range

Chest X-ray and ultrasonography

Chest X-rays were performed in 18 patients – in 8 (44.4%) inflammatory changes were found. In almost all of these cases, the pleural fluid was additionally visualized using ultrasound. One child with a normal radiograph showed isolated fluid in the pleural cavities. In 5 (26.3%) children, due to clinical indications, CT of the chest was additionally performed – in 4 cases pulmonary parenchyma involvement was visible.

Abdominal ultrasound was performed in 17 (89.4%) patients. 12 of them had some abnormalities, including images of acute appendicitis, swelling of the intestinal wall, mesenteric lymphadenopathy, abnormal image of the gallbladder, hepato- or splenomegaly and free fluid (which was the most common sign seen in nearly half of cases). In one child, abdominal ultrasound detected features of liver damage (correspond with the clinical status and laboratory tests), another patient had an abnormal kidney image – the obliteration of the corticomedullary differentiation. Table 3 and 4 shows in detail abnormalities found on the ultrasound examination.

Table 3. Abdominal ultrasound findings (n=17)

	Number of patients (%)
Ascites	8 (47.1)
Mesenteric lymphadenitis	5 (29.4)
Hepato/splenomegaly	5 (29.4)
Bowel wall thickening	3 (17.6)
Abnormal image of the gallbladder	2 (11.8)
Changes suggestive of acute appendicitis	2(11.8)

Table 4. Chest ultrasound findings (n=9)

	Number of patients (%)
Pleural fluid	8/9 (88.9)
Subpleural consolidations	4/9 (44.4)

Echocardiography

Each patient underwent echocardiography. The most common deviation present in nearly half of children were fluid in the pericardial sac and left ventricular systolic dysfunction (in 52.6 and 47.4% respectively). 21.1% of patients had coronary artery abnormalities: dilatation of the left coronary artery in two children (in one of them with a visible hyperechoic wall), and of the right coronary artery in the other two. Table 5 presents characteristics of the echocardiography findings.

Treatment

All children were treated with intravenous immunoglobulin infusion (IVIG) – 2 of them were given a dose of 1 g/kg, 13 – 2 g/kg and 4 received 4 g/kg. Glucocorticosteroids pulses and/or an additional dose of 2 g/kg IVIG was administered due to the lack or partial response of first line treatment (IVIG alone). Indications for escalation of treatment were: severe or worsening general condition of the child, features of shock, persistent fever 24-36 hours after the end of the IVIG infusion or its relapse.[17] Patients had the implemented treatment on an average of 5.5 days (range 4-7 day) since the onset of the fever and after an average of 2.5 (range 1-5) days of hospitalization.

Table 5. Echocardiography abnormalities (n=19)

	Number of patients (%)
Pericardial effusion	10 (52.6)
Left ventricular dysfunction	9 (47.4)
Mitral valve regurgitation	6 (31.6)
Tricuspid regurgitation	4 (21.1)
Coronary artery dilation	4 (21.1)
Enlarged left chamber	2 (10.5)
Enlarged right chamber	1 (5.3)

Intravenous methylprednisolone pulse therapy at the daily dose of 20 mg/kg for 3 days followed by 2 mg/kg/day was additionally administered in over half of the patients.[20,21] It was given on average after another 24 hours of having a fever (in 5-8 day of fever). Two (10.5%) children received 2 mg/kg/day methylprednisolone from the start of hospitalization. By the time of discharge, patients were transitioned to an equivalent dose of oral prednisone and then tapered off over two to three weeks. In one case steroid therapy was complicated by steroid-induced hyperglycemia.

Five (26.3%) children had symptoms of shock and had to be treated with dopamine and/or milrinone. Passive oxygen therapy was used in 5 children (26.3%), 3 of which required treatment in the ICU and assisted ventilation – high flow nasal cannula.

All children received antithrombotic treatment. Enoxaparin (at a dose of 1 mg/kg) and acetylsalicylic acid (at a dose of 3-5 mg/kg or 30-50 mg/kg in the case of coronary arterial involvement) were used. Table 6 presents the characteristics of the applied treatment.

Table 6. Applied treatment (n=19)

	Number of patients (%)
IVIG	19 (100)
Pulses of methylprednisolone	11 (57.9)
Dopamine and/or milrinone	5 (26.3)
Cephalosporins II/III generation	19 (100)
Another antibiotic	6 (31.6)
Betablokers	4 (21.1)
Enoxaparin	8 (42.1)
Aspirin	14 (73.7)
Furosemide	9 (47.4)
Albumin	2 (10.5)

The mean hospitalization length was 12 days (range 8-18). All children were discharged in good general condition (no symptoms, with normal echocardiography results). Only 2 patients had sinus bradycardia, the rest recovered without sequelae. No deaths were recorded.

Discussion

In the last weeks of 2020 pediatricians in our region were overwhelmed by a large number of seriously ill children with PIMS-TS. Within several weeks, children suspected of this disease were admitted to the hospital almost every day, some of whom required oxygen treatment and/or intensive care. As in other regions of the world, the rise in the number of PIMS-TS cases was preceded by a wave of SARS-COV2 infections. In our region, the increased wave of SARS-COV2 infections was delayed but it arrived with high intensity in the fall of 2020.

As it is emphasized in the previous reports the clinical picture of PIMS-TS overlaps with the symptoms present in KD. In both diseases, it can be observed multiform rash, mucosal symptoms, conjunctivitis, cervical lymphadenopathy, and hand/foot swelling. However less than 50% of children with final diagnosis of PIMS-TS meet clinical criteria for the diagnosis of KD.[22] In PIMS-TS the most common complication in the cardiovascular system is left ventricular systolic dysfunction, in KD it is dilatation/aneurysm of the coronary arteries.[23-26,34]

Symptoms of shock are more common in subjects with PIMS-TS, while similar life threatening symptoms are reported in less than 10% of children with KD (Kawasaki shock syndrome).[23,27] Gastrointestinal and neurological symptoms are undoubtedly more common in PIMS-TS.[28] Due to the frequent occurrence of gastrointestinal symptoms, especially severe abdominal pain, PIMS-TS may resemble acute appendicitis or enteritis.[29] Frequent neurological symptoms may raise the suspicion of neuro infection.[30] Due to a diverse clinical picture, the differential diagnosis of PIMS-TS is extensive and the correct diagnosis is often difficult.

In children diagnosed with PIMS-TS, increased concentrations of CRP, PCT, ferritin, D-dimers, fibrinogen, troponin, Nt-proBNP are observed. Hypoalbuminemia, anaemia, thrombocytopenia, neutrophilia and lymphopenia are commonly detected. The same laboratory abnormalities occur in KD, however, lower levels of inflammatory parameters and myocardial injury markers as well as higher leukocytes and platelets counts are found.[31,32] Both, in KD and in PIMS-TS, high serum PCT concentrations are detected. This parameter is considered to be a marker of systemic bacterial infection, while no bacterial infection can be found in both of these diseases. Probably the high PCT is the result of uncontrolled and excessive release of pro-inflammatory cytokines - a cytokine storm.[33,34]

Because the clinical picture of PIMS-TS has some similarities to KD, similar treatment methods are used.[35] All children in presented group received immunoglobulin infusions, majority were treated with intravenous corticosteroids. Intravenous antibiotic therapy was administered in all cases, until the results of negative microbiological tests (blood, urine, rectal smear culture) were obtained, which, according to the previously mentioned WHO and CDC criteria, allowed the diagnosis to be confirmed.

Conclusion

In summary, the reported group of children with PIMS-TS presented similar features as previously described. Due to the multitude of possible clinical symptoms, PIMS-TS can mimic KD, appendicitis, meningitis and other disease entities characterized by a systemic inflammatory response. It may also coexist with other diseases. Because of the overlapping clinical manifestations between PIMS-TS and KD, patients with PIMS-TS are treated with the therapeutic protocols used in KD. Further research is needed to establish the optimal therapeutic approach to a child with PIMS-TS.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, P.O.Z., P.M., K.P. and B.K.; Methodology, P.O.Z. and B.K.; Software, P.O.Z.; Validation, P.O.Z.; Formal Analysis, P.O.Z.; Investigation, P.M. and K.P.; Resources, P.M. and K.P.; Data Curation, K.P. and B.K.; Writing – Original Draft Preparation, P.O.Z., K.W. and B.K.; Writing – Review & Editing, P.O.Z.; Visualization, P.O.Z.; Supervision, K.P. and B.K..; Project Administration, P.O.Z.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

Ethics approval

Bioethics Committee at University of Rzeszow (resolution 9/01/2021).

References

1. Nakra NA, Blumberg DA, Herrera-Guerra A, et al. Multi-System Inflammatory Syndrome in Children (MIS-C) Following SARS-CoV-2 Infection: Review of Clinical Presentation, Hypothetical Pathogenesis, and Proposed Management. *Children (Basel)*. 2020;7(7):69.

2. Dhar D, Dey T, Samim MM. et al. Systemic inflammatory syndrome in COVID-19-SISCoV study: systematic review and meta-analysis. *Pediatr Res*. 2021;18:1-16.
3. Pouletty M, Borocco C, Ouldali N, et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa--COVID-19): a multicentre cohort. *Annals of the rheumatic diseases*. 2020;79(8):999-1006.
4. Choi SH, Kim HW, Kang JM, et al. Epidemiology and clinical features of coronavirus disease 2019 in children. *Pediatr Infect Vaccin*. 2020;27:11-23.
5. Dufort EM, Koumans EH, Chow EJ, et al. Multisystem Inflammatory Syndrome in Children in New York State. *N Engl J Med*. 2020;383:347-358.
6. Belot A, Antona D, Renolleau S, et al. SARS-CoV-2-related paediatric inflammatory multisystem syndrome, an epidemiological study, France, 1 March to 17 May 2020. *Euro Surveill*. 2020;25(22): 2001010.
7. Gkoutzourelas A, Bogdanos D, Sakkas I. Kawasaki Disease and COVID-19. *Mediterr Journal of Rheumatology*. 2020;31(supl 2):268-274.
8. Valverde I, Singh Y, Sanchez-de-Toledo J, et al. Acute Cardiovascular Manifestations in 286 Children With Multisystem Inflammatory Syndrome Associated With COVID-19 Infection in Europe. *Cirrculation*. 2021;143(1):21-32.
9. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi*. 1967;16(3):178-222.
10. Esteve-Sole A, Anton J, Pino-Ramírez RM, et al. Similarities and differences between the immunopathogenesis of COVID-19-related pediatric inflammatory multisystem syndrome and Kawasaki disease. *J Clin Invest*. 2021;131(6):e144554.
11. Consiglio CR, Cortugno N, Sardh F, et al. The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell*. 2020;183:1-14.
12. Kabeerdoss J, Pilania RK, Karkhele R, et al. Severe COVID-19, multisystem inflammatory syndrome in children, and Kawasaki disease: immunological mechanisms, clinical manifestations, and management. *Rheumatol Int.* 2021; 41:19-32.
13. Rowley, A. H. Understanding SARS-CoV-2-related multisystem inflammatorysyndrome in children. *Nat Rev Immunol.* 2020;20:453-454.
14. Gruber CN, Patel RS, Trachtman R, et al. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). *Cell*. 2020;183(4):982-995.e14.
15. Suzuki J, Abe K, Matsui T, et al. Kawasaki Disease Shock Syndrome in Japan and Comparison With Multisystem Inflammatory Syndrome in Children in European countries. *Front Pediatr.* 2021;9:625456.
16. Roe K. A viral infection explanation for Kawasaki disease in general and for COVID-19 virus-related Kawasaki disease symptoms. *Inflammopharmacology*. 2020;28:1219-1222.
17. Henderson LA, Canna SW, Friedman KG, et al. American College of Rheumatology Clinical Guidance for Pediatric Patients with Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with SARS-CoV-2 and Hyperinflammation in COVID-19. Version 2. *Arthritis Rheumatol.* 2020;72:1791-1805.
18. World Health Organization. Multisystem inflammatory syndrome in children and adolescents with COVID-19. https://www.who.int/news-room/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19. Accessed on January 29, 2021.
19. Centers for Disease Control and Prevention (CDC). Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019 (COVID-19).
20. https://emergency.cdc.gov/han/2020/han00432.asp. Accessed on January 29, 2021.
21. Harwood R, Allin B, Jones CE, et al. PIMS-TS National Consensus Management Study Group. A national consensus management pathway for paediatric inflammatory multisystem syndrome temporally associated with COVID-19 (PIMS-TS): results of a national Delphi process. *Lancet Child Adolesc Health*. 2020;S2352--S4642(20):30304-30307.
22. de Graeff N, Groot N, Ozen S, et al. European consensus--based recommendations for the diagnosis and treatment of Kawasaki disease – the SHARE initiative. *Rheumatology (Oxford).* 2019;58(4):672-682.
23. Yasuhara J, Watanabe K, Takagi H, Naokata S, Kuno T. COVID-19 and multisystem inflammatory syndrome in children: A systematic review and meta-analysis. *Pediatr Pulmonol.* 2021;56(5):837-848.
24. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association [published correction appears in *Circulation*. 2019;140:e181–e184]. *Circulation*. 2017; 135:927-999.
25. Dhar D, Dey T, Samim MM, et al. Systemic inflammatory syndrome in COVID-19–SISCoV study: systematic review and meta-analysis. *Pediatr Res.* 2021. doi: 10.1038/s41390-021-01545-z
26. Suzuki J, Abe K, Matsui T, et al. Kawasaki Disease Shock Syndrome in Japan and Comparison With Multisystem Inflammatory Syndrome in Children in European countries. *Front Pediatr.* 2021;19:625456.
27. Kabeerdoss J, Pilania RK, Karkhele R, et al. Severe COVID-19, multisystem inflammatory syndrome in children, and Kawasaki disease: immunological mechanisms, clinical manifestations, and management. *Rheumatol Int*. 2021;41:19-32.
28. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet*. 2020;395(10239):1771-1778.

29. Fouriki A, Fougère Y, De Camaret C, et al. Case Series of Children With Multisystem Inflammatory Syndrome Following SARS-CoV-2 Infection in Switzerland. *Front Pediatr.* 2021;8:594127.
30. Miller J, Cantor A, Zachariah P, Ahn D, Martinez M, Margolis K. Gastrointestinal Symptoms as a Major Presentation Component of a Novel Multisystem Inflammatory Syndrome in Children That Is Related to Coronavirus Disease 2019: A Single Center Experience of 44 Cases. *Gastroenterology.* 2020;159:1571-1574.
31. Chen TH. Neurological involvement associated with COVID-19 infection in children. *J Neurol Sci.* 2020;418:117096.
32. Fernández-Cooke E, Grasa CD, Domínguez-Rodríguez S, et al. Prevalence and Clinical Characteristics of SARS-CoV-2 Confirmed and Negative Kawasaki Disease Patients During the Pandemic in Spain. *Front Pediatr.* 2021;8:617039.
33. Yeo WS, Ng QX. Distinguishing between typical Kawasaki disease and multisystem inflammatory syndrome in children (MIS-C) associated with SARS-CoV-2. *Med Hypotheses*. 2020;144:110263.
34. Okada Y, Minakami H, Tomomasa T, et al. Serum procalcitonin concentration in patients with Kawasaki disease. *J Infect.* 2004;48(2):199-205.
35. Korczowski B, Mroczkowska-Juchkiewicz A, Papierkowski A. High serum procalcitonin concentration in Kawasaki disease. *Med Sci Monit.* 1998;4(6):CS1072-1074.
36. Belhadjer Z, Auriau, J, Méot M et al. Addition of Corticosteroids to Immunoglobulins Is Associated With Recovery of Cardiac Function in Multi-Inflammatory Syndrome in Children. *Circulation.* 2020;142:2282-2284.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.3

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 18–27

ORIGINAL PAPER

Piotr Choręza [1], Artur Filipecki [2], Małgorzata Kowalska [3], Aleksander J. Owczarek [4]

Healthcare services granted to patients with diagnosed acute initial or subsequent myocardial infarction in the Silesian voivodeship (Poland)

[1] Department of Statistics, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Poland
[2] 1st Department of Cardiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland
[3] Department of Epidemiology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland
[4] Health Promotion and Obesity Management Unit, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland

ABSTRACT

Introduction and aim. Cardiovascular diseases remains the leading cause of death in most of devoloped societies, including Poland. The study aimed to assess the changes in the number, duration, and costs of hospitalizations due to myocardial infarction in Silesian voivodeship (Poland) in 2009-2014.

Material and methods. Data were obtained from the Silesian Voivodeship Branch of the National Health Fund. The number, costs, and duration of healthcare services granted during an inpatient hospital stay to patients with acute initial or subsequent myocardial infarction in 2009-2014 were processed and analyzed quarterly for the whole Silesian voivodeship and its subregions.

Results. From 54826 patients aged 66±12, the majority were males (62.3%) and 63.4% of 80866 hospitalizations were granted to them. We observed a decreasing trend for the total number of healthcare services granted in 2009-2014 that varied depending on the subregion. Simultaneously, we found that in most subregions the costs of services and the number of invasive services increased over time. We observed that treating patients above 80 years with acute initial or subsequent myocardial infarction generated lower costs of hospitalization but was extended in time.

Conclusion. Increased number and costs and accompanying reduced duration of hospitalizations granted in 2009-2014, especially in the range of invasive cardiology and cardiac surgery, results from implementing international guidelines and recommendations for acute myocardial infarction procedures. Lower cost and extended time of hospitalization for patients older than 80 years most likely result from using conservative (non-invasive) methods of treatment.

Keywords. acute myocardial infarction, cardiology, healthcare services, Silesian Voivodeship

Corresponding author: Piotr Choręza, e-mail: pchoreza@sum.edu.pl

Received: 20.11.2021 / Accepted: 30.11.2021 / Published: 30.03.2022

Choręza P, Filipecki A, Kowalska M, Owczarek AJ. *Healthcare services granted to patients with diagnosed acute initial or subsequent myocardial infarction in the Silesian voivodeship (Poland).* Eur J Clin Exp Med. 2022;20(1):18–27. doi: 10.15584/ejcem.2022.1.3.

Introduction

Cardiovascular disease (CVD) remains in the field of interest of western medicine since the second half of the 18th century when British physician William Heberden described unstable angina in 1768.[1] CVD also remains the leading health problem of the European societies despite the public health and health promotion programs focused on reducing the cardiovascular risk factors and despite the progress made in medicine.

The CVD costs incurred by the economies of the European Union members constantly increase. In 2015, the costs of inpatient treatment incurred by the European healthcare systems constituted 51% of the EUR 111 billion dedicated to CVD treatment. The largest proportion of the CVD expenditures was noted in Austria (66%) and Sweden (60%) while the lowest proportion (31%) was noted in Croatia and Slovenia.[2] In the OECD's European region member states, the average time of hospitalization due to myocardial infarction was 6.7 days and it varied strongly depending on the country. The longest hospitalization time, over 10 days, was noted in Germany, while the shortest hospitalization times were noted in Scandinavia: 4.7 days in Sweden, 4.0 days in Norway, and 3.9 days in Denmark.[3] Similarly, the ratio of inpatient treatment per 100,000 citizens for patients suffering from acute coronary syndromes (ACSs) highly varied between the countries. According to the European Statistical Office (Eurostat) data, in 2014 in Poland, bear 180 hospitalizations per 100,000 citizens were caused by the ACSs. At the same time, in other Central European countries, this coefficient was higher: 206 for the Czech Republic, 207 for Hungary, 220 for Slovakia, 276 for Lithuania, 288 for Sweden, and 290 for Germany. What is interesting, in the same year 17.7% of the Polish population complained of cardiovascular ailments, while for other countries this percentage was lower: 12.8% in Germany, 7.8% in Sweden, and 5.0% in the Czech Republic.[3] However, it is alarming that the number of hospitalizations caused by ACSs has been constantly increasing since 2006 in all countries of the region, besides Sweden.[4]

Silesian voivodeship is the most urbanized and the most densely populated (urbanization level: 76.7%; population density: 367.6 people per km^2) area of Poland.[5] According to the Central Statistical Office data, the mortality due to ischemic heart disease in the Silesian voivodeship population noted in 2014 was one of the highest in Poland (79.6 per 100,000) and it was higher than Europe's average.[4] These facts make analyses of the healthcare services granted to the patients in the Silesian voivodeship necessary.

Aim

The study aimed to analyze the number of healthcare services granted during an inpatient hospital stay to the patients with acute initial or subsequent myocardial infarction (diagnosis code I21-I22 according to the ICD-10 classification) in Silesian voivodeship in 2009-2014. It also aimed to assess the temporal and spatial variability of the hospitalization costs and hospitalization time over the same period.

Material and methods

Ethical approval and consent to participate

All experimental protocols were approved by Bioethical Commission of the Medical University of Silesia (permission Nº KNW/0022/KB/68/17). All methods were carried out in accordance with relevant guidelines and regulations as well as respecting the confidentiality of biomedical data.

The range of the analyzed data

Secondary epidemiological, depersonalized data was obtained from the Silesian Voivodeship Branch of the National Health Fund of Poland (NFZ) in Katowice.

We analyzed the healthcare services in the range of pulmonology, internal medicine, nephrology, geriatrics, cardiology/invasive cardiology, and cardiac surgery provided to patients with acute initial or subsequent myocardial infarction during their inpatients' hospital stay in the Silesian voivodeship in the 2009-2014 period. Patients with acute initial or subsequent myocardial infarction were identified based on the main cause of hospitalization reported by the healthcare providers and settled by the NFZ. We analyzed the data using the ecological study model.

The database contained 123,075 healthcare services granted to 64,472 patients in the Silesian voivodeship in the 2009-2014 period. The data were aggregated before the analyzes due to the way they had been processed by the NFZ during the billing process.

Because of a small number of services granted to patients with AMI in the 2009-2014 period in the pulmonology, geriatrics, and nephrology units, they were analyzed together with the internal medicine hospitalizations and created a group of *Internal Medicine*. Similarly, the services granted to patients in the range of cardiac surgery and invasive cardiology were pooled together and created a group of *Invasive Cardiology & Cardiac Surgery.*

The following services included Ordinance of the President of National Health Fund of Poland Nº32/2008/DSOZ of the day June 11, 2008, were included: E10 – ACS-invasive diagnostics, E11 – ACS-two-step invasive treatment > 3 days, E12 – ACS-complex invasive treatment, E13 – Invasive treatment, E14 – ACS-invasive treatment < 4 days, E23 – Coronary angioplasty with single DES stent implantation, E24 – Angioplasty with implantation of not less than 2 stents or multi-vessel, E25 – Single-stent angioplasty and other procedures, E26 – Balloon coronary angioplasty.

The services with hospitalization exceeding 30 days, the services granted to patients below 25 years of age

or living outside the Silesian voivodeship's area were excluded from the data pool.

Finally, after initial data processing, 82,276 healthcare services granted to 55,143 patients were enrolled for analyses. Detailed information about the range of the enrolled services is presented in Table 1.

Table 1. The groups and the number of healthcare services in each range of services granted to patients with diagnosed acute or subsequent myocardial infarction in whole Silesian voivodeship and its subregions in 2009-2014 that were enrolled in the study

Group of healthcare services	Range of healthcare services	N (%)
Internal Medicine	Pulmonology – hospitalization Internal medicine – hospitalization Geriatrics – hospitalization Nephrology – hospitalization	15,201 (18.57%)
Invasive Cardiology & Cardiac Surgery	Cardiac surgery – hospitalization Cardiology[a]: – hospitalization, E11, E12, E13, E14 Cardiology[b]:– hospitalization, E10, E11, E12, E13, E14 Cardiology: – hospitalization, E23, E24, E25, E26	50,350 (61.5%)
Cardiology	Cardiology – hospitalization	16,315 (19.93%)
	TOTAL:	**81,866 (100%)**

[a] In the National Health Fund of Poland (NFZ) billings in the 2009-2012 period a homogeneous patients group included the services listed in the catalog as: E11, E12, E13, E14.
[b] In the National Health Fund of Poland (NFZ) billings in the 2013-2014 period a homogeneous patients group included the services listed in the catalog as: E11, E12, E13, E14, and additionally the invasive diagnostic services (E10).
Legend: Table contains the healthcare codes according to the Ordinance of the President of National Health Fund of Poland N°32/2008/DSOZ of the day June 11, 2008.
E10 – ACS-invasive diagnostics, E11 – ACS-two-step invasive treatment > 3 days, E12 – ACS-complex invasive treatment, E13 – Invasive treatment, E14 – ACS-invasive treatment < 4 days, E23 – Coronary angioplasty with single DES stent implantation, E24 – Angioplasty with implantation of not less than 2 stents or multi-vessel, E25 – Single-stent angioplasty and other procedures, E26 – Balloon coronary angioplasty
Legend abbreviations: ACS – Acute Coronary Syndrome, DES – Drug Eluting Stent

Cost assessment

The cost of hospitalizations' was based on the valuation of healthcare services by NFZ provided to patients qualified for homogeneous therapeutic groups, according to the Ordinance of the President of National Health Fund of Poland N°32/2008/DSOZ of the day June 11, 2008. The healthcare services were priced in Polish złoty (PLN) which is the official currency and legal tender of Poland. To enable comparison of the costs of therapy to other countries we present the costs of granted healthcare services in euro (€), according to the average PLN/euro exchange rate in 2009-2014, based on the archival data of the National Bank of Poland (1 € = 4.168 PLN).

Statistical analysis

The statistical analyzes were performed with the R Cran x64 v. 3.3.1 software (Lucent Technologies FR, Vienna, Austria, www.R-project.org).

The number, costs, and duration of the healthcare services in the Silesian voivodeship in the 2009-2014 period were analyzed quarterly and assigned to Silesian's subregions (*Nomenclature of Territorial Units for Statistics-3*) according to the patients' domicile. The linear regression models for obtained results were performed, talking appropriate requirements. The standardized regression coefficient (β) with the 95% confidence interval (95% CI) and the coefficient of determination (r^2) were presented. To assess the relationship between the age group of patients' and the obtained therapeutic strategy the χ^2 test was used. Statistical significance was set at a *p-value* below 0.05.

Results

Study group demography

The demographic structure of the patients is presented in Figure 1. More than half of the healthcare services were granted to men (63.4%), who constituted 62.3% of patients in the database. Accordingly, 29,945 out of 81,866 healthcare services (36.6%) were granted to women. They constituted 37.7% of patients (20,658 out of 54,826) enrolled in the study. The average age of patients receiving healthcare services was 66±12 years. Women were about 6.5 years older than men (70±12 years *vs.* 64±12 years, respectively).

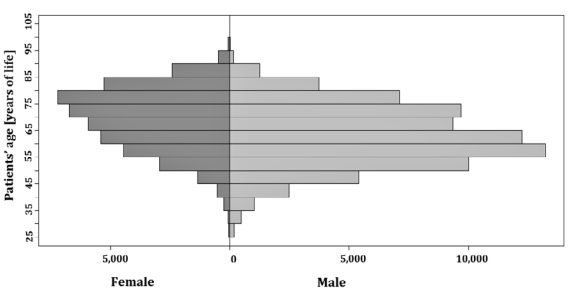

Fig. 1. Demographic structure of patients with diagnosed acute initial or subsequent myocardial infarction that received healthcare services in Silesian voivodeship in 2009-2014

Number, cost, and duration of healthcare services

Our analyses showed that the cost of granted healthcare services constantly increased over the analysed 2009-2014 period. The sudden reduction of the average cost of healthcare services observed in the third quarter of 2013, accompanied by a reduction in their number, most likely indicates a change in the billing process by the NFZ. We also found that the average duration of services tended to decrease over the analysed 2009-2014 period. The number, the average cost, and the average duration of all analyzed healthcare services granted to patients with diagnosed acute initial or subsequent myocardial infarction (diagnosis I21, I22 according to the ICD-10) in the whole Silesian voivodeship in each quarter of 2009-2014 period are presented in Figure 2.

We noted a decreasing trend of the number of granted healthcare services granted over the 2009-2014 period in all analysed ranges in the whole Silesian voivodeship, but it slightly varied between subregions. Simultaneously, we found that the costs of healthcare services increased over time. The observed trends differed between the analysed groups of services. In the group of *Internal Medicine,* we noted decreasing trends in the number, average cost, and duration of services for all analysed subregions. On the contrary, in the group of *Invasive Cardiology & Cardiac Surgery,* we found increasing trends for these variables for the whole Silesia voivodeship and analysed subregions, except for the Sosnowiec subregion. The values of the standardized regression coefficient b (with 95% CI) for each range of healthcare services granted to patients with acute initial or subsequent myocardial infarction in Silesian voivodeship and its subregions over 2009-2014 are presented in Table 2 and 3.

Cost and duration of healthcare services concerning the patient's age

The data analysis showed that the average cost of healthcare services granted in the 2009-2014 period in the Silesian voivodeship for patients under 36 years of age increased by 80.6 € with each next one year of life (YOL). In the group of patients aged 37-79, the average cost of granted healthcare services remained constant (2,085.7±68.0 €). In the group of patients over 80 years of age, the cost of services decreased by 50.2 € per one YOL. The relationship between the average costs of healthcare services concerning patients' age is presented in Figure 3.

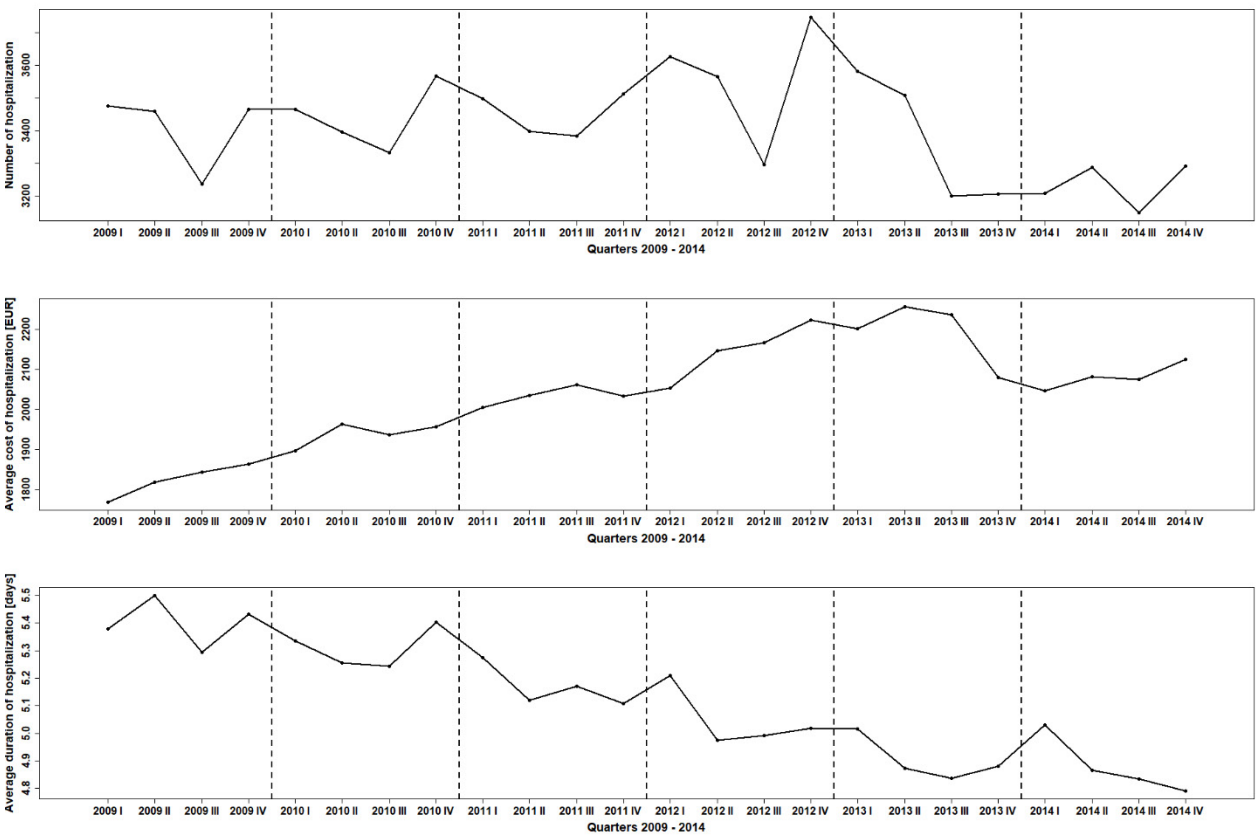

Fig. 2. Characteristics of healthcare services granted quarterly to patients with diagnosed acute initial or subsequent myocardial infarction in Silesian voivodeship in 2009-2014: A) the number of hospitalizations; B) the average cost of hospitalizations; C) the average duration of hospitalizations

Table 2. Linear regression models β coefficients and 95% confidence interval (95% CI) for the number, the average cost, and the average duration of healthcare services granted in each range to patients with diagnosed acute or subsequent myocardial infarction in whole Silesian voivodeship and its subregions in 2009-2014

Region	Total healthcare services			Hospitalizations in the group of *Internal Medicine*		
	Number	Average cost	Average duration	Number	Average cost	Average duration
Silesian voivodeship	-7.21 (-15.85 ÷ 1.43)	15.52*** (10.82 ÷ 20.22)	-0.03*** (-0.03 ÷ -0.02)	-21.31*** (-23.56 ÷ -19.06)	-2.51*** (-3.04 ÷ -1.98)	-0.09*** (-0.11 ÷ -0.07)
	0.11	0.66	0.88	0.94	0.80	0.86
Bielsko subregion	1.94* (0.18 ÷ 3.70)	10.89*** (7.07 ÷ 14.71)	-0.09*** (-0.11 ÷ -0.07)	-3.31*** (-4.02 ÷ -2.60)	-2.12 (-4.84 ÷ 0.60)	-0.15*** (-0.21 ÷ -0.09)
	0.18	0.59	0.84	0.79	0.10	0.54
Bytom subregion	1.52 (-0.34 ÷ 3.38)	13.37*** (8.55 ÷ 18.19)	0.00 (-0.002 ÷ 0.002)	-1.36*** (-1.87 ÷ -0.85)	-1.73* (-3.04 ÷ -0.42)	-0.06** (-0.10 ÷ 0.02)
	0.10	0.57	0.00	0.56	0.23	0.33
Częstochowa subregion	-2.33 (-4.82 ÷ 0.16)	19.88*** (13.82 ÷ 25.94)	0.01 (-0.01 ÷ 0.02)	-3.25*** (-3.88 ÷ -2.62)	0.00 (-1.51 ÷ 1.51)	-0.03 (-0.07 ÷ 0.01)
	0.13	0.65	0.03	0.83	0.00	0.13
Gliwice subregion	1.02 (-0.20 ÷ 2.24)	12.14*** (7.50 ÷ 16.79)	-0.03*** (-0.05 ÷ 0.01)	-1.53*** (-2.02 ÷ - 1.04)	-3.68*** (-5.50 ÷ -1.86)	-0.07** (-0.11 ÷ -0.03)
	0.11	0.54	0.40	0.62	0.41	0.38
Katowice subregion	-1.33 (-4.00 ÷ 1.34)	10.57** (4.38 ÷ 16.76)	-0.07*** (-0.08 ÷ -0.06)	-2.48*** (-3.50 ÷ -1.46)	-4.41*** (-5.76 ÷ -3.06)	-0.09*** (-0.13 ÷ -0.05)
	0.04	0.34	0.86	0.51	0.65	0.52
Rybnik subregion	1.23 (-1.38 ÷ 3.84)	23.70*** (14.91 ÷ 32.48)	-0.02** (-0.03 ÷ 0.00)	-5.43*** (-6.39 ÷ -4.47)	-1.37* (-2.33 ÷ -0.41)	-0.04** (-0.06 ÷ -0.02)
	0.04	0.56	0.25	0.85	0.26	0.28
Sosnowiec subregion	-6.53*** (-8.74 ÷ -4.32)	17.45*** (11,94 ÷ 22.96)	0.02** (0.01 ÷ 0.03)	-1.94*** (-2.57 ÷ -1.31)	-1.57 (-3.24 ÷ 0.10)	-0.09*** (-0.13 ÷ -0.05)
	0.60	0.64	0.33	0.62	0.14	0.45
Tychy subregion	-2.73*** (-4.12 ÷ -1.34)	20.51*** (13.45 ÷ 27.57)	-0.05*** (-0.07 ÷ -0.03)	-1.94*** (-2.57 ÷ -1.31)	-5.36*** (-8.04 ÷ -2.67)	-0.18*** (-0.24 ÷ 0.12)
	0.40	0.60	0.56	0.62	0.41	0.60

*** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

Moreover, our data analysis showed that the average duration of healthcare services increased with patients' age by the power function ($r^2 = 0.83$). The average duration of hospitalizations in relation to patients' age is presented in Figure 4. We observed a longer duration of the healthcare services (> 6 days) in a group of patients over 80 years of age and simultaneous reduction of costs of healthcare services that suggest using conservative (non-invasive) treatments or comorbidities in that group of patients compared to patients aged below 80.

The χ^2 test results confirm this supposition. We found that 63.4% of 70,949 healthcare services granted to patients below 80 years of life were invasive meanwhile in the group aged over 80 it was only 49.9% of 11,424 services, $p<0.001$. Patients aged below 80 years of life had more than a 1.74 greater chance to get invasive treatment compared to the elderly group (95% CI: 1.67 – 1.81).

Discussion

Demographic structure

The available literature data emphasize that the risk of morbidity and mortality due to cardiovascular diseases increase with patients' age. The studies based on the *Ryzyko program* (eng. *Risk program)* algorithm show that the risk of cardiovascular diseases is over 20% higher in the group of patients aged over 70 years old, compared to a group of patients ten years younger.[6] There is also the relationship between the extension of the average length of life and an increase in society's healthcare needs and incurred medical costs.[7, 8, 9] According to the demographic prognosis for developed societies, by 2050 more than 50% of the population will be 65 years old and above.[10, 11]

In our study, women constituted 30.73% of patients enrolled in the study, and 36.64% of healthcare services were granted to them. They were also on average 6.5 years older than the men enrolled in this study (70±12 years *vs.* 64±12 years). This can be explained by the car-

Table 3. Linear regression models β coefficients and 95% confidence interval (95% CI) for the number, the average cost, and the average duration of healthcare services granted in each range to patients with diagnosed acute or subsequent myocardial infarction in whole Silesian voivodeship and its subregions in 2009-2014

Region	Hospitalizations in the group of *Cardiology*			Hospitalizations in the group of *Invasive Cardiology & Cardiac Surgery*		
	Number	Average cost	Average duration	Number	Average cost	Average duration
Silesian voivodeship	2.20* (0.26 ÷ 4.14)	4.71*** (4.02 ÷ 5.40)	-0.08*** (-0.10 ÷ -0.06)	11.90* (3.57 ÷ 20.23)	2.88 (-1.00 ÷ 6.76)	0.01* (0.00 ÷ 0.02)
	0.18	0.89	0.81	0.26	0.09	0.21
Bielsko subregion	4.19*** (3.35 ÷ 5.03)	6.23*** (5.01 ÷ 7.45)	-0.09*** (-0.11 ÷ -0.07)	1.07 (0.01 ÷ 2.13)	13.64*** (10.92 ÷ 16.36)	-0.02*** (-0.03 ÷ -0.01)
	0.81	0.82	0.65	0.15	0.81	0.59
Bytom subregion	0.41* 0.06 ÷ 0.76)	4.69** (1.50 ÷ 7.88)	-0.02 (-0.06 ÷ 0.02)	2.49** (1.20 ÷ 3.78)	-1.50 (-6.44 ÷ 3.44)	0.02 (-0.00 ÷ 0.04)
	0.19	0.27	0.07	0.39	0.02	0.11
Częstochowa subregion	-0.38 (-0.91 ÷ 0.15)	1.83* (0.22 ÷ 3.44)	-0.15*** (-0.23 ÷ -0.07)	1.31 (-0.51 ÷ 3.13)	1.40 (-5.03 ÷ 7.83)	0.05*** (0.03 ÷ 0.07)
	0.08	0.18	0.45	0.08	0.01	0,70
Gliwice subregion	0.66** (0.21 ÷ 1.11)	-1.18 (-2.83 ÷ 0.47)	-0.06** (-0.10 ÷ -0.02)	1.88*** (1.10 ÷ 2.66)	2.62 (-2.10 ÷ 7.34)	-0.01 (-0.03 ÷ 0.01)
	0.27	0.08	0.36	0,50	0.05	0.05
Katowice subregion	-1.05 (-2.07 ÷ -0.03)	1.09 (-0.69 ÷ 2.87)	-0.17*** (-0.21 ÷ -0.13)	2.20* (0.44 ÷ 3.96)	-1.78 (-6.21 ÷ 2.65)	-0.04*** (-0.06 ÷ -0.02)
	0.16	0.06	0.74	0.21	0.03	0.65
Rybnik subregion	2.21*** (1.60 ÷ 2.82)	8.05*** (4.72 ÷ 11.38)	-0.03 (-0.07 ÷ 0.01)	4.45** (1.92 ÷ 6.98)	-2.95 (-7.52 ÷ 1.62)	-0.04*** (-0.06 ÷ -0.02)
	0.69	0.51	0.14	0.35	0.07	0.50
Sosnowiec subregion	-2.94*** (-3.90 ÷ -1.98)	6.77*** (4.99 ÷ 8.55)	-0.06*** (-0.08 ÷ -0.04)	-1.66 (-3.56 ÷ 0.24)	9.68*** (5.49 ÷ 13.87)	0.08*** (0.06 ÷ 0.10)
	0.62	0.72	0.67	0.12	0.48	0.81
Tychy subregion	-0.91** (-1.44 ÷ -0.38)	6.79*** (4.83 ÷ 8.75)	-0.06** (-0.10 ÷ -0.02)	0.16 (-0.99 ÷ 1.32)	2.30 (-2.76 ÷ 7.36)	-0.01 (-0.03 ÷ 0.01)
	0.33	0.68	0.38	0.03	0.03	0.05

*** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

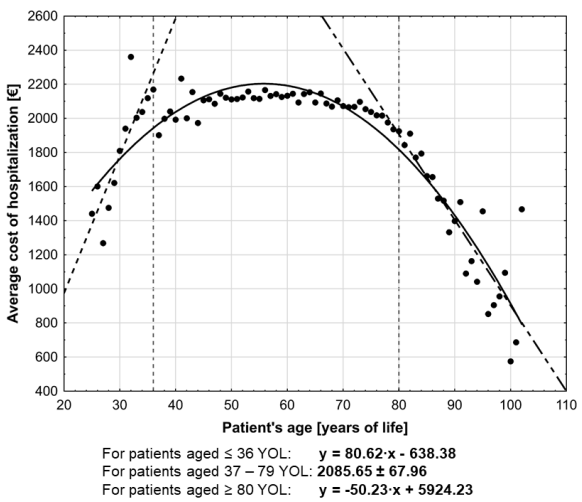

Fig. 3. Cost of healthcare services granted to patients with diagnosed acute initial or subsequent myocardial infarction in Silesian voivodeship in 2009-2014 in relation to their age

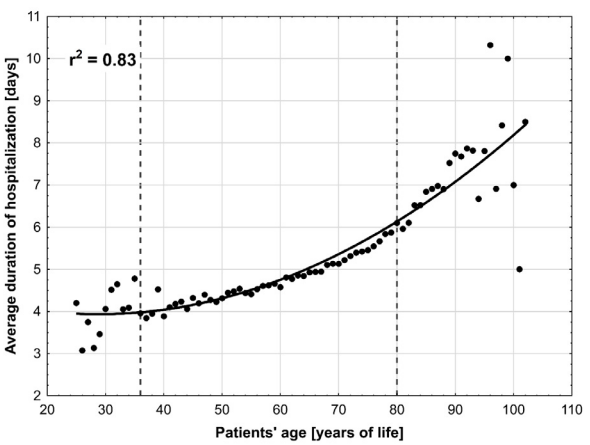

Fig. 4. Time of hospitalization of patients with diagnosed acute or subsequent myocardial infarction in Silesian voivodeship in 2009-2014 in relation to their age

dioprotective effect of estrogens and increased burden cardiovascular risk factors in the group of men.[12] The data analysis showed no significant differences in the demographic structure of patients between individual subregions of the Silesian voivodeship. These results correspond with available literature data. According to the National Institute of Hygiene (PZH) report, the average age of patients, with myocardial infarction being the main cause of hospitalization or direct cause of death in 2009-2012, was 63 years for males and 74 years for female patients.[13] According to Loundon et al., the average age of patients with diagnosed myocardial infarction in the United Kingdom in the 2000-2013 period was 67±14 years and males constituted 64.2% of patients.[14] Some studies confirm our observation that females diagnosed with myocardial infarction are about 7 years older comparing to males.[15,16]

Levels of education and income are considered the factors influencing the risk of cardiovascular disease (CVD) development. According to the 2011 General Census, 18.5% of the Silesian voivodeship population over 25 years of age had tertiary education, 32.3% had secondary education and 43.6% had primary or vocational education. The education level of the Silesian voivodeship citizens did not differ significantly from the education level of populations of other regions of Poland and the national average (20.0%, 31.4%, and 43.1%, respectively). According to the Polish Central Statistical Office, the average monthly gross salary of the Silesian voivodeship inhabitants in the 2009-2014 period, slightly exceeded the national average.[5] Since both factors were at the comparable level as the national average we may conclude that the Silesian voivodeship population wasn't more prone to CVD development when taking into account these two factors.

The number of granted healthcare services

The demand for healthcare services depends on many factors like the aging of the population, increase of wealth, and an increase in the number of concluded commercial health insurance contracts. The last one mentioned is seen as the marker of increased health awareness, which is associated with more frequent use of healthcare resources.[9] The availability of services, comprehensiveness, continuity, effectiveness and high quality of services provided are measures of the effective healthcare system.[10] Moreover, control of the number of hospitalizations may be treated as the quality and effectiveness of primary care measures.[17]

In our study, we found that the number of services granted slightly decreased in all analysed groups of healthcare services in the Silesian voivodeship over the analysed period of 2009-2014. We also found that the number of healthcare services granted slightly varied between Silesian voivodeship's subregions. Unfortunately, the direct comparison with the literature data may be difficult for this indicator. Most studies analyse cases in which acute myocardial infarction was the leading diagnosis and was a direct cause of hospitalization meanwhile in our study we analysed healthcare services granted to patients with myocardial infarction apart from the primary diagnosis. An analysis based on assigning patients to homogeneous therapeutic groups, patients with many comorbidities may be omitted.[18] For example, in the United States, the number of healthcare services granted to patients with myocardial infarction increased from 28% to 40% in the 2002-2011 period. Simultaneously, the number of hospitalizations per 100,000 citizens due to acute myocardial infarction being the leading diagnosis, decreased from 1,067 to 677.[18]

In our study, we found that in the *Internal Medicine* group the number of hospitalizations significantly decreased in the Silesian voivodeship and all analysed subregions. Simultaneously, the number of services granted in the *Invasive Cardiology & Cardiac Surgery* group tended to increase in the Silesian voivodeship and all of the subregions, except for the Sosnowiec subregion. This may have been related to the fact, that the patients with AMI over time more often received healthcare services in specialized units of invasive cardiology centres. The network of invasive cardiology centres in Poland has been developing since 1981 and in 2016 the number of these centres corresponded to the recommendations of international scientific societies.[19] The increase of accessibility of the new therapeutic strategies in Poland, including cardiac surgery and invasive cardiology services contributed to the greatest reduction in the number of deaths due to cardiovascular diseases among Central European countries. It was estimated that the increase in the availability of new therapies caused a 37% reduction in deaths due to cardiovascular diseases.[19] This estimation was especially optimistic as according to Häkkinen et al. acute myocardial infarction was the reason for 2.3±7.3% of hospitalizations in Poland in 2009 and this percentage was higher than in other analysed Western Europe countries.[20]

Observed since the mid 2010 increase in the number of services granted in the *Invasive Cardiology & Cardiac Surgery* group in the Rybnik subregion most likely results from opening the Department of Cardiology, Electrocardiology, and Angiology in Racibórz Medical Centre on September 8, 2010. Similarly, the increasing number of the same services observed in the Bielsko subregion may result from launching the Department of Cardiac Surgery in the American Heart of Poland hospital in Bielsko-Biała in 2012. The trend of the increasing number of invasive services noted in Gliwice and Katowice subregions may result from the considerable availability of invasive cardiology units, including highly specialized academic centres.

The analysis showed the decreasing trend in the number of healthcare services granted in each analysed group of services for the Sosnowiec subregion what in comparison with the increased burden of deaths due to acute or subsequent myocardial infarction noted in our previous study is wondering.[21] The observed phenomenon may result from insufficient availability of highly specialized services in the field of cardiology, invasive cardiology, and cardiac surgery for the Sosnowiec subregion inhabitants. The above-mentioned increased burden of deaths due to AMI also noted for this subregion might support this hypothesis.[21] As of 2014, none of the medical entities operating in the Sosnowiec subregion have established a cardiac surgery department, and additionally, both Będzin and Zawiercie counties (that belong to this subregion) lack a cardiology department. Some authors interpret that a decrease in the number of hospitalizations due to acute myocardial infarction in conjunction with an increase in the costs of the granted healthcare services may be the marker of treatment improvement. But these conclusions may be unauthorized, and they usually overestimate the population health status.[18]

Cost and the duration of the hospitalizations

Acute myocardial infarction remains the leading cause of hospitalization in the majority of the developed countries.[22] The annual medical costs incurred by EU member states due to cardiovascular diseases amounted to EUR 106 billion in 2009. The largest part of this sum was inpatient treatment costs, which constituted between 34% to 76% of the expenditures.[23]

In our study, we noted the increase of the average cost of healthcare services granted in *Cardiology*, *Invasive Cardiology & Cardiac Surgery* groups in the whole Silesian voivodeship as well as in each of the analysed subregions. Our results correspond with available literature sources that also notice the general trend of increasing healthcare costs and explain it with the use of new therapeutic strategies recommended by the international scientific societies.[7] On the other hand, the problem of reporting the homogeneous group, which was the highest-rated by the National Health Fund, even if it was not related to the leading medical problem during hospitalization, is well known in Poland.

Some data show that the average cost of healthcare services granted to patients with myocardial infarction in the EU member states in the 2007-2012 period was USD 5,966 and it significantly varied between member states (USD 547-10,435). Moreover, the average cost of services granted in Eastern European countries was USD 992 and it was significantly lower than for "old EU member states".[24]

Zhao and Winget noted that the costs of healthcare services granted to patients with diagnosed myocardial infarction increase with patients' age.[7] Also, there is a relationship between an optimal CVD risk profile and reduction of healthcare expenditures, and the inverse relationship between risk profile and the healthcare expenditures among the elderly.[25] Silesian voivodeship is one of the most urbanized and densely populated Polish regions (urbanization level: 76.7%, population density: 367.6 people per km^2) which creates adverse environmental conditions that increase the risk of CVD.[5] Our results show that the average cost of healthcare services granted to patients aged 33-80 years of age is constant meanwhile among patients over 80 years of age it decreases. Our findings do not contradict the information cited above, but they indicate different treatment strategies in elderly patients. The advanced age of patients is also a factor influencing the average duration of hospitalization but the gender impact on the duration of hospitalization is not clearly defined. However, for sure, the comorbidities and an emergency admission may be the reason for the extension of an inpatient stay.[15,25] The duration of hospitalization frequently serves as the simplest indicator of the healthcare system's effectiveness.[26] The average duration of granted services increased in the 2008-2012 period in most European countries, besides Sweden and Finland.[20]

We noted a slight decrease in the average duration of healthcare services granted in the 2009-2014 period. This might be related to the reduction of the number of inpatient treatments within the *Internal Medicine* and *Cardiology* groups with slight variation between each of the subregions.

The average time of healthcare services granted in the *Invasive Cardiology & Cardiac Surgery* group remained constant for the whole Silesian voivodship and most of the analysed subregions. The decreasing trends of average hospitalization time, observed in the Katowice and Rybnik subregions, may result from the effectiveness of healthcare services granted in highly specialized academic centres and new-formed commercial centre in Racibórz. Interestingly, the significant increase in the average hospitalization time and the accompanying decrease in the number of granted healthcare services were noted for the Sosnowiec subregion, including for the *Cardiology* and the *Invasive Cardiology & Cardiac Surgery* groups. That finding is alarming, especially in conjunction with a confirmed considerable burden of deaths due to acute initial or subsequent myocardial infarction among Sosnowiec subregion's citizens'.[21] The Sosnowiec subregion is one of the most heterogeneous of Silesian voivodeship's subregions both in terms of environmental conditions and resources of the health care system. In our opinion, the connection between the observed death burden and the structure of the healthcare services granted to patients in the Sosnowiec subregion requires further analyses.

The average duration of hospitalization due to AMI noted in our study is insensibly lower, compared to the national average, as well as to most of the Central European countries (data for 2011).[4] It must be mentioned that the average duration of inpatients' stay in countries of the region varies from 4.8 days in Sweden to 8 days in Germany. The reduction in the average duration of inpatient stay in Poland occurs jointly with the increasing percentage of patients with diagnosed myocardial infarction. But at the same time, longer than the national average inpatient stays are frequent in many Polish hospitals.[15,20] This agrees with the observation that the duration of hospitalization is significantly longer when conservative treatment (non-invasive) is used.[7, 25] We observed a decrease in the average cost of services and an increase in the average duration of hospitalization for patients with diagnosed acute initial or subsequent myocardial infarction older than 80 years of age. These results may indicate that they were treated using conservative (non-invasive) methods of treatment. Some studies show that a longer time of hospitalization is observed in highly specialized academic centres when compared to hospitals with a lower level of reference but transferring patients from lower to high reference level centres may explain this phenomenon.[15,25] Also, a longer time of healthcare services granted is observed in cases ended with the patient's death.[25] Since the obtained data didn't include information such as the reference level of the healthcare services provider or the healthcare services' final outcome, we were not able to determine if that was true for the analysed data from Silesian voivodeship for the 2009-2014 period.

Limitation

Although this study is based on the most reliable and complete data obtained from the National Health Fund of Poland it has some limitations.

First of all, our database includes secondary epidemiological data obtained after the billing medical services process completion, thus the authors did not have access to the original medical documentation of patients enrolled in the study.

The pattern of healthcare services and incurred costs may differ between STEMI and NSTEMI patients. Unfortunately, we do not know what type of MI was diagnosed in each case (MI with or without ST elevation), which makes data analysis difficult. Moreover, we do not know if the patients were admitted to the invasive cardiology unit within the "time window" entitled to perform the angioplasty which affects the selected therapeutic strategy and the treatment costs incurred.

Conclusions

In the 2009-2014 period, we observed the increase in numbers and costs of healthcare services granted to patients and the reduction of hospitalization time of patients with acute initial or subsequent myocardial infarction from the Silesian voivodeship in the range of invasive cardiology and cardiac surgery. We believe that this results from implanting the guidelines and recommendations of international scientific societies for acute myocardial infarction proceedings. We observed the decrease in the average cost of services and the increase in the average hospitalization time in the group of patients older than 80 years of age. We believe that they were treated using conservative (non-invasive) methods which are cheaper but require a longer recovery time when compared to a more modern approach.

The decreasing number of healthcare services granted to patients with acute initial or subsequent myocardial infarction from the Sosnowiec subregion requires further analyses.

Declarations

Funding

The project was funded by Medical University of Silesia grants no. KNW-1-162/N/9/Z and PCN-1-119/K/0/Z.

Author contributions

Conceptualization, P. Ch. and A.O.; Methodology, P.Ch. and A.O.; Software, P.Ch.; Validation, P.Ch., A.F. and A.O.; Formal Analysis, P.Ch.; Investigation, P.Ch., A.F. and A. O.; Data Curation, P. Ch..; Writing – Original Draft Preparation, P.Ch., M.K. and A.O.; Writing – Review & Editing, P.Ch. A.F, M.K. and A.O.; Visualization, P.Ch.; Supervision, A.F. and A.O.; Project Administration, P.Ch.; Funding Acquisition, A.O.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

Secondary epidemiological, depersonalized data was obtained from the Silesian Voivodeship Branch of the National Health Fund of Poland (NFZ) in Katowice after the healthcare services settlement process. Disclosure of data requires the consent of the National Health Fund of Poland, as data administrator.

The datasets analysed during the current study are not publicly available but there is possible to obtain them through access to public information. For this purpose, a written request addressed to the Director of Silesian Voivodeship Branch of the National Health Fund of Poland in Katowice should be made.

Ethics approval

All experimental protocols were approved by Bioethical Commission of the Medical University of Silesia (permission Nº KNW/0022/KB/68/17).

References

1. Kline KP, Conti CR, Winchester DE. Historical perspective and contemporary management of acute coronary syndromes: from MONA to THROMBINS2. *Postgrad Med*. 2015;127(8):855-862.
2. Wilkins E, Wilson L, Wickramasinghe K, et al. *European Cardiovascular Disease Statistics*. Brussels: European Heart Network. 2017.
3. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J.* 2016;37(42):3232-3245.
4. European Statistical Office. URL: http://ec.europa.eu/eurostat; Accessed February 9, 2021.
5. Bank Danych Lokalnych Głównego Urzędu Statystycznego/ Local Data Bank of Statistics Poland. URL: https://bdl.stat.gov.pl/BDL/start; Accessed February 9, 2021.
6. Trzeciak BG, Siebert J, Gutknecht P, Molisz A, Filipiak KJ, Wożakowska-Kapłon B. Cardiovascular risk factors determined via internet in 2 perdiods of time: 2004-2009 and 2010-2015 in Poland. *Int J Occup Med Environ Health.* 2017;30(3):499-510.
7. Harding A, Pritchard C. UK and twenty comparable countries GDP-expenditure-on-health 1980-2013: the historic and continued low priority of UK health related expenditure. *Int J Health Policy Manag.* 2016;5(9):519-523.
8. Zelený T, Bencko V. Healthcare system financing and profits: all that glitters in not gold. *Cent Eur J Public Heath.* 2015;23(1):3-7.
9. Kucharska E. System opieki zdrowotnej wobec wieloproblemowości w pomocy społecznej. *Przegl Lek.* 2012;69(9);698-702.
10. Hulme C. The cost of health care resources in cardiovascular disease. *Resuscitation*. 2013;84(7):865-866.
11. Stanhewicz AE, Wenner MW, Stachenfeld NS. Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Prysiol Heart Circ Physiol.* 2018;315(6):H1569-1588.
12. Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny, Śląski Uniwersytet Medyczny, Gdański Uniwersytet Medyczny, Warszawski Uniwersytet Medyczny. Raport: *Występowanie, leczenie i prewencja wtórna zawałów serca w Polsce.* Warszawa, Zabrze, Gdańsk; 2014.
13. Loudon BL, Gollop ND, Carter PR, Uppal H, Chandran S, Potluri R. Impact of cardiovascular risk factors and disease on length of stay and mortality in patients with acute coronary syndromes. *Int J Cardiol.* 2016;220:745-749.
14. Yin J, Lurås H, Hagen TP, Dahl FA. The effect of activity-based financing on hospital length of stay for elderly patients suffering from heart diseases in Norway. *BMC Health Serv Res.* 2013;13:172.
15. Lloyd-Jones D, Adams RJ, Brown TM et al. Heart disease and stroke statistics - 2010 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation.* 2010;121:e1-e170.
16. Lentsck MH, Latorre Mdo R, Mathias TA. Trends in hospitalization due to cardiovascular conditions sensitive to primary health care. *Rev Bras Epidemiol.* 2015;18(2):372-384.
17. Sacks NC, Ash AS, Ghosh K, Rosen AK, Wong JB, Rosen AB. Trends in acute myocardial infarction hospitalizations are we seeing the role picture? *Am Heart J.* 2015;170(6):1211-1219.
18. Kobza J, Geremek M. Explaining the Decrease in Deaths from Cardiovascular Disease in Poland. The Top-Down Risk Assessment Approach, from Policy to Health Impact. *Postepy Hig Med Dosw (Online).* 2016;70:295-304.
19. Häkkinen U, Chiarello P, Cots F, Peltola M, Rättö H. Patient classification and hospital costs of care for acute myocardial infraction in nine European countries. *Health Econ.* 2012;21(Suppl 2):19-29.
20. Choręza P, Filipecki A, Kowalska M, Owczarek A. Mortality due to acute myocardial infarction in the Silesian voivodeship (Poland) in 2009-2014. *Int J Occup Med Environ Health.* 2021;34(3):363-372.
21. Bacci MR, Fonseca FL, Nogueira LF, et al. Predominance of STEMI and severity of coronary artery disease in a cohort of patients hospitalized with acute coronary syndrome: a report from ABC Medical School. *Rev Assoc Med Bras.* 2015;61(3):240-243.
22. Tarride J, Lim M, DesMeules M, et al. A review of cardiovascular disease. *Can J Cardiol.* 2009;25:195-202.
23. Willis BL, DeFina LF, Bachmann JM, et al. Association of ideal cardiovascular health and long-term healthcare costs. *Am J Prev Med.* 2015;49(5):678-685.
24. Khosravizadeh O, Vatankhah S, Bastani P, Kalhor R, Alirezaei S, Doosty F. Factors affecting length of stay in teaching hospitals of a middle-income country. *Electron Physician.* 2016;8(10):3042-3047.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.4

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 28–35

ORIGINAL PAPER

Clara Italiano Monteiro [1], Rodrigo Polaquini Simões [1,2], Alessandro Domingues Heubel [1], Claudio Donisete da Silva [1], Paula Angélica Ricci [1], Ariane Petronilho[1], Audrey Borghi-Silva [1], Renata Gonçalves Mendes [1]

Arterial stiffness can predict cardiorespiratory fitness in type 2 diabetic patients?

[1] Physiotherapy Department, Federal University of Sao Carlos, São Carlos, São Paulo, Brazil
[2] Physiotherapy Department, Federal University of Alfenas, Alfenas, Minas Gerais, Brazil

ABSTRACT

Introduction and aim. Arterial stiffness (AS) has been associated with reduced cardiorespiratory fitness (CRF). The aim of this study was to verify if there is a relationship between augmentations index (AIx), as an index for AS assessment, and CRF in individuals with type 2 Diabetes Mellitus (T2DM).

Material and methods. Observational cross-sectional study including 32 individuals diagnosed with T2DM who performed two evaluations: 1. Arterial stiffness assessment using SphygmoCor and 2. CRF throughout a cardiopulmonary exercise test on a treadmill ergometer. Oxycon Mobile® device was used to obtain oxygen uptake consumption at peak ($\dot{V}O_{2peak}$); oxygen uptake efficiency slope (OUES) determined by linear regression in reason of the logarithmic transformation of the ventilation and $\dot{V}O_2$ obtained every minute of exercise test. Statistical analysis comprised Pearson's Correlation and linear regression analysis performed in SigmaPlot.

Results. There was a significant correlation between AS and CRF: AIx and OUES; AIx@75 and; OUES. In linear regression, AIx was determinant for $\dot{V}O_{2peak}$ and OUES – AIx and; AIx@75 and $\dot{V}O_{2peak}$.

Conclusion. AS was associated with CRF in individuals with T2DM. These results contribute to the body of evidence linking arterial functional properties to CRF and suggests greater attention for this important index.

Keywords. augmentation index, cardiorespiratory fitness, type 2 diabetes

Introduction

Arterial stiffness (AS) has been widely recognized as a clinically relevant and independent prognostic cardiovascular biomarker.[1] Comorbidities such as hypertension, obesity and diabetes negatively impacts AS, as they accelerate the inflammatory process, increasing the vascular damage.[2] Hemodynamically, AS leads to an increased pulse wave velocity (PWV) and an early- reflected pressure wave to the heart.[3] As a consequence there is an overloaded systolic peak pressure and a decreased myocardial perfusion pressure.[3]

An alternative measure of the load inflicted on the central arterial and ventricular walls[2] is the augmentation index (AIx), an indirect measure that translates how much the central pulse pressure is responsible for the reflected pulse wave.[3] It is an index influenced by the diameter and elasticity of small arteries and arterioles.[3]

Individuals diagnosed with type 2 diabetes mellitus (T2DM) have a high prevalence rate of cardiovascular disease. Evidence of accelerated increase in AS and significant changes in central hemodynamics, cardiac function and structure have been observed in T2DM.[4]

Corresponding author: Renata Gonçalves Mendes, e-mail: renatamendes@ufscar.br

Received: 1.11.2021 / Revised: 8.12.2021 / Accepted: 11.12.2021 / Published: 30.03.2022

Monteiro CI, Simões RP, Heubel AD et al. *Arterial stiffness can predict cardiorespiratory fitness in type 2 diabetic patients?* Eur J Clin Exp Med. 2022;20(1):28–35. doi: 10.15584/ejcem.2022.1.4

Advanced glycation end products in the elastic artery walls have been attributed as a causal factor of vascular dysfunction and increased AS.[4]

Wilkerson et al. respectivelyhave also demonstrated vascular function and skeletal muscle compromised in T2DM with vasodilation and increased blood flow impaired and reduced capillary density and dysfunctional capillary hemodynamics.[5]respectively Additionally, Baldi et al. have found T2DM associated with a reduced cardiorespiratory fitness (CRF) and lower peak oxygen uptake ($\dot{V}O_{2peak}$).[6] In this sense, vascular system is a component of the body´s ability to deliver oxygen to skeletal muscles during physical exercise contributing to CRF.

In addition to $\dot{V}O_{2peak}$, oxygen uptake efficiency slope (OUES) has been investigated as a function of the cardiopulmonary reserve that indicates the efficiency with which oxygen is extracted and taken to the body, with the advantage of not being affected by the intensity of the exercise.[7] Gajanand et al. showed that OUES can offer a valid submaximal measure for CRF for individuals diagnosed with T2DM integrating cardiovascular, musculoskeletal and respiratory functions.[8]

More specifically, association between increased AS and decreased CRF was previously verified in young, elderly and obese population.[3,9] Integrated responses are activated in physiologic systems to provide or improve the ability to sustain the physical exercise.[10] Cardiovascular response by maximal cardiac output directly correlates with CRF and $\dot{V}O_{2peak}$ and better CRF reflected in a lower systolic load and lower AIx.[3] Higher CRF and its beneficial anti-inflammatory also favorably affects vascular structure and function.[3]

Aim

Therefore, there seems to be a bidirectional association between AS and CRF, which has not yet been explored for the diabetic population. This study aimed to assess the relationship between AS and CRF in individuals diagnosed with T2DM. The hypothesis of the study is a presence of a negative association between AS and CRF in individuals with T2DM.

Material and methods

Study design and participants

A cross-sectional, observational, descriptive study was conducted from November 2018 to March 2019 in a sample of 32 individuals diagnosed with T2DM based on plasma glucose criteria according to American Diabetes Association, aged between 35 to 75 years old of both genders and who were invited through print and digital media and contact the researchers to voluntarily to participate in the study.[11] The non-inclusion criteria were individuals with a history of clinically proven cardiopathy and/or through examinations, vascular surgery of the carotid, femoral or aortic arteries, uncontrolled hypertension, cognitive disorders that interfere with the understanding of the experimental procedure, pregnant women and users of non-licit drugs. The exclusion criteria were factors potentially detrimental to the quality of the measurements or to make wave recording unreliable with low signal pickup, individuals who do not complete all assessments. This study was in accordance with the principles expressed in the Helsinki Declaration. The study was approved by the Human Research Ethics Committee of University (process number 2.814.754) and all individuals read and signed the free and informed consent form. The individuals were instructed to avoid alcoholic drinks, coffee or any other stimulating drink the night before and the day of data collection; do not perform activities that required moderate to heavy physical effort the day before data collection and do not speak unnecessarily during the assessment to avoid interference during signal acquisition.

Study protocol

General evaluation

Personal data, clinical history and physical examination were assessed in all patients. Individuals were also asked about medications, family and previous history and physical activity level. The level of physical activity was considered according to the ACSM: practice less than 30 minutes of moderate activity five days a week or intense physical activity for 20 minutes on three days a week were considered sedentary.[12] Dyslipidemia and hypertension were attested by previous clinical diagnosis and Brazilian guidelines.[13,14] Body mass index (BMI) >30 kg/m^2 was considered as obesity.[15]

Arterial stiffness assessment

Pulse waves were obtained transcutaneously by SphygmoCor® device (AtCor Medical Pty Ltd, Australia) with transducers in the topography of the right carotid and right femoral arteries. Measurements were taken after a 10-minute of rest in the supine position. To determine the carotid-femoral pulse wave velocity, two pressure-sensitive transducers were placed on the skin, more specifically on prominent parts of the right common carotid and right femoral artery. The software used identifies R wave of the ECG and the base of the pulse wave to calculate the time and the speed in m/s that the wave takes to go through this stretch, which is, the distance traveled by the waves between the right carotid artery and the right femoral. Two measurements were performed with no difference greater than 5% between them and the mean of the two measurements was considered the cfPWV.[1]

Pulse wave analysis was also performed by the same device and were obtained systolic and diastolic central blood pressure (SBP and DBP, respectively) and central pulse pressure (PP) to obtain augmentation index

(AIx). AIx is defined as the difference between the second and the first peak of systolic pressure, denominated augmentation pressure (AP), expressed as a percentage of the pulse pressure (PP: the difference between systolic and diastolic pressure) (AIx% = [AP/PP] × 100). This index measures the increase in the AP during systole due to the reflex of the pressure waves that travel forward of the peripheral circulation. The AP can be considered an indirect measure of arterial elasticity, being obtained by the difference between the second systolic peak (referring to the reflected wave) and the first peak (wave resulting from ventricular systole). To avoid interindividual variability secondary to heart rate, AIx could be corrected for a heart rate of 75 beats per minute (AIx@75).[16]

Cardiopulmonary exercise test (CPET)

CPET was performed on a separate day and in the afternoon, avoiding any influence of the circadian rhythm. The test was performed in a treadmill ergometer (Super ATL, Porto Alegre, Rio Grande do Sul, Brasil) with incremental protocol in steps of Bruce and in the presence of a cardiologist.[17]using an open circuit technique, during the last 2 to 4 minutes of a multistage treadmill test of maximal exercise in 151 men and 144 women of 29 to 73 years of age. $\dot{V}o_{2max}$ was higher in men than in women (P < 0.0001 Ventilatory and metabolic variables were recorded using a portable system Oxycon Mobile® (Mijnhardt/Jäger, Würzburg, Alemanha). Individuals were encouraged to perform the test until exhaustion and the criteria for test interruption were as described by Baladi.[18] $\dot{V}O_{2peak}$ was identified as the highest value observed during the final 30 seconds of the test.[18] Oxygen uptake efficiency slope (OUES) was determined by linear regression in reason of the logarithmic transformation of the ventilation (VE) and oxygen consumption ($\dot{V}O_2$) obtained every minute of CPET using the following equation ($\dot{V}O_2$ = a log VE + b), providing an accurate mathematical model for the analysis of respiratory gas exchange during incremental exercise.[7] In this equation, the constant 'a' represents the OUES coefficient and 'b' represents the intercept. Glucose levels were monitored before and after CPET and to values lower than 100 mg/dl, 15 g to 30 g of carbohydrate was offered before exercise. When blood glucose was greater than 250 mg/dl, the test was rescheduled.[19]

Statistical analysis

A posteriori power analysis was performed using GPower® *3.1 (Kiel University, Germany). Considering our sample size of* 32 individuals and a 5% error, statistical power was calculated to be 80% with and effect size of 0.46. The descriptive data were presented as mean and standard deviation. The Shapiro-Wilk test verified the data distribution. Pearson's Correlation were used to investigate relationship between AS measurements (AIx, AIx@75, and PWV) and $\dot{V}O_{2peak}$ and between AS measurements and OUES. Univariate and multiple regression analysis (adjusted for age and BMI) were also performed to identify vascular determinants for CRF ($\dot{V}O_{2peak}$ and OUES). A logarithmic transformation for the BMI was applied in order to fulfill the assumption of normality. Intraclass Correlation Coefficient was applied according Hopkins (2000) classification: 0 to 0.3 was considered a small correlation, 0.31 to 0.49 moderate, 0.50 to 0.69 large, 0.70 to 0.89 very large and 0.90 to 1.00 near perfect.[19] All tests were made in SigmaPlot 11.0 (Systat Software Inc., USA) and significant values were considered when p<0.05.

Table 1. Clinical, anthropometric, comorbidity characteristics and medications in T2DM individuals[a]

General Features	**N = 32**
Age, years	54.25 (9.07)
Men, n (%)	22 (66)
Weight, kg	85.55 (18.02)
Height, m	1.72 (0.11)
BMI, kg/m²	29.00 (5.07)
Diagnosis, months	83.97 (70.71)
Menopause, n (%)	7 (21.9)
Hb1Ac, %	8.03 (1.76)
Risk Factors	
Obesity, n (%)	13 (40.6)
Smoker, n (%)	3 (9.4)
Sedentary, n (%)	20 (62.5)
Hypertension, n (%)	14 (42.7)
Dyslipidemia, n (%)	18 (56.2)
Medications	
SGLT 2 Inhibitors, n (%)	7 (21.9)
Sulfonylurea, n (%)	10 (31.2)
Biguanide, n (%)	23 (71.9)
Glyptin, n (%)	4 (12.5)
Insulin, n (%)	8 (25.0)
Diuretic, n (%)	5 (15.6)
Angiotensin-Converting Enzyme Inhibitors, n (%)	1 (3.1)
Angiotensin II Receptor Blockers, n (%)	8 (25.0)
Beta-Blockers, n (%)	3 (9.4)
Lipid reducer, n (%)	8 (25.0)

[a] The data presented are described as mean (standard deviation) and in percentage of individuals in the sample. BMI: body mass index; SGLT-2: sodium/glucose cotransporter 2

Results

A total of fifty-two individuals were initially recruited, however, seventeen were excluded for not having completed the tests, one was excluded for presenting outliers and 2 were excluded for having a diagnosis of prediabetes. Therefore, thirty-two individuals were included in

the final sample of this study. The Table 1 shows sample characterization regarding general features, risk factors and medications. More than half of our population was classified as sedentary, diagnosed with dyslipidemia and has no controlled glycemia, as indicated by an analysis of glycated hemoglobin (Hb1Ac).

Table 2. Variables of arterial stiffness and peak CPET[a]

Arterial stiffness and hemodynamics measurements	
ASP, mmHg	123.28 (16.11)
ADP, mmHg	83.22 (8.75)
PP, mmHg	39.56 (11.68)
MAP, mmHg	99.19 (11.6)
HR, bpm	71.09 (10.45)
AP, mmHg	10.44 (5.7)
AIx, %	25.25 (8.79)
AIx@75, %	23.81 (7.53)
BSP, mmHg	135.50 (18.99)
BDP, mmHg	82.31 (8.62)
PPA, mmHg	12.22 (4.88)
PWV, m/s	8.33 (1.53)
PTT, ms	48.56 (8.49)
Cardiorespiratory exercise test variables (peak)	
HR, bpm	156.19 (20.2)
% HR_{max}	92.95 (19.6)
$\dot{V}O_2$, mL/kg^{-1}.min^{-1}	22.40 (3.56)
Systolic BP, mmHg	200.71 (30.74)
Diastolic BP, mmHg	101.19 (13.42)
VE/VCO_2 slope	40.54 (7.66)
OUES	2.00 (0.5)
RER	1.15 (0.12)
O_2P, mL/beat	12.61 (2.91)

[a] The data presented are described as mean (standard deviation). ASP: Aortic Systolic Pressure; ADP: Aortic Diastolic Pressure; PP: Pulse Pressure; MAP: Medium Arterial Pressure; HR: Heart Rate; AP: Augmentation Aortic Pressure; AIx: Augmentation index; AIx@75: Augmentation Index at the heart rate of 75 beats min^{-1}; BSP: Brachial Systolic Pressure; BDP: Brachial Diastolic Pressure; PPA: pulse pressure amplification; PWV: Pulse Wave Velocity; PTT: Pulse Transit Time; $\dot{V}O_{2peak}$: Oxygen Uptake; VE/VCO_2 slope: linear relation between minute ventilation and carbon dioxide production; OUES: oxygen uptake efficiency slope; RER: respiratory exchange ratio; % HRmax: maximum heart rate reached in percentage; O_2P: oxygen pulse

The results of the AS and CPET are outlined in Table 2 and it is possible to note that the individuals are considered with poor CRF.[21] According to the Brazilian Society of Hypertension, 6 individuals were considered to be at stage 1, 2 and 3 of hypertension (1, 2 and 1, respectively). Regarding the amplification of pulse pressure, characterized by the difference in brachial systolic pressure and aortic systolic pressure, individuals are within the normal range.[22,23]

The Figure 1 shows the correlation between AS with $\dot{V}O_{2peak}$ and OUES. Considering significant correlations found between CRF and AS measurements, a simple linear regression analysis was performed (Table 3) and a small correlation was identified. There was a significant correlation between both AS variables with OUES; however, predicting results of analysis to OUES with Aix was better than Aix75. In the OUES estimation model based on the AIx, it was able to explain 28% of OUES variance, and the following predictive equation was obtained: OUES = 2.76 – (0.03 × AIx). The regression coefficient associated with AIx was 0.03 suggesting that each one-unit increase in AIx is associated a 0.03 unit decrease in OUES.

To $\dot{V}O_{2peak}$ estimation model based on the AIx@75, this variable was able to explain 23% of $\dot{V}O_{2peak}$ variance, and the following predictive equation was obtained: $\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1}) = 27.82 – (0.23 × AIx@75). The regression coefficient associated with AIx@75 was 0.23 suggesting that each one-unit increase in AIx@75 is associated a 0.23 unit decrease in $\dot{V}O_{2peak}$.

Discussion

The aim of this study was to evaluate the association between AS and CRF, namely $\dot{V}O_{2peak}$ and OUES in individuals diagnosed with T2DM. The main findings of the study were: I) there was an association between arterial stiffness (AIx and AIx @ 75) and CRF ($\dot{V}O_{2peak}$ and OUES); II) AIx was determinant for $\dot{V}O_{2peak}$ and OUES.

As mentioned before, AIx is an indirect measurement of AS that represents the difference between the first and the second systolic peaks expressed as a percentage of pulse pressure and it is explained by the reflected pulse wave.[2,16,24] In this way, although PWV has been considered as the standard measure for AS, AIx is a substitute measure for the load inflicted on central arterial and ventricular walls.[2]

In addition, AIx is also important as an independent marker of premature coronary artery disease, a strong independent predictor of congestive heart failure and all-cause mortality in healthy and clinical populations.[24] In an apparently healthy population, Janner et al. found an average value of AIx: 21.8 for men and 30.0 for women.[24] In addition, Solanki et al. observed the following results for apparently healthy individuals from 35 to 44 years old AIx@75 results – 30.03 (9.88), from 45 to 54 years old - 29.38 (11.37), 55 to 65 years old - 32.10 (11.85).[24] Our findings regarding AIx corroborate these values found in the literature. Given the importance that AIx has gained in the literature, it deserves to be more investigated.

In people with T2DM, the study by Brooks et al. reveals an direct effect of the disease in AIx.[4] The author explains that this influence may be due to changes in the stiffness of the artery wall due to glycation, calcification

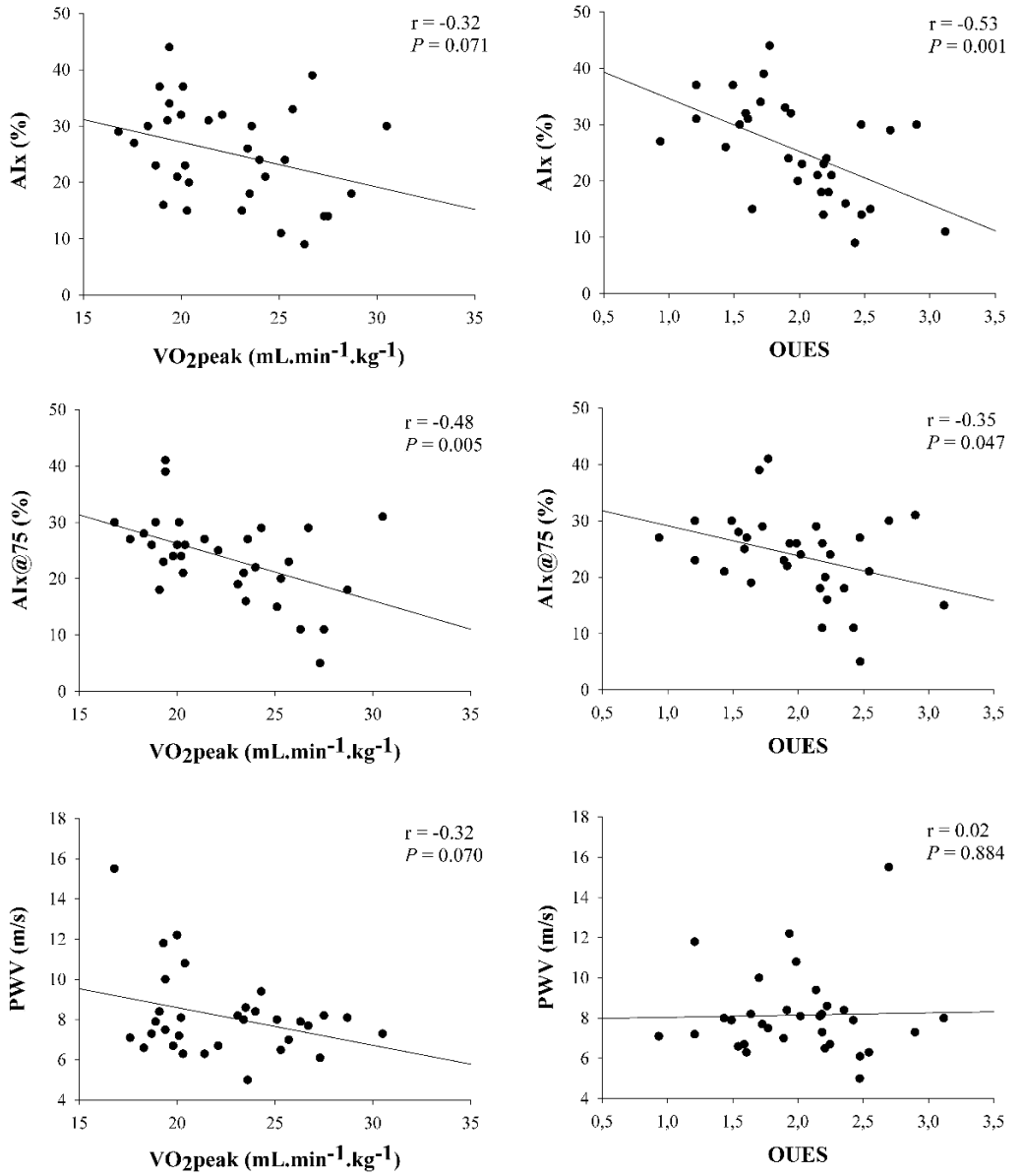

Fig. 1. Correlations between arterial stiffness measurements and oxygen uptake ($\dot{V}O_{2\,peak}$) on the right and between arterial stiffness measurements and oxygen uptake efficiency slope (OUES) on the left. AIx: augmentation index; AIx@75: augmentation index standardized to a heart rate of 75 beats per minute; PWV: pulse wave velocity

and changes in the composition of the extracellular matrix. In addition to negatively influencing vascular measurement, diabetes is also an independent predictor of cardiovascular disease and is associated with low CRF, which is another cardiovascular risk factor.[6]

The relationship of AIx with cardiorespiratory variables in individuals with T2DM is still poorly studied, however some studies showed the influence of AIx on $\dot{V}O_{2peak}$ in other populations.[3,16] Binder et al. evaluated asymptomatic men and obtained as a result that AIx is significant and inversely related to $\dot{V}O_{2peak}$ before and after confounding adjustments such as age, heart rate, body mass index and others. Denham et al. compared endurance and control athletes and concluded that there is a difference in the AIx values between the groups, but this difference is attenuated when the values were normalized by the $\dot{V}O_{2peak}$ value.[16] In addition, they concluded that moderate levels of CRF help achieve lower AIx values, avoiding adverse loading on the heart and large arteries and mitigating the risk of future cardiovascular events and all-cause mortality.

Denham et al. explains that the improvement in $\dot{V}O_{2peak}$ reflects cardiovascular adaptations associated with physical exercise.[16] Exercise increases circulating endothelial progenitor cells known to maintain the integrity of the internal arterial wall. High laminar shear stress,

subsequent release of endothelial nitric oxide by nitric oxide synthase and increased circulating EPC caused by repeated and prolonged resistance exercise sessions, influence lower blood pressure and AIx in athletes. Corroborating these data, another study explains that CRF has an anti-inflammatory and antithrombotic effect, which may affect the vascular structure and function.[3] These studies help to explain the relationship found between the variables of AS and CRF.

Table 3. Univariate regression: $\dot{V}O_2$peak estimation model based on AIx@75 (Model 1) and OUES estimation model based on AIx (Model 2)[a]

Variable	Coefficient	Standard error	P Value
Model 1 – $\dot{V}O_2$peak			
R^2 0.231			
Constant	27.82	1.89	<0.001
AIx@75 (%)	-0.23	0.076	0.00
Model 2 – OUES			
R^2 0.284			
Constant	2.76	0.23	<0.001
AIx (%)	-0.03	0.01	0.00

[a] $\dot{V}O_2$: oxygen uptake; AIx@75: augmentation index standardized to a heart rate of 75 beats per minute; OUES: oxygen uptake efficiency slope; AIx: augmentation index

Table 4. Multivariate regression: $\dot{V}O_2$peak estimation model based on AIx@75 (Model 1) and OUES estimation model based on AIx (Model 2)[a]

Variable	Coefficient	Standard error	P value
Model 1 – $\dot{V}O_{2peak}$			
Age (years)	-0.10	0.06	0.12
BMI* (kg/m^2)	-12.96	7.82	0.11
AIx@75 (%)	**-0.17**	**0.08**	0.04
Model 2 – OUES			
Age (years)	-0.01	0.01	0.29
BMI* (kg/m^2)	3.21	0.89	0.00
AIx (%)	**-0.02**	**0.01**	0.00

[a] $\dot{V}O_2$: oxygen uptake; AIx@75: augmentation index standardized to a heart rate of 75 beats per minute; OUES: oxygen uptake efficiency slope; AIx: augmentation index, *Natural logarithm transformed variables, Model 1: R^2 = 0.336; Adjusted R^2 = 0.265; P = 0.009; Model 2: R^2 = 0.536; Adjusted R^2 = 0.486; P < 0.001.

Despite our findings regarding AIx corroborating the literature, the results related to PWV are still contradictory. Augustine et al. obtained an inversely significant result between PWV and $\dot{V}O_{2máx}$ in obese middle-aged women.[9] On the other hand, some studies have also found no significant relationship between the cardiorespiratory and AS variables.[27,28]

Some studies show that AIx and PWV are influenced by different anatomical and physiological properties[24], what can explain this difference found in our study. The increased reflection and stiffness of the arterial waves cause an increase in the systolic load in the heart, limit the cardiac output during exercise and, thus, can reduce $\dot{V}O_2$.[3] In addition, Wilkinson et al. shows that the PWV is not affected by changes in heart rate, while AIx is influenced by this variable.[27] This explanation helps to reinforce our findings and the importance of adjusting AIx by heart rate. Our findings show that only AIx@75 was determinant for $\dot{V}O_{2peak}$ and it can be explained by the relationship between heart rate and AIx that occurs due to the reduction in ejection duration, causing a change in the wave reflected in the diastole.[4]

Another possible explanation is that some studies indicate that PWV increases with age, while AIx tends to stabilize after 60 years old.[24] This plateau can be explained by the reduction of impedance incompatibility between the peripheral and central arteries, decreasing the amplitude of the reflection and altering the location of the reflection of the pulse wave distally.[24] However, although the population studied was diagnosed with diabetes, the PWV was not yet characteristic of AS, that is, above 10 m/s as established by Van Bortel et al.[1] In addition, the association of AS and CRF is stronger in individuals with more advanced clinical cases and the average diagnosis time for our population is 6 years.[28]

Regarding OUES, it is known that it is a variable that reflects the integration of function and health of the skeletal, cardiovascular and pulmonary muscular systems and indicates the effectiveness with which oxygen is absorbed and distributed to the body.[7]"container-title":"Journal of the American College of Cardiology"",""-DOI":"10.1016/S0735-1097(96[10] A possible explanation for the association between AIx and OUES found in our study is metabolic acidosis, which acts on the inflammatory process of the arterial wall, releasing cytokines that can induce vascular calcification. The development of metabolic acidosis is one of the physiological bases of OUES, as it controls the distribution of blood to skeletal muscles.[7]"container-title":"Journal of the American College of Cardiology"",""DOI":"10.1016/S0735-1097(96

Although the study subjects have an OUES value within the expected, according to Myers et al., the minute ventilation/carbon-dioxide output value is slightly above the established, showing a poor prognosis and a moderate risk of cardiovascular events.[31] On the other hand, our findings regarding this variable corroborate the findings of Gürdal et al., because it is known that exercise capacity is impaired in the population diagnosed with T2DM.[32]

Conclusion

The AIx could be considered a predictive variable of CRF, since our results showed an association between AIx with OUES and AIx@75 with $\dot{V}O_{2peak}$ in individuals diagnosed with T2DM. Despite the small sample, which

is a possible limitation of our study, these findings are clinically important, AIx is easily accessible and non-invasive and has been shown to be an important measure of AS. As an important predictor of cardiovascular impairment and with proved cardiorespiratory performance association, greater attention is suggested for this important vascular variable. These results contribute to the body of evidence linking arterial functional properties to cardiorespiratory fitness.

Acknowledgments

We thank all the lab colleagues for useful discussions. This study was supported by CAPES (Code 001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of manuscript.

Declarations

Funding

CAPES - Coordination for the Improvement of Higher Education Personnel - Brazil (Financial Code 001).

Author contributions

Conceptualization, R.G.M., C.I.M. and R.P.S.; Methodology, R.G.M., C.I.M. and A.D.H.; Formal Analysis, C.I.M., A.D.H. and C.D.S.; Investigation, C.I.M., C.D.S., P.A.R., and A.P.; Resources, R.G.M., and A.B.S.; Data Curation, C.I.M.; Writing – Original Draft Preparation, C.I.M.; Writing – Review & Editing, C.I.M., R.G.M., R.P.S., A.D.H., and C.D.S.; Project Administration, R.G.M.; Funding Acquisition, R.G.M., and C.I.M.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author, RGM. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Ethics approval

The study was approved by the Human Research Ethics Committee of University (process number 2.814.754) and all individuals read and signed the free and informed consent form.

References

1. Van Bortel LM, Laurent S, Boutouyrie P, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens*. 2012;30(3):445-448. doi:10.1097/HJH.0b013e32834fa8b0
2. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006;27(21):2588-2605. doi:10.1093/eurheartj/ehl254
3. Binder J, Bailey K, Seward J, et al. Aortic Augmentation Index Is Inversely Associated With Cardiorespiratory Fitness in Men Without Known Coronary Heart Disease. *Am J Hypertens*. 2006;19(10):1019-1024. doi:10.1016/j.amjhyper.2006.02.012
4. Brooks BA, Molyneaux LM, Yue DK. Augmentation of central arterial pressure in Type 2 diabetes. *Diabet Med*. 2001;18(5):374-380.
5. Wilkerson DP, Poole DC, Jones AM, et al. Older Type 2 diabetic males do not exhibit abnormal pulmonary oxygen uptake and muscle oxygen utilization dynamics during submaximal cycling exercise. *Am J Physiol-Regul Integr Comp Physiol*. 2011;300(3):R685-R692. doi:10.1152/ajpregu.00479.2010
6. Baldi JC, Aoina JL, Oxenham HC, Bagg W, Doughty RN. Reduced exercise arteriovenous O_2 difference in Type 2 diabetes. *J Appl Physiol*. 2003;94(3):1033-1038. doi:10.1152/japplphysiol.00879.2002
7. Baba R, Nagashima M, Goto M, et al. Oxygen uptake efficiency slope: A new index of cardiorespiratory functional reserve derived from the relation between oxygen uptake and minute ventilation during incremental exercise. *J Am Coll Cardiol*. 1996;28(6):1567-1572. doi:10.1016/S0735-1097(96)00412-3
8. Gajanand T, Wallen M, Dias K, Keating SE, Coombes JS. Utility of the oxygen uptake efficiency slope in participants with overweight/obesity and type 2 diabetes. *Obes Res Clin Pract*. 2019;13(1):80-81. doi:10.1016/j.orcp.2016.10.226
9. Augustine JA, Yoon ES, Choo J, Heffernan KS, Jae SY. The Relationship Between Cardiorespiratory Fitness and Aortic Stiffness in Women with Central Obesity. *J Womens Health*. 2016;25(7):680-686. doi:10.1089/jwh.2015.5314
10. Arena R, Arrowood JA, Fei DY, Helm S, Kraft KA. Maximal Aerobic Capacity and the Oxygen Uptake Efficiency Slope as Predictors of Large Artery Stiffness in Apparently Healthy Subjects: *J Cardiopulm Rehabil Prev*. 2009;29(4):248-254. doi:10.1097/HCR.0b013e3181a3338c
11. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes—2019*. *Diabetes Care*. 2019;42(Supplement 1):S13-S28. doi:10.2337/dc19-S002
12. Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*. 2007;116(9):1081-1093. doi:10.1161/CIRCULATIONAHA.107.185649
13. Faludi A, Izar M, Saraiva J, et al. ATUALIZAÇÃO DA DIRETRIZ BRASILEIRA DE DISLIPIDEMIAS E PREVENÇÃO DA ATEROSCLEROSE - 2017. *Arq Bras Cardiol*. 2017;109(1). doi:10.5935/abc.20170121
14. Sociedade Brasileira de Cardiologia, ed. 7th Brazilian Guidelines of Hypertension. 2016;107:103.

15. World Health Organization, ed. *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*. World Health Organization; 2000.
16. Van Bortel LM, Laurent S, Boutouyrie P, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity: *J Hypertens*. 2012;30(3):445-448. doi:10.1097/HJH.0b013e32834fa8b0
17. Denham J, Brown NJ, Tomaszewski M, Williams B, O'Brien BJ, Charchar FJ. Aortic augmentation index in endurance athletes: a role for cardiorespiratory fitness. *Eur J Appl Physiol*. 2016;116(8):1537-1544. doi:10.1007/s00421-016-3407-x
18. Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J*. 1973;85(4):546-562. doi:10.1016/0002-8703(73)90502-4
19. Balady GJ, Arena R, Sietsema K, et al. Clinician's Guide to Cardiopulmonary Exercise Testing in Adults: A Scientific Statement From the American Heart Association. *Circulation*. 2010;122(2):191-225. doi:10.1161/CIR.0b013e3181e52e69
20. SBD. Diretrizes da Sociedade Brasileira de Diabetes 2019-2020 | Request PDF. Disponível em www.diabetes.org.br. Published 2019. Accessed March 21, 2020. www.diabetes.org.br
21. Will G Hopkins. New View of Statistics: Effect Magnitudes. Published 2000. Accessed September 10, 2020. http://www.sportsci.org/resource/stats/effectmag.html
22. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. Lippincott Williams & Wilkins; 2013.
23. Sociedade Brasileira de Cardiologia, ed. 7ª Diretriz Brasileira de Hipertensão Arterial. Published online 2016:103.
24. Avolio, A. P., Van Bortel Luc M., Boutouyrie Pierre, et al. Role of Pulse Pressure Amplification in Arterial Hypertension. *Hypertension*. 2009;54(2):375-383. doi:10.1161/HYPERTENSIONAHA.109.134379
25. Janner JH, Godtfredsen NS, Ladelund S, Vestbo J, Prescott E. Aortic Augmentation Index: Reference Values in a Large Unselected Population by Means of the SphygmoCor Device. *Am J Hypertens*. 2010;23(2):180-185. doi:10.1038/ajh.2009.234
26. Chirinos JA, Kips JG, Jacobs DR, et al. Arterial Wave Reflections and Incident Cardiovascular Events and Heart Failure. *J Am Coll Cardiol*. 2012;60(21):2170-2177. doi:10.1016/j.jacc.2012.07.054
27. Solanki JD, Mehta HB, Shah CJ. Aortic pulse wave velocity and augmentation index@75 measured by oscillometric pulse wave analysis in Gujarati nonhypertensives. *Vasc Investig Ther*. 2018;1(2):50. doi:10.4103/VIT.VIT_10_18
28. Haapala EA, Lee E, Laukkanen JA. Associations of cardiorespiratory fitness, physical activity, and BMI with arterial health in middle-aged men and women. *Physiol Rep*. 2020;8(10). doi:10.14814/phy2.14438
29. Namgoong H, Lee D, Hwang MH, Lee S. The relationship between arterial stiffness and maximal oxygen consumption in healthy young adults. *J Exerc Sci Fit*. 2018;16(3):73-77. doi:10.1016/j.jesf.2018.07.003
30. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol*. 2000;525(Pt 1):263-270. doi:10.1111/j.1469-7793.2000.t01-1-00263.x
31. Green DJ, Spence A, Halliwill JR, Cable NT, Thijssen DHJ. Exercise and vascular adaptation in asymptomatic humans: Exercise training and the vasculature. *Exp Physiol*. 2011;96(2):57-70. doi:10.1113/expphysiol.2009.048694
32. Myers J, Arena R, Dewey F, et al. A cardiopulmonary exercise testing score for predicting outcomes in patients with heart failure. *Am Heart J*. 2008;156(6):1177-1183. doi:10.1016/j.ahj.2008.07.010
33. Gürdal A, Kasikcioglu E, Yakal S, Bugra Z. Impact of diabetes and diastolic dysfunction on exercise capacity in normotensive patients without coronary artery disease. *Diab Vasc Dis Res*. 2015;12(3):181-188. doi:10.1177/1479164114565631

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 36–43

ORIGINAL PAPER

Aysel Topan [1], Aylin Kurt [2,3], Merve Yanik [4], Nilüfer Tatoğlu [4], Musa Özsavran [4]

The knowledge and behaviors of mothers with children 0–3 aged about pacifier use – a cross-sectional study

[1] Zonguldak Bülent Ecevit University, Faculty of Health Sciences, Pediatric Nursing, Kozlu, Zonguldak, Turkey
[2] Bartın University, Faculty of Health Sciences, Pediatric Nursing, Bartın, Turkey
[3] Bartın University, Child Education Application and Research Center, Bartın, Turkey
[4] Zonguldak Bülent Ecevit University, Institute of Health Sciences, Pediatric Nursing, Zonguldak, Turkey

ABSTRACT

Introduction and aim. The use of pacifier is a very common practice in the early childhood period in the world. In recent years, the harms of pacifiers have been discussed rather than their benefits. We aimed to determine the knowledge and behaviors of mothers with children aged 0-3 about the use of pacifiers.

Material and methods. A cross-sectional study was conducted with 363 mothers between January 10th and November 31st, 2020 in Zonguldak, Turkey. The data were collected with a web-based questionnaire. This article was prepared following STROBE guidelines.

Results. The mothers started using the pacifier for the first time when their children were at an average of 2.86±3.31 months old, and they used it for at an average of 12.06±9.13 months. Of the mothers, 36.4% were found to clean the pacifier every month and 30.6% to have the behavior of dipping the pacifier into a product such as sugar, honey, molasses, and jam. The mothers with undergraduate degrees had the behavior of cleaning pacifiers more than those with postgraduate degrees ($p<0.001$).

Conclusion. Mothers preferred to give pacifiers to babies at a high rate and had misinformation about the use of pacifiers that may harm their children's health.

Keywords. behaviors, children, mothers, pacifiers, pediatric nursing

Introduction

The use of pacifier is a very common practice in the early childhood period in the world. It is thought to meet the natural sucking needs of babies, especially in low and middle-income countries. It is a method that has deep cultural bases in our society and has been used to relieve the children for centuries.[1]

The American Academy of Paediatrics suggests delaying pacifier usage until one month of age for breastfed babies to ensure that breastfeeding is performed firmly, and using a pacifier after the establishment of breastfeeding.[2] The use of pacifier is considered to be appropriate as it reduces the risk of sudden infant death syndrome and relieves the pain in medical procedures and calms the child.[3,4] The American Academy of Pediatrics recommends that parents consider offering pacifiers to infants one month and older at the onset of sleep to reduce the risk of sudden infant death syndrome. In addition to the benefits of pacifier use, the harms are discussed.[2]

Corresponding author: Aylin Kurt, e-mail: aylinkurt67@gmail.com

Received: 8.01.2022 / Revised: 25.01.2022 / Accepted: 26.01.2022 / Published: 30.03.2022

Topan A, Kurt A, Yanik M, Tatoğlu N, Özsavran M. *The knowledge and behaviors of mothers with children 0–3 aged about pacifier use – a cross-sectional study.* Eur J Clin Exp Med. 2022;20(1):36–43. doi: 10.15584/ejcem.2022.1.5

Potential complications of pacifier use, particularly with prolonged use, include a negative effect on breastfeeding, dental malocclusion, otitis media, and starting smoking in adolescence. Adverse dental effects can be evident after two years of age, but mainly after four years.[2] The use of pacifiers may lead to oral motor dysfunction in the babies, babies' rejection of the mother's breast and their inability to receive sufficient breast milk. The babies who cannot get sufficient breast milk may be deprived of the protective effect of breast milk on the intestinal mucosa.[5] At the same time, the use of pacifiers may cause the baby not to be able to perform the sucking movements that should be done normally and the lower jaw to be left behind due to dysfunction, and its long-term use may lead to mouth construction deformity and early tooth decay. When the pacifier is not disinfected under appropriate conditions, it can cause diarrhoea in the baby.[1,6]

Mothers/caregivers are important in preventing harm that may occur due to pacifier use. There are a limited number of studies examining mothers' pacifier use in Turkey. In these studies, it is stated that mothers usually use pacifiers when they are 0-1 months old, they use pacifiers to keep them quiet when they cry, they think that using pacifiers is harmful, they find pacifiers harmful in terms of teeth development, and they would not use pacifiers for their babies if they had sufficient knowledge about it. These studies did not examine how mothers use the pacifiers.[1,7]

In our country, the reasons for mothers' use of pacifiers and their usage patterns can be affected by traditional methods. Due to these traditional methods (not changing pacifiers regularly, not cleaning pacifiers regularly, usign with honey foods etc.), babies can be harmed by the use of pacifiers. It is necessary to determine the wrong practices, to correct the practices of the families and to inform them for the future studies.

Aim

Thus, we aimed to determine the knowledge and behaviours of mothers with children aged 0-3 about the use of pacifier in Zonguldak, Turkey. In line with this general purpose, the research questions are as follows: (1) How are the behaviours of mothers' use of pacifier? (2) What are the thoughts of mothers about the use of pacifier? (3) Is there a difference in the behaviour of mothers' use of pacifier according to their level of education? (4) Is there a difference in finger sucking behaviour of children according to their first time to start pacifier and the duration of pacifier use?

Material and methods

Study design

The study used a cross-sectional study design in which the data were collected via an electronic questionnaire and analysed using descriptive and analytical statistics. This study is reported in accordance with strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies.[8]

Setting

The study used a cross-sectional study design in which the data were collected via an electronic questionnaire between January 10th and November 31st, 2020 with 363 mothers and analyzed using descriptive statistics in Zonguldak, Turkey. A Community Health Care nurse (a researcher) with an MSc degree, who was responsible for organizing the data collection. After the mothers had signed the consent form, they received, via email, a separate electronic code which they could use to enter the research electronic study. This study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies.[8]

Participants

The longest use of pacifiers is recommended between 0-3 years of age.[2] Therefore, mothers with children between the ages of 0-3 were included in the study. A convenience sample of the Zonguldak Maternity and Children's Hospital and the Family Health Centers, with a population whose medical records can be accessed, was used. An e-mail was sent to all mothers whose contact information was available and met the inclusion criteria. Inclusion criteria of mothers for the study are as follows: (1) Having a child between the ages of 0-3 (2) Using the pacifier for their child (3) Agreeing and being willing to participate in the study (4) Living in Zonguldak, Turkey. The mothers were informed about the purpose of the study, the benefits to be obtained from the research, the time to spend for the interview and their consent was obtained.

The sample size was calculated with G*Power version 3.1.2 software.[9] A post hoc power analysis was based on the correlation coefficient between psychosocial adjustment and adherence to medication. A power of 0.80 was determined at a 0.5 effect size and an alpha of 0.05.

Data collection

In the collection of data, a questionnaire form that consisted of 18 open and closed-ended questions prepared in line with the literature.[1,6] This questionnaire evaluated the socio-demographic characteristics of the mothers and their thoughts about the use of pacifier. The data were collected with the web-based questionnaire method due to the isolation and protection led by the pandemic period.

Data analysis

The data were evaluated using the Statistical Package for the Social Sciences (SPSS) for Windows 22.0 statistical program. The variables were examined using descriptive statistics (frequencies, percentages, averages and standard deviations). Cross-tabulation was performed to describe nursing encounters in relation to patient age groups. The chi-squared test was used to estimate significant differences in characteristics. The origin of the differences was detected with the Fisher

Table 1. The characteristics of mothers (n=363)

		Mean ± SD	Min - Max
Age of mother		31.04 ± 5.86	18 - 53
Age of the child included in the study (month)		17.60 ± 11.6	0 - 36
		n	%
Number of children in the family	1 child	197	54.3
	2 children	120	33.1
	3 and above children	46	12.6
Education level of mother	Primary School	46	12.7
	High School	93	25.6
	University	199	54.8
	Postgraduate	25	6.9
Employment status of mother	Unemployed	179	49.3
	Employed	184	50.7
Income level of the family	The income is less than the expenditures	46	12.7
	The income is equal to the expenditures	225	62
	The income is more than the expenditures	92	25.3

Table 2. The behaviours of mothers' use of pacifier (n=363)

The behaviours of mothers' use of pacifier			Mean ± SD	Min – Max
The first time of the child's use of pacifier (month)			2.86 ± 3.31	0 – 24
The child's duration of pacifier use (month)			12.06 ± 9.13	0 – 48
			n	%
Type of Pacifier*	Orthodontic		279	73.6
	Traditional		100	26.4
Use of pacifier chain	Yes		190	52.3
	No		173	47.7
Type of pacifier chain*	With metal clips		24	9.1
	With plastic clips		96	36.2
	Long		36	13.6
	Short		49	18.5
	Rope part decorated with swinging toy		60	22.6
Pacifier cleaning		Yes	355	97.8
		No	8	2.2
Frequency of pacifier cleaning		Monthly	132	36.4
		Every two month	104	28.7
		3 months and over	88	24.2
Pacifier change		Yes	315	86.8
		No	48	13.2
Frequency of pacifier change		Monthly	132	36.4
		Every two month	104	28.7
		3 months and over	88	24.2
Behaviour of dipping the pacifier into a product		Yes	111	30.6
		No	252	69.4
The product that the pacifier is dipped into*		Sugary products such as honey, molasses, jam	110	88
		Herbal tea	6	4.8
		Breast milk	9	7.2

* More than one option has been marked

Table 3. The thoughts of Mothers about the use of pacifier (n = 363)

Thoughts of mothers about the use of pacifier		n	%
Reasons to use pacifiers	Calming the baby	196	19.4
	Getting a comfortable sleep	42	4.2
	Organising the duration between meals	68	6.7
	Being able to keep the baby comfortable and quiet	111	11
	Preventing finger sucking	118	11.7
	Helping during teething	82	8.1
	Facilitating separation from the breast after feeding	31	3.1
	Reducing the pain when there is pain	8	0.8
	Because family elders want	353	35
Thinking that pacifier is harmful for the baby	Yes, it is harmful.	129	35.5
	No, it is not harmful.	234	64.5
The harms of the pacifier *	It damages the tooth development.	94	27.8
	It damages the chin development.	108	32
	It damages the palate structure.	91	26.9
	It prevents / reduces the baby's breast milk intake.	45	13.3

* More than one option has been marked

Exact test. A p-value of < 0.05 was considered significant.

Ethical considerations

Ethical consent was obtained from Zonguldak Bülent Ecevit University Human Research Ethics Committee for the study, dated 08/01/2020 and numbered 2020/01. Necessary written permissions were obtained from the management of the institution where the research was conducted. Data collection was based on the voluntary participation of the mothers who were included in the study. The mothers were informed about the purpose of the study and confidentiality of all data and their consent was obtained.

Results

The mean age of the mothers was 31.08±6.04 (18-53) and of the children was 17.60±11.6 (0-36) months. Of mothers, 54.3% had 1 child, 33.1% had 2 children and 12.6% had 3 and above children. Of the mothers, 54.8% were university graduates, 50.7% were employed, and the ratio of equal to income expense was 62.0% (Table 1).

The children started using the pacifier for the first time when they were at an average of 2.86±3.31 months old, and they used it for at an average of 12.06±9.13 months. 73.6% of the mothers used orthodontic pacifiers, 52.3% used pacifier chains, 36.2% used plastic clips, 97.8% cleaned the pacifier, 36.4% cleaned the pacifier every month, 36.4% changed pacifier every month, 30.6% had the behaviour of dipping the pacifier into a product, and the products that the pacifier was dipped into were sugary ones with a percentage of 88% such as honey, molasses and jam (Table 2).

In this study, 19.4% of the mothers stated that they used pacifiers to calm their babies and 35% used them as their family elders wanted. 35.5% of the mothers stated that using a pacifier was harmful. Of the mothers, 32% and 27.8% stated that the use of pacifier gave harm to the development of the jaw and the tooth development, respectively (Table 3).

There was a statistically significant difference between the behaviours of mothers' pacifier cleaning and their educational level (p=0.01). The mothers with undergraduate degrees cleaned pacifiers more than those with postgraduate degrees (p<0.001). It was found that there was no statistically significant difference between the educational level of mothers and the behaviours of thinking that the pacifier was harmful, the first time to start the pacifier, the duration of pacifier use, the frequency of pacifier cleaning, the pacifier change, the frequency of pacifier change, and the behaviour of dipping pacifier into a product (p> 0.05) (Table 4).

When finger sucking behaviours of children were compared according to their first time to start pacifier (p=0.03) and the duration of pacifier use (p=0.001), a statistically significant difference was found between them. The children who started using pacifier at 0-5 months old displayed more finger sucking behaviour than those who started it at 11-15 months and 16-24 months old (p=0.03). The children using pacifiers for 0-10 months displayed more finger sucking behaviour than those using it for 31-48 months (p=0.001) (Table 5).

Discussion

This study was carried out in Zonguldak city centre to determine the knowledge and behaviours of mothers with children aged 0-3 about the use of pacifier. In the study, the children started using the pacifier for the first time when they were at an average of 2.86±3.31 months, and they used it for at an average of 12.06±9.13 months.

Table 4. The differences in the behaviours of mothers' use of pacifier according to their level of education (n = 363)

The behaviours of mothers' use of pacifier	Educational level of mothers							
	Primary school		High school		Undergraduate		Postgraduate	
	n	%	n	%	n	%	n	%
Thinking that the pacifier is harmful								
Yes, it is.	19	14.7	29	22.5	75	58.1	6	4.7
No, it is not.	27	11.5	64	27.4	124	53	19	8.1
Statistical analysis*	$\chi2$= 3.384 p = 0.343							
The first time to start using pacifier (months)								
0-5	37	12.1	81	26.5	170	55.6	18	5.9
6-10	6	14.0	10	23.3	23	53.5	4	9.3
11-15	2	20	0	0	5	50	3	30
16-24	1	25	2	50	1	25	0	0
Statistical analysis*	$\chi2$= 13.360 p = 0.112							
Duration of Pacifier use (month)								
0-10	13	7.5	46	26.4	102	58.6	13	7.5
11-20	15	13.3	32	28.3	59	52.2	7	6.2
21-30	15	26.3	13	22.8	25	43.9	4	7.0
31-48	3	25.0	2	16.7	7	58.3	0	0.0
Statistical analysis*	X^2 16.540 p = 0.056							
Cleaning pacifiers	42	11.8	92	25.9	197	55.5	24	6.8
Statistical analysis*	**$\chi2$** = 11.245 p = 0.010							
Post hoc **	3-4 (p < 0.001)							
Frequency of cleaning pacifiers								
Monthly	21	9.1	57	24.8	133	57.8	19	8.3
Every two month	15	16.3	28	30.4	45	48.9	4	4.3
3 months and over	4	12.5	7	21.9	19	59.4	2	6.3
Statistical analysis*	X^2 = 6.513 p = 0.364							
Changing pacifiers	36	11.4	84	26.7	171	54.3	24	7.6
Statistical analysis*	$\chi2$ = 6.194 p = 0.103							
Frequency of changing pacifiers								
Monthly	13	9.8	30	22.7	79	59.8	10	7.6
Every two month	17	16.3	32	30.8	50	48.1	5	4.8
3 months and over	8	9.1	22	25	49	55.7	9	10.2
Statistical analysis*	$\chi2$ = 7.566 p = 0.263							
The behaviour of dipping the pacifier into a product	22	19.8	29	26.1	54	48.6	6	5.4
Statistical analysis*	$\chi2$ = 7.656 p = 0.54							

* $\chi2$: Chi-square test, ** Post hoc: Fisher exact test

In this study, 19.4% of the mothers stated that they used pacifiers to calm their babies and 35% used them as their family elders wanted, and 35.5% of the mothers stated that using a pacifier was harmful.

The mothers in this study started giving pacifiers to their babies in the first months of their lives. Contrary to this practice, it is clearly stated in the World Health Organization's Ten Steps for Successful Breastfeeding Principles that "no baby bottles or fake pacifiers should be given to breastfed babies". In order not to reduce the breast milk intake of babies, it is recommended not to give pacifiers to babies who are only breastfed (for six months).[10] Mothers can concern that pacifiers may interfere with breastfeeding.[11] However, some current studies showed that early versus late recommendation of pacifier introduction did not affect the proportion of breastfeeding at six months.[12,13] Exclusive breastfeeding is associated with reduced pacifier sucking in children at 12 months. Promotion of exclusive breastfeeding may reduce the use of pacifiers and their potential deleterious effects.[14]

In this study, 73.6% of the mothers used orthodontic and 26.4% traditional pacifiers. Of the mothers, 35.5% stated that using a pacifier was harmful. The mothers in the study try different types of pacifiers in order to reduce the harms of the pacifier. In the study by Sezici and

Table 5. The differences in finger sucking behaviour of children according to their first time to start pacifier and the duration of pacifier use (n = 363)

			The child's finger sucking behaviour	Statistical analysis*	Post hoc **
The child's first time to start pacifier (month)	0-5 (1)	n	62		
		%	78.5		
	6-10 (2)	n	11	**χ2** = 5.300 p = 0.021	1-3 (p = 0.03) 1-4 (p = 0.03)
		%	13.9		
	11-15 (3)	n	3		
		%	3.8		
	16-24 (4)	n	3		
		%	3.8		
The duration of pacifier use (months)	0-10 (1)	n	51		
		%	65.4	**χ2** = 11.666 p = 0.09	1-4 (p = 0.001)
	11-20 (2)	n	19		
		%	24.4		
	21-30 (3)	n	7		
		%	9.0		
	31-48 (4)	n	1		
		%	1.3		

* χ2: Chi-square test, **Post hoc: Fisher exact test

Yiğit[7], the rate of orthodontic pacifier use was found to be as 68% and traditional pacifier use as 31.4%. On the other hand, in another study, the rates of traditional and orthodontic pacifier use were determined to be as 13.3% and 24%, respectively.[15] The use of orthodontic pacifier is thought to stimulate muscle contraction, tongue position and nasal breathing, similar to breastfeeding, thus not interfering with facial growth and occlusion. Therefore, orthodontic pacifiers are preferred more frequently by mothers.[16,17]

In this study, 30.6% of the mothers in this study had the behaviour of dipping the pacifiers into products, and the products were mostly the sugary ones (88%) such as honey, molasses and jam. Dipping the pacifier into a nutrient and giving it to the child is a traditional practice in our country and learned from the elders of the family. However, this situation may cause the child to stop breastfeeding in the early period. The history of the pacifier is based on the use of sugary products or honey to calm the new-born.[18,19] Sezici and Yiğit[7] stated in their study that 53.4% of the mothers gave the pacifier to their babies with sugary food.

There was a statistically significant difference between the mothers' pacifier cleaning and their level of education (p=0.01). Among the mothers, those with undergraduate degrees cleaned pacifiers more than those with postgraduate degrees (p<0.001). Behaviour of thinking that the pacifier was harmful, the first time to start the pacifier, the duration of pacifier use, the frequency of pacifier cleaning, the pacifier change, the frequency of pacifier change, and the behaviour of dipping the pacifier into a product did not differ regarding the educational level of the mothers (p>0.05). The maternal education level stands out as the leading factor affecting the use of pacifiers. The more the educational level of mother got, the less the duration of pacifier use became. The number of dentist visits in the children of mothers with high educational level was also high.[20]

In this study, starting to use pacifiers at 0-5 months, when sucking behaviour was seen mostly, caused finger sucking behaviour in children. The children using pacifiers for 0-10 months displayed more finger sucking behaviour than those using it for 31-48 months (p=0.001). According to the results, children sustain the situation of having satisfaction from the pacifier with the behaviour of finger sucking. In cases where breastfeeding cannot occur, the pacifier improves the sucking reflex by providing the neurobehavioral organization of the baby.[21] According to Freud's psychoanalytic theory, 0-5 months corresponds to the oral period. In this period, children display sucking behaviour intensely and reach oral pleasure.[22] However, Butler et al. stated that infants in the pacifier users were reported to have more night wakings, to sleep longer during the day, and to shorter stretches of nighttime sleep than finger suckers into 0-6 months.[23] Using pacifiers and finger sucking can be used to relax and help to children to sleep.

Limitations

There are some limitations of our study. Firstly, the data were collected via an electronic questionnaire. The fact that mothers cannot be reached directly may limit

whether the questionnaires are filled in by the right people (convincing/certainty). In future studies, authors can use any video app or telephone calls for interviews, rather than just an electronic questionnaire.

This research was performed according to a cross-sectional study design in Zonguldak, Turkey. The data were and represent the perceptions of those mothers who were invited to participate in that region in Turkey. Thus, generalizability of the findings is limited. The questionnaire was used for the first time in this study, and no statistical testing was conducted to prove the reliability and validity of the entire questionnaire. The last limitation of this study is that the authors did not take into account clinical data, e.g. newborn/premature infant, child's weight, weight gain, etc. In future studies, it is recommended to examine the impact of such data on pacifier use.

Conclusion

The mothers gave a pacifier to their children up to an average of 1 year old, mothers had misinformation about the use of pacifiers, which would harm the health of their children, and the level of education was important in the occurrence of this situation. It is necessary to determine the wrong practices (such as not changing pacifiers regularly, not cleaning pacifiers regularly, using with honey foods) to correct the practices of the families and to inform them for the future studies.

Considering the important roles of pediatric nurses in the treatment and follow-up of children, the current situation needs to be improved. In line with the results obtained from the research, it is recommended to increase the effectiveness and efficiency of breastfeeding education led by pediatric nurses given within the scope of "Promoting Breastmilk and Baby-Friendly Health Institutions Program" to mothers and mother candidates in health institutions, and to inform especially about the harms of pacifier use.

Acknowledgements

The authors thank all parents who participate in this study.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, A.T., A.K., M.Y., N.T and M.Ö.; Methodology, A.T., A.K., M.Y., N.T and M.Ö.; Software, A.T., A.K., M.Y., N.T and M.Ö.; Validation, A.T., A.K., M.Y., N.T and M.Ö.; Formal Analysis, A.T., A.K., M.Y., N.T and M.Ö.; Investigation, A.T., A.K., M.Y., N.T and M.Ö.; Resources, A.T., A.K., M.Y., N.T and M.Ö.; Data Curation, A.T., A.K., M.Y., N.T and M.Ö.; Writing – Original Draft Preparation, A.T., A.K., M.Y., N.T and M.Ö.; Writing – Review & Editing, A.T., A.K., M.Y., N.T and M.Ö.; Visualization, A.T., A.K., M.Y., N.T and M.Ö.; Supervision, A.T., A.K., M.Y., N.T and M.Ö.; Project Administration, A.T., A.K., M.Y., N.T and M.Ö.; Funding Acquisition, A.T., A.K., M.Y., N.T and M.Ö.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

Data available on request from the authors.

Ethics approval

Ethical consent was obtained from Zonguldak Bülent Ecevit University Human Research Ethics Committee for the study, dated 08/01/2020 and numbered 2020/01.

References

1. Uğurlu ES, Çakal MM, Avşar A. Annelerin 0-1 yaş arası bebeklerinde emzik kullanma durumları. *ACU SAğlık Bilim Derg*. 2017;1:10-15.
2. Moon RY, Darnall RA, Feldman-Winter L, Goodstein MH, Hauck FR. SIDS and other sleep-related infant deaths: Updated 2016 recommendations for a safe infant sleeping environment. *Pediatrics*. 2016;138(5).
3. Efendi D, Rustina Y, Gayatri D. Pacifier and swaddling effective in impeding premature infant's pain score and heart rate. *Enferm Clin*. 2018;28:46-50.
4. Jin H, Kim IA, Jeong MH, Park KY, Kim BS. Salivary Cortisol and Pain Scoring to Compare the Efficacy of Oral Dextrose and Pacifier for Neonatal Pain Control. *Perinatology*. 2018;29(2):83.
5. Kaye EC, Friebert S, Baker JN. Early integration of palliative care for children with high-risk cancer and their families. *Pediatr Blood Cancer*. 2016;63:593-597.
6. Doğramacı EJ, Rossi-Fedele G. Establishing the association between nonnutritive sucking behavior and malocclusions: A systematic review and meta-analysis. *J Am Dent Assoc*. 2016;147(12):926-934.e6.
7. Sezici E, Yiğit D. Knowledge, Attitude, and Behavior about the Pacifier use of Mothers of Hospitalized. *J Curr Res Heal Sect*. 2018;8(1):29-40.
8. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-349.
9. Erdfelder E, FAul F, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods*. 2009;41(4):1149-1160.
10. World Health Organization and UNICEF. Baby-friendly hospital initiative : revised, updated and expanded for integrated care. Geneva, Switzerland; 2009.

11. Rocha CR, Verga KE, Sipsma HL, Larson IA, Phillipi CA, Kair LR. Pacifier use and breastfeeding: A qualitative study of postpartum mothers. *Breastfeed Med*. 2020;15(1):24-28.
12. Hermanson Å, Åstrand LL. The effects of early pacifier use on breastfeeding: A randomised controlled trial. *Women and Birth*. 2020;33(5):e473-e482.
13. Jaafar SH, Ho JJ, Jahanfar S, Angolkar M. Effect of restricted pacifier use in breastfeeding term infants for increasing duration of breastfeeding. *Cochrane Database Syst Rev*. 2016;2016(8):CD007202.
14. Gomes-Filho IS, Pinheiro SMS, Vieira GO, Alves TDB, Cruz SS da, Ana Claudia Morais Godoy Figueiredo, Eduardo Luiz Andrade Mota NF de O, et al. Exclusive breast-feeding is associated with reduced pacifier sucking in children: Breast-feeding and pacifier-sucking habit. *J Am Dent Assoc*. 2019;150(11):940-947.
15. Poureslami H, Hoseinifar R, Hosseinifar R, Sharifi H, Poureslami P. The frequency of using different types of pacifier and bottle nipple among 1-24 months old children. *J Oral Heal Oral Epidemiol*. 2016;5(1):13-16.
16. Lima AA dos SJ, Alves CMC, Ribeiro CCC, Pereira ALP, da Silva AAM, Silva LFG e., et al. Effects of conventional and orthodontic pacifiers on the dental occlusion of children aged 24–36 months old. *Int J Paediatr Dent*. 2017;27(2):108-119.
17. Maurya RK, Singh H, Malyala D, Niranjan B, Dubey A. Evaluation of effect of orthodontic pacifiers in prevention of sudden infant death syndrome: A finite element method and questionnaire based study. *J Clin Pediatr Dent*. 2021;45(2):74-82.
18. Santos TR dos, Buccini G dos S, Sebastião LT. Factors associated with pacifier use among children of working women with childcare in the workplace. *Rev CEFAC*. 2017;19(5):654-663.
19. Buccini G dos S, Pérez-Escamilla R, Paulino LM, Araújo CL, Venancio SI. Pacifier use and interruption of exclusive breastfeeding: Systematic review and meta-analysis. *Matern Child Nutr*. 2017;13(3):1-19.
20. Kakti AA, Alabdullah AS, Abrar Alahmed ZA, Alswailem E, Alhassan H. Prevalence of pacifier use and the impact of maternal education and regularity of dental visits on the age of pacifier withdrawal. *J Indian Soc Pedod Prev Dent*. 2019;37(1):8-11.
21. Lubbe W, ten Ham-Baloyi W. When is the use of pacifiers justifiable in the baby-friendly hospital initiative context? A clinician's guide. *BMC Pregnancy Childbirth*. 2017;17(1):1-10.
22. Gündoğdu YB. The possibility of development and change by psychoanalytic theory of personality. *J Turkish Stud*. 2016;11(17):373-373.
23. Butler R, Moore M, Mindell JA. Pacifier use, finger sucking, and infant sleep. *Behav Sleep Med*. 2016;14(6):615-623.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.6

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 44–48

ORIGINAL PAPER

Naciye Kış [1], Hanife Gülden Düzkalır [2], Mehmet Oğuzhan Ağaçlı [3], Berrin Erok [1], Zeynep Gamze Kılıçoğlu [4]

Contribution of diffusion weighted MRI to the differential diagnosis of renal masses

[1] Department of Radiology, University of Medical Sciences, Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey
[2] Department of Radiology, University of Medical Sciences, Kartal Education and Research Hospital, İstanbul, Turkey
[3] Department of Radiology, University of Medical Sciences, Umraniye Education and Research Hospital, İstanbul, Turkey
[4] Department of Radiology, University of Medical Sciences, Haydarpaşa Education and Research Hospital, İstanbul, Turkey

ABSTRACT

Introduction and aim. We aimed to assess the usefulness of diffusion weighted imaging (DWI) and apparent diffusion coefficients (ADCs) for characterizing renal masses.

Material and method. In this retrospective study we measured the ADC values of renal masses at b=0, b=500 and b=1000. Measurements were made by placing a circular region of interest with a diameter of 1 cm.ADC values from normal renal parenchyma were taken to define the ADC and to compare with the ADC values of the lesions.

Results. A total of 72 lesions of 54 patients were included.40 of the masses were benign and 32 were malignant. The ADC values of benign lesions at both b values were significantly higher than malignant lesions. We found the lowest values in angiomyolipomas (AMLs) and oncocytomas and the highest values in Bosniac type I cysts. Similarities was found between the ADC values of some AMLs and the RCCs. In terms of statistical results, the inclusion of AMLs in the analysis did not significantly affect the difference between malignant and benign lesions.

Conclusion. In our study, the ADC values of benign renal masses were higher than those of normal renal parenchyme, which is higher than those of malignant renal masses.The lowest ADC values were observed in AMLs and oncocytomas.

Keywords. apparent diffusion coefficient, diffusion weighted imaging, renal neoplasms

Introduction

Benign and malignant kidney lesions can originate from different tissues. Characterization of renal masses is needed in the treatment planning. Renal cell carcinoma (RCC) is the most common malignant kidney tumor in adults.[1] There are three major subtypes of RCC: clear cell RCC, papillary RCC and chromophobe RCC. Since these subtypes have different prognoses and responses to molecular therapy, subtyping is important and cross-sectional imaging is essential for the detection and characterization.[2-4] Magnetic resonance imaging (MRI) has many advantages including high contrast resolution, absence of ionizing radiation, less toxicity of its contrast agents compared to iodinated contrast agents. Diffusion

Corresponding author: Berrin Erok, e-mail: drberrinerok@hotmail.com

Received: 15.09.2021 / Revised: 15.11.2021 / Accepted: 22.11.2021 / Published: 30.03.2022

Kış N, Düzkalır HG, Ağaçlı MO, Erok B, Kılıçoğlu ZG. *Contribution of diffusion weighted MRI to the differential diagnosis of renal masses*. Eur J Clin Exp Med. 2022;20(1):44–48. doi: 10.15584/ejcem.2022.1.6

weighted imaging (DWI) is a method that enables the characterization of biological tissues based on the diffusion properties of water molecules. However, since this method is sensitive to cardiac, respiratory and peristaltic movements, its use in the early days was limited in brain examination. Nowadays, with the development of fast MRI sequences such as echo-planar imaging (EPI), it has started to be used in other body areas effectivelly. Since the sequences used in DWI is also T2 weighted, "apparent diffusion coefficient" (ADC) maps with only diffusion effect are created to erase the T2 effect.[5]

Aim

The main purpose of our study is to determine DWI findings of various renal masses and to investigate their contribution to the diagnosis regarding benign and malignant differentiation by presenting the characteristic features and calculating the ADC values that may be useful in differential diagnosis.

Material and methods

In this retrospective study we measured the ADC values of renal masses of 54 cases at b=0, b=500 and b=1000. Measurements were made by placing a circular region of interest (ROI) with a diameter of 1 cm on the lesions. In the relatively homogeneous lesions larger than 2 cm, the average of 3 separate ROI measurements in the same slice was calculated. On the other hand, for the lesions with heterogeneous internal structure, the measurements were made from the solid parts that enhances on postcontrast images and shines most on DWI. The ADC value of the lesions with a diameter of 1 cm was made by using a single ROI. In addition, the average of 3 different ADC values from normal renal parenchyma were taken to define the ADC and to compare with the ADC values of the lesions.

Statistical analysis

Data were analyzed using the IBM SPSS Statistics 22 (IBM SPSS, Turkey). The conformity of normal distribution of data was evaluated by Shapiro-Wilk test. In addition to descriptive statistical methods (mean, standad deviation), in the comparison of parametes in two goups student t test was used. ROC curve analysis was used to establish a cut-off point and the significance level for the study was set as $p<0.05$.

Results

A total of 72 lesions of 54 patients (35 males and 19 females), aged between 26 and 86 (mean 59.5±15.7 years) were included in this study. 40 of the masses were benign (19 Bosniac type 1 cysts, 12 Bosniac type 2 cysts, 3 oncocytomas and 6 angiomyolipomas) and 32 were malignant (31 RCC and 1 transitional cell carcinoma).

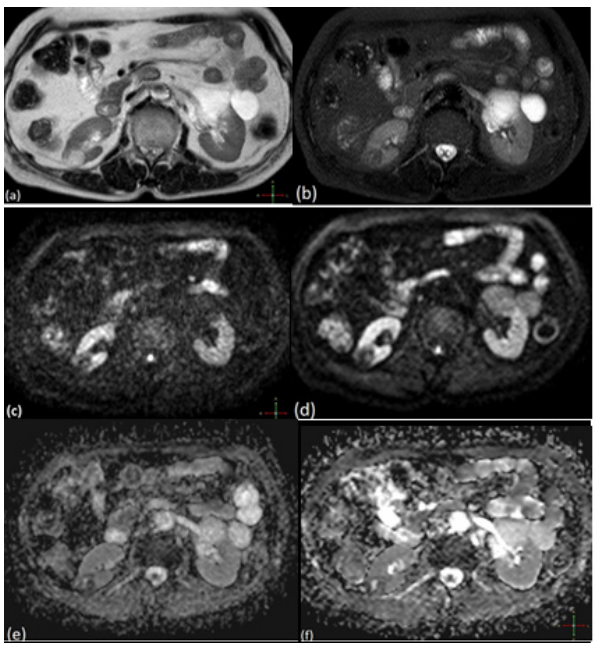

Fig. 1. a-e) Angiomyolipoma of the right kidney in a 78-year-old female patient, which is hyperintense on T 2-w image (a); losing signal in the fat suppressed sequence (b); showing locally restricted diffusion on DWI (c-e); with ADC values measured as 1.21x10^{-3} mm^2/s at b=1000 (d) and 1.67x10^{-3} mm^2/s at b=500 (e)

The average ADC values of all masses without distinguishing between malignant and benign masses were

Table 1. ADC values of benign-malignant lesions

	Malignant (n=32) (31 RCC and 1 transitional cell carcinoma)		**Benign (n=40)** (19 Bosniac type 1 cysts, 12 Bosniac type 2 cysts, 3 oncocytomas and 6 AMLs)		**p**
	mean x10^{-3} mm^2/s	**SD**	**mean** x10^{-3}mm^2/s	**SD**	
ADC value of the lesion at b=1000	1.206**	0.386	2.328	0.713	<0.0001
ADC value of the lesion at b=500	1.413**	0.441	2.524	0.782	<0.0001
ADC value of the normal parenchyma at b=1000	1.920	0.180	1.939	0.164	0.653
ADC value of the normal parenchyma at b=500	2.227	0.252	2.230	0.274	0.954

** p < 0.01

found to be 1.72±0.81x10^{-3} mm^2/s at b=1000 and was 1.95±0.82x10^{-3} mm^2/s at b=500 .The average ADC values of 32 malignant masses (1.2x10^{-3} mm^2/s at b=1000 and 1.41x10^{-3} mm^2/s at b=500) were significantly lower than the ADC values of the normal renal parenchyma which was statistically significant (p<0.01). The average ADC values of benign masses were higher than the ADC values of normal renal parenchyma (1.92x10^{-3} mm^2/s at b=1000 and 2.23x10^{-3} mm^2/s at b=500) and it was statistically significant.The ADC values of benign lesions at both b=1000 and at b=500 were significantly higher than the ADC values of malignant lesions (p<0.01). These differences between benign-malignant lesions did not differ significantly between images obtained at b=500 and b=1000 images (Table 1).

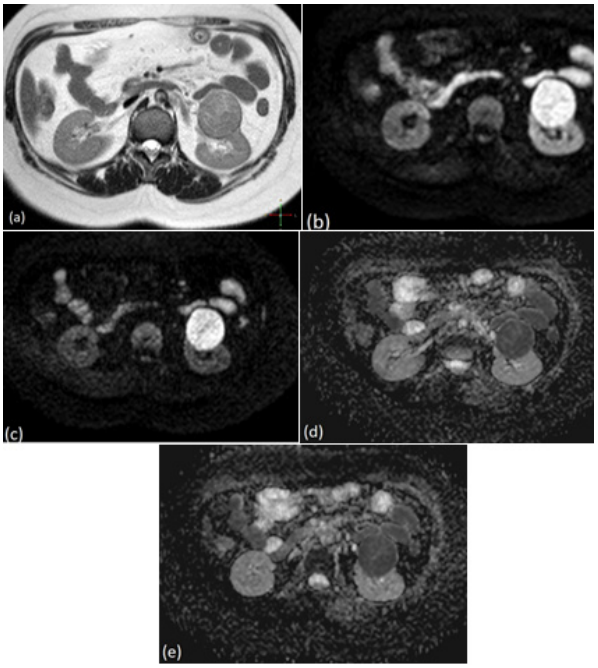

Fig. 2. a-e) Oncocytoma in a 26-year-old female patient. T2-w image shows a hypointense lesion with central linear hyperintity and smooth contours (a); significant restriction on diffusion-weighted images is shown (b-d); with ADC values measured as 0.9x10^{-3} mm^2/s at b=1000 (c) and 1.11x10^{-3} mm^2/s at b=500 (d)

Table 2. ADC values of the AMLs and oncocytomas

	ADC (mean, x10^{-3} mm^2/s)	SD
ADC value of the AMLs at b=1000 (n=6)	1.048	0.510
ADC value of the AMLs at b=500 (n=6)	1.486	0.195
ADC value of the oncocytomas at b=1000, (n=3)	1.286	0.362
ADC value of the oncocytomas at b=500 (n=3)	1.450	0.310

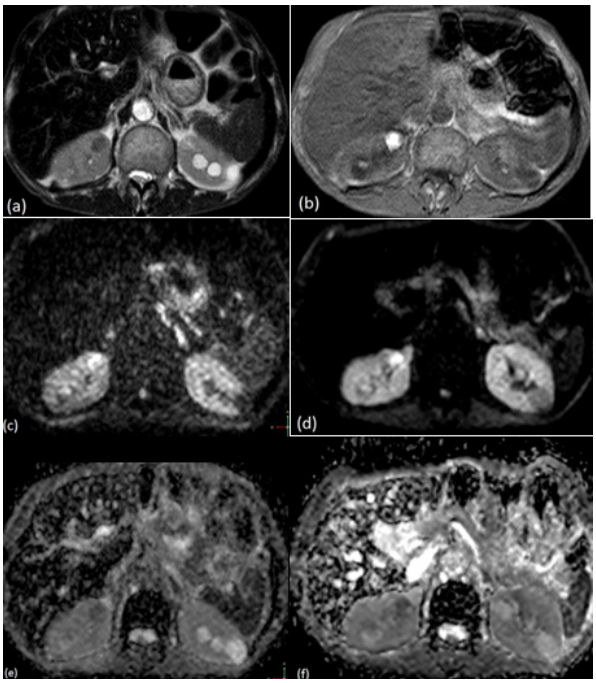

Fig. 3. a-e). Hemorrhagic cyst in the right kidney and simple cysts in the left kidney in a 69-year-old male patient. On T2w images, one hypointense lesion in the right kidney and a few hyperintense lesions in the left kidney are shown (a); On T1w images, the lesion on the right is hyperintense, and the lesions on the left are hypointense (b); While restricted diffusion was observed in the right lesion, it was not observed in the left lesions on DWI (c-f); The ADC value of the right lesion was measured as 1.57x10^{-3} mm^2/s at b=1000 (e) and 1.58x10^{-3} mm^2/s at b=500 (f). The ADC value of the largest left lesion was measured as 3.09x10^{-3} mm^2/sn at b=1000 (e) and 3,50x10^{-3} mm^2/s at b=500 (f)

Among benign masses, we found the lowest values in angiomyolipoma (Fig. 1) and oncocytomas (Fig. 2), and the highest values in Bosniac type I cysts (Fig. 3), (Table 2). It was found similarities between the ADC values of some angiomyolipomas and the ADC values of RCCs (Fig. 4). In terms of sensitivity in distinguishing benign-malignant lesions, AML was first excluded from the analysis. It was then analyzed again by adding AMLs. In terms of statistical results, it was revealed that the inclusion of AMLs in the analysis did not significantly affect the difference between malignant and benign lesions.

Discussion

Randomized movements of molecules depending on their kinetic energies are called as diffusion. Diffusion-weighted MRI is an MRI technique used to show molecular diffusion which is Brownian motions of spins in biological tissues. In conventional MRI, the molecular motion of water contributes a very small amount to the image. With the use of strong gradients, the tissues become sensitive to diffusion of water and DWI can be

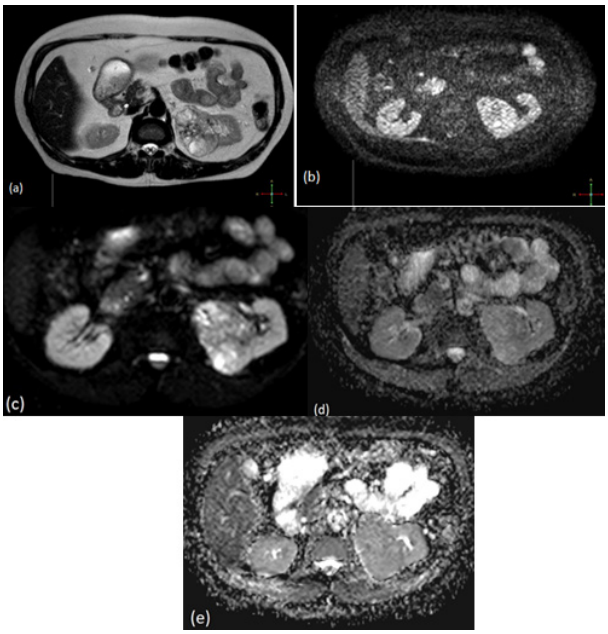

Fig. 4. a-e). Clear cell RCC in a 56-year-old male patient. Heterogeneous hyperintense lesion containing areas of cystic necrosis in the left kidney on T2W images (a); showing restriction on DWI (b-e); the ADC values were measured as 1.68x10^{-3} mm^2/s at b=1000 (d) and 2.05x10^{-3} mm^2/s at b=500 (e)

performed. The kidneys are very suitable for diffusion studies with its high blood flow and its basic function in fluid transport.[6,7] Because of their complex anatomical and physiological structures, kidneys attract a great attention for DWI.[8,9] In a cross-sectional study conducted on 30 patients having renal masses a statistically significant difference was found in mean of ADC values in relation to different types of malignant RCCs, while no statistically significant difference in relation to different types of benign renal masses was found.[10] Although there is no clear consensus on which b value to use in the evaluation of the renal lesions, the recommended b value is between 600 and 1000 s/mm^2.[11] We measured all the ADC values in our study at b value of both 500 s/mm^2 and 1000 s/mm^2. In the study conducted by Cova et al. with 39 cases, ADC values were measured from normal renal parenchyma, areas of the lesion and dilated collecting system. The study found the followings; higher ADC values in simple renal cysts and renal pelvis of hydronephrotic kidneys compared to normal renal parenchyma, lower ADC values in solid kidney tumors and the lowest ADC values in the renal pelvis of pyonephrotic kidneys. [12] Similarly, in our study, the highest ADC values measured at both b values were belonged to simple renal cysts. In a retrospective study including 66 renal tumors of which 33 were clear cell RCC, 9 were papillary RCC, 4 were chromophobe RCC, 11 were oncocytoma and 9 were AML, oncocytomas were found to have the highest ADC values, significantly higher than AMLs and all RCC subtypes.[13] In the study of Taouli et al., ADC measurements of 109 kidney masses at b=400 and b=800 values, 81 of which were benign and 28 of which were malignant were analyzed.[14] They found the average ADC values of AMLs (n=10) lower than RCCs. In the study of Zhang et al., 1 AML case had an ADC value of 1.23x10-3 ^{3} mm^2/s. In the same study, the average ADC value of RCCs was 2.03x10-3 mm^2/s.[15] Doğanay et al. analyzed ADC values by adding and subtracting AML in their study and found a lower average ADC value in AMLs.[16] Kılıçkesmez et al. found that ADC values of AMLs were higher than RCCs and they reported that as the fat content of AMLs increased, ADC values decreased. [17] In our study, ADC values of AMLs were found also to be higher than malignant lesions with no significant difference in terms of statistical results and as the fat content of the AMLs increased, the ADC values decreased in our AML cases. All measurements in our study performed at 2 different b values made from solid parts of malignant tumors and were found to be lower than simple cysts. On the other hand, in some studies in the literature, ADC values of cystic parts of malignant lesions and ADC values of benign cystic lesions were also compared.[14-18] Among these studies, except for those of Taouli et al. [14] ADC values of cystic parts of malignant lesions were lower than ADC values of benign cystic lesions. Zhang et al. reported that although benign cysts and cystic/necrotic renal tumors may look similar in conventional MRI, there is a significant difference in ADC values.[15] There are some limitations of our study including the limited number of the lesions and the limited pathologies (e.g. absence of metastases).In addition, for the malignant lesions the ADC measurements were made from the solid parts and the ADC values of the cystic/necrotic components of the malignant lesions could not be compared.

Conclusion

DWI is useful in differentiating benign and malignant renal masses.In our study, the ADC values of benign renal masses were higher than those of normal renal parenchyma, which is higher than those of malignant renal masses. Among the benign lesions the lowest ADC values were observed in AMLs and oncocytomas. The ADC values of AMLs were higher than those of RCCs.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, N.K. and B.E.; Methodology, N.K. and Z.G.K.; Validation, N.K., B.E., and H.G.D., Formal Analysis, N.K., B.E. and Z.G.K.; Investigation, N.K., B.E. and M.O.A.; Data Curation, N.K., B.E., M.O.A. and

H.G.D.; Writing – Original Draft Preparation, N.K. and B.E.; Writing – Review & Editing, N.K., B.E. and Z.G.K., Supervision, N.K. and Z.G.K.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Ethics approval was obtained from the Hospital's Ethical Committee.

References

1. Eble JN , Sauter G, Epstein JI , Sesterhenn IA , eds. *Pathology and genetics: tumours of the urinary system and male genital organs.* Lyon, France: *IARC.* 2004;65.
2. Escudier B , Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*. 2007;356:125-134.
3. Motzer RJ , Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*. 2007;356:115-124.
4. Kış N, Erok B. Diffusion weighted imaging in differentiation of the clear cell RCC from the major non-clear cell RCC subtypes. *Eur J Clin Exp Med.* 2021;19(3):215-220.
5. Jones DK, Fundementals of diffusion MR imaging. Section 1 Physiological MRTechniques. Clinical MR Neuroimaging: diffusion, Perfusion, Spectroscopy. *Cambridge universty Pres.* 2005;54-86.
6. Squillaci E, Manenti G, Di Stefano F, et al. Correlation of diffusion-weighted MR imaging with cellularity of renal tumours. *Anticancer Res.* 2004;24:4175-4179.
7. Kiliçkesmez O, Yirik G, Bayramoğlu S, et al. Non-breath-hold high b-value diffusion weighted MRI with parallel imaging technique: apparent diffusion coefficient determination in normal abdominal organs. *Diagn Interv Radiol.* 2008;14:83-87.
8. Fukuda Y, Ohashi I, Hanafusa K, et al. Anisotropic diffusion in kidney: apparent diffusion coefficient measurements for clinical use. *J Magn Reson Imaging.* 2000;11:156-160.
9. Namimoto T, Yamashita Y, Mitsuzaki K, et al. Measurement of the apparent diffusion coefficient in diffuse renal disease by diffusion-weighted echo planar MR imaging. *J Magn Reson Imaging.* 1999;9:832-837.
10. ElSayed AM, Abd-elazizElFiki IM, Salem AF, AlaaEldin AM. Added Value of Apparent Diffusion Coefficient and Diffusion Weighted Imaging in Characterization of Renal Masses. *Annals of the Romanian Society for Cell Biology*. 2021;25(6):15004-15013.
11. Taouli B, Beer AJ, Chenevert T, et al. Diffusion-Weighted Imaging outside the brain: Consensus statement from an ISMRM-Sponsored Workshop. *J Magn Reson Imaging.* 2016;44(3):521-540.
12. Cova M, Squillaci E, Stacul F, et al. Diffusion-weighted MRI the evaluation of renal lesions: preliminary results. *Br J Radiol.* 2004;23:39-45.
13. de Silva S, Lockhart KR, Aslan P, *et al.* The diagnostic utility of diffusion weighted MRI imaging and ADC ratio to distinguish benign from malignant renal masses: sorting the kittens from the tigers. *BMC Urol*. 2021;21:67.
14. Taouli B, Thakur RK, Manelli L, et al. Renal lesions: characterization with diffusion weighted imaging versus contrast-enhanced MR imaging. *Radiology.* 2009;251:398-407.
15. Zhang J, Tehrani YM, Wang L, et al. Renal masses: characterization with diffusion weighted MR imaging- a preliminary experience. *Radiology*. 2008;247:458-464.
16. Doğanay S, Kocakoç E, Çiçekçi M, et al. Ability and utility of diffusion-weighted MRI with different b values in the evaluation of benign and malignant renal lesions. *Clin Radiol.* 2011;66:420-425.
17. Kiliçkesmez O, İnci E, Atilla S, et al. Diffusion-weighted imaging of the renal and adrenal lesions. *J Comput Assist Tomogr.* 2009;33:828-833.
18. Sandrasegaran K, Sundaram CP, Ramaswamy R, et al. Usefulness of diffusion-weighted imaging in the evaluation of renal masses. *AJR Am J Roentgenol.* 2010;194:438-445.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.7

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 49–55

ORIGINAL PAPER

Vanisha Aroon Hirani [1], Sira Stanslaus Owibingire [2], Jeremiah Robert Moshy [2], Karpal Singh Sohal [2]

The incidence and pattern of non-odontogenic orofacial pain conditions at a tertiary hospital in Tanzania

[1] Department of Oral and Maxillofacial Surgery, Kenyatta National Hospital, Nairobi, Kenya
[2] Department of Oral and Maxillofacial Surgery, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

ABSTRACT

Introduction and aim. Non-odontogenic orofacial pain (NOFP) is a result of pathology, or injury to the structures in the orofacial region including the muscles, temporomandibular joint, neurovascular structures, and glands. This multi-diverse aetiopathogenesis poses a challenge in the diagnosis and management of NOPF. To determine the incidence and trend of various non-odontogenic orofacial pain conditions at a tertiary hospital in Tanzania.

Material and methods. This cross-sectional study was conducted at the Muhimbili National Hospital (MNH) for 6 months. The information gathered included socio-demographic characteristics of participants, characteristics of pain, and cause of pain. Pain intensity was assessed using the Visual Analogue Scale (VAS).

Results. The incidence of NOFP was 3.3%. The male to female ratio was 1.7:1 and the mean age of patients was 44.2 ± 17.4 years. The mean intensity of the pain using the VAS was 47.27 ± 5.66. Most (36.7%) patients experienced sharp pain. The common causes of NOFP were trauma (43.3%) and malignant lesions (38.3%). A statistically significant association between the age and sex of the patients and the causes of non-odontogenic pain was observed ($p < 0.05$).

Conclusion. The incidence of non-odontogenic orofacial pain is low. Trauma and malignant conditions were the leading causes of NOFP.

Keywords. incidence, inflammation, non-odontogenic pain, pattern, Tanzania

Introduction

Orofacial pain (OFP) which has been defined as pain whose origin is below the orbito-meatal line, above the neck, and anterior to the ears, including pain within the mouth is a common affliction that affects between 10 and 50% of the population.[1,2] The pain in the facial region can be classified broadly based on its origin as odontogenic (arising from the tooth and its supporting structures) or non-odontogenic (which can arise from musculoskeletal, neurological, or vascular structures around the head and neck).[3,4]

When pain occurs in the orofacial area, it is very important to identify and diagnose its cause.[5] Odontogenic pain is the most common orofacial pain and it can be managed well and predictably.[3,6] However, non-odontogenic orofacial pain (NOFP) represents a challenge to the clinician since the pain can arise from many sources with overlapping signs and symptoms.[2,7]

The clinical presentation of non-odontogenic pain varies and can be very mild and intermittent or severe, sharp, and continuous depending on the etiology/source.[6,8] Obtaining a detailed history from the patient

Corresponding author: Karpal Singh Sohal, e-mail: karpal@live.com

Received: 24.09.2021 / Revised: 02.11.2021 / Accepted: 06.11.2021 / Published: 30.03.2022

Hirani VA, Owibingire SS, Moshy JR, Sohal KS. *The incidence and pattern of non-odontogenic orofacial pain conditions at a tertiary hospital in Tanzania.* Eur J Clin Exp Med. 2022;20(1): 49–55. doi: 10.15584/ejcem.2022.1.7.

including the location, duration, frequency, periodicity, character, and quality of pain assists in reaching to correct diagnosis.[9] This is of key importance to guide the delivery of appropriate treatment and avoiding unnecessary treatment without eliminating the source.[8]

Currently in Africa there is a paucity of studies on orofacial pain especially of the non-odontogenic type. [2] and Tanzania is not exceptional. This makes it a challenging task to quantify the extent of the problem in our population. It is important to have local epidemiological information regarding non-odontogenic pain in Tanzania instead of basing on western literature due to considerable differences in sociocultural and environmental factors. With local information at hand, it will become easier for clinicians to have a high suspicion index for the cause when a person with orofacial pain present to them for treatment.

Aim

This study was conducted to determine the incidence and trend of various non-odontogenic orofacial pain conditions at a tertiary hospital in Tanzania.

Material and methods

This was a cross-sectional hospital-based study that was conducted at the Muhimbili National Hospital (MNH) from September 2019 up to February 2020. A convenient method of sampling was used whereby all patients with orofacial pain were received and screened. Only those with non-odontogenic OFP were further physically examined, investigated and those who consented were included in the study. Patients who were mentally challenged, aged under 18 years, and/or had been previously diagnosed with non-odontogenic orofacial pain and in whom management had already been instituted before the commencement of the study were excluded. All patients who qualified were recruited until the end of the data collection period (i.e Feb 2020).

A specific questionnaire that was translated to the Swahili language was used for this study. It captured information regarding the socio-demographic characteristics of participants, the characteristics of pain (intensity, nature, aggravating factors, and relieving factors), and the cause of pain (which was obtained after a thorough examination of the patient to ascertain diagnosis). The questionnaire used was reviewed by specialists in the field of oral and maxillofacial surgery and medicine for the assessment of its validity. A pilot study was conducted to ascertain the clarity of the questions asked.

Pain intensity was assessed using the Visual Analogue Scale (VAS) which is a continuous scale comprised of a horizontal line of 10 centimeters (100 mm) in length, anchored by "no pain" (score of 0) and "pain as bad as it could be" or "worst imaginable pain" (score of 100 [100-mm scale]). The respondent was asked to place a line perpendicular to the VAS line at the point that represents their pain intensity and that was measured with a ruler. The VAS scale was graduated into ten intervals for ease of understanding for the patients. Before administering the questionnaire, patients were given an explanation and demonstration of how to score the VAS scale.

The management of NOFP in this study followed the protocol of managing orofacial pain in the department on oral and maxillofacial surgery - MNH. All patients were managed on the same day of reporting after establishing the cause of pain. Chronic pain due to malignancy was managed initially by acetaminophen and non-steroidal anti-inflammatory drugs (diclofenac and/or ibuprofen). The 2nd line of treatment included weak opioids (tramadol), and strong opioids (morphine) depending on the severity of pain. Pain due to trauma and inflammatory conditions was managed by either acetaminophen alone or in combination with non-steroidal anti-inflammatory drugs (NSAIDs). The neuropathic pain was managed by anticonvulsants (carbamazepine) and antidepressants (amitriptyline). The myofascial pain and temporomandibular joint disorders were managed by the use of acetaminophen and/or NSAIDs and antidepressants/ muscle relaxants (diazepam).

Age was grouped into 18-40, 41-60, and > 60 years. Marital status was dichotomized into those without a partner (single, divorced, and widowed) and those with a partner (cohabiting and married). Education was dichotomized into lower (no formal education and primary education) and higher education (secondary, college education, and above). The occupation was dichotomized into those with a stable income (civil servants, businesspersons, the self-employed, and those who were employed privately were considered to have a stable income) and with unstable income (no formal employment, students, peasants, petty traders). The pain intensity was categorized into no pain (0–4 mm), mild pain (5–44mm), moderate pain (45–74 mm), and severe pain (75–100 mm).[10]

The data obtained from this study were coded, entered into the computer program, and analyzed using SPSS software version 23.0. Data was presented in the form of the mean for continuous variables and percentages for categorical variables. The probability level of $p<0.05$ was selected for statistical significance.

The study was approved by the Institution Review Board of the Muhimbili University of Health and Allied Sciences (Ref.No.287/298/01A) and permission was granted by the MNH. Participation was voluntary and for each participant, a signed informed consent form was obtained before data collection. The participants were assured of confidentiality and their right to par-

ticipate or withdraw without any conditions. No names were used to avoid identification. All patients were treated according to the established protocol in MNH, and refusal to participate or withdraw from the study did not compromise the management of the patient.

Results

A total of 3651 patients with orofacial pain (OFP) were attended to at the oral and maxillofacial clinic throughout the study period. Those who had orofacial pain due to non-odontogenic causes were 120 (3.3%). Most (75, 62.5%) were male, with a male to female ratio of 1.7:1. The age of the patients ranged from 19 to 89 years, with a mean age of 44.2 ± 17.4 years. Almost half (61, 50.8%) of the patients were aged ≤ 40 years, most (82, 68.3%) had a low level of education and a few had a stable source of income (Table 1).

Table 1. Distribution of patients with non-odontogenic orofacial pain according to socio-demographic features

Socio-demographic characteristics	Gender		Total n (%)
	Male n (%)	Female n (%)	
Age (years)			
18-40	46 (61.3%)	15 (33.3%)	61 (50.8%)
41-60	17 (22.7%)	13 (28.9%)	30 (25.0%)
>60	12 (16.0%)	17 (37.8%)	29 (24.2%)
Marital status			
Without partner	32 (42.7%)	20 (44.4%)	52 (43.3%)
With partner	43 (57.3%)	25 (55.6%)	68 (56.7 %)
Education			
Lower level	51 (68.0%)	31 (68.9%)	82 (68.3%)
Higher level	34 (32.0%)	14 (31.1%)	38 (31.7%)
Occupation			
Stable income	40 (53.3%)	16 (35.6%)	56 (46.7%)
Unstable income	35 (46.7%)	29 (64.4%)	64 (53.3%)

The onset of the pain in the majority (91, 75.8%) was gradual and the remaining ones (29, 24.2%) reported a sudden onset. Pain radiation to the other side was reported by 47 (39.2%) patients. The intensity of the pain using the visual analogue score (VAS) ranged from 13 to 100 with a mean of 47.27 ± 5.66. Most (44, 36.7%) patients experienced sharp pain (Fig 1). The factors that aggravated pain included chewing (105, 87.5%), tooth brushing (70, 58.3%), speaking/talking (63, 52.5%), and washing face (14, 11.7%). Most (72, 60%) patients took medication for pain relief, other methods included resting (59, 49.2%) and massaging the painful region (4, 3.3%). The common causes of non-odontogenic orofacial pain were trauma (52, 43.3%), malignant lesions (46, 38.3%), inflammatory conditions (11, 9.2%), neuralgia (7, 5.8%) and myofascial pain disorder (4, 2.3%) (Fig. 2).

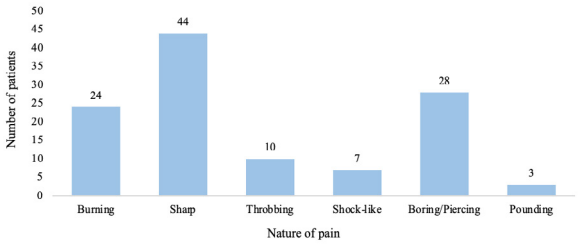

Fig. 1. Distribution of patients with non-odontogenic orofacial pain according to the nature of pain experienced

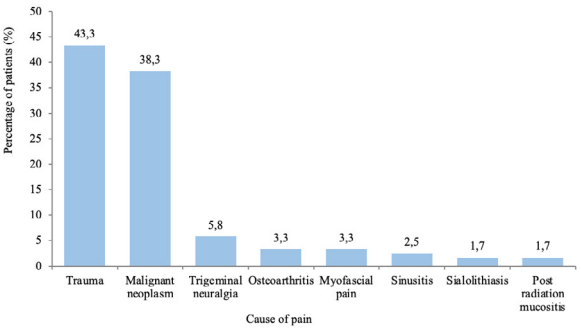

Fig. 2. Percentage distribution of patients according to the cause of non-odontogenic orofacial pain

A statistically significant association between causes of non-odontogenic pain and socio-demographic characteristics of the patients was observed ($p < 0.05$). Trauma was the most common cause of pain orofacial pain in males, individuals aged less than 40 years, and those with a stable source of income. Malignant conditions were the cause of pain in females and those aged above 40 years. Myofascial pain disorder was predominantly noted in females (Table 2). With an exception of neuralgia, non-odontogenic pain due to all causes was mostly of gradual onset, with mild to moderate intensity and aggravated by chewing (Table 3).

Discussion

Non-odontogenic orofacial pain (NOFP) is a result of pathology, or injury to the structures in the orofacial region including the muscles, temporomandibular joint, neurovascular structures, and the glands.[6] Thus, the aetiology of non-odontogenic pain can be trauma to the facial structures, idiopathic (burning mouth syndrome), orofacial neoplasms, inflammatory conditions (sinusitis), and psychogenic.[6,11] The pain of non-odontogenic cause is not a common entity, with a reported prevalence of less than 10% from different studies, similarly in the current study a low incidence (3.3%) was obtained.[2,12,13]

The findings of this study depicted that the common causes of NOFP in our setting were trauma, neoplastic conditions, and inflammatory conditions. This is contrary to findings from Nigeria which reported infective process, neoplasia, and psychological stress as the

Table 2. Causes of non-odontogenic pain in respect to the sociodemographic characteristics of the patients

Socio-demographic characteristics	Causes of non-odontogenic pain					p-value
	Trauma n (%)	Malignancies n (%)	Inflammatory conditions n (%)	Neuralgia n (%)	Myofascial pain n (%)	
Sex						
Male	48 (64%)	19 (25.3%)	6 (8%)	2 (2.7%)	-	<0.001
Female	4 (8.9%)	27 (60%)	5 (11.1%)	5 (11.1%)	4 (8.9%)	
Age group						
≤ 40 years	44 (72.1%)	11 (18%)	4 (6.6%)	1 (1.6%)	1 (1.6%)	<0.001
41-60 years	8 (26.7%)	14 (46.7%)	4 (13.3%)	3 (10%)	1 (3.3%)	
> 60 years	-	21 (72.4%)	3 (10.3%)	3 (10.3%)	2 (6.9%)	
Income						
Stable	33 (58.9%)	13 (23.2%)	6 (10.7%)	2 (3.6%)	2 (3.6%)	0.01
Unstable	19 (29.7%)	33 (51.6%)	5 (7.8%)	5 (7.8%)	2 (3.1%)	
Education						
Low level	32 (39%)	41 (50%)	4 (4.9%)	4 (4.9%)	1 (1.2%)	0.001
High level	20 (52.6%)	5 (13.2%)	7 (18.4%)	3 (7.9%)	3 (7.9%)	

Table 3. Distribution of patients according to clinical characteristics of pain from different causes

Clinical characteristics	Causes of non-odontogenic pain				
	Trauma n (%)	Malignancies n (%)	Inflammatory conditions n (%)	Neuralgia n (%)	Myofascial pain n (%)
Onset					
Gradual	39 (75%)	39 (84.8%)	9 (81.8%)	1 (14.3%)	3 (75%)
Sudden	13 (25%)	7 (15.2%)	2 (18.2%)	6 (85.7%)	1 (25%)
Nature of pain					
Burning	-	22 (47.8%)	2 (18.2%)	-	-
Sharp	39 (75%) (26.7%)	3 (6.5%)	2 (18.2%)	-	-
Throbbing	3 (5.8%)	5 (10.9%)	2 (18.2%)	-	-
Shock-like	-	-	-	7 (100%)	-
Boring/Piercing	9 (17.3%)	15 (32.6%)	4 (36.3%)	-	-
Pounding	1(1.9%)	1 (2.2%)	1 (9.1%)	-	-
Dull	-	-	-	-	4 (100%)
Intensity					
Mild	40 (76.9%)	17 (37%)	5 (45.5%)	-	2 (50%)
Moderate	12 (23.1%)	15 (32.6%)	2 (18.2%)	1 (14.3%)	1 (25%)
Severe	-	14 (30.4%)	4 (36.4%)	6 (85.7%)	1 (25%)
Aggravating factor					
Chewing	49 (94.2%)	37 (80.4%)	9 (81.8%)	6 (85.7%)	4 (100%)
Brushing	36 (69.2%)	24 (52.2%)	3 (27.3%)	6 (85.7%)	1 (25%)
Speaking	30 (57.7%)	21 (45.7%)	4 (36.4%)	7 (100%)	1 (25%)
Washing face	2 (3.8%)	5 (10.9%)	-	7 (100%)	4 (100%)
Reliving factor					
Medication	43 (87.2%)	21 (45.7%)	4 (36.4%)	2 (28.6%)	2 (50%)
Resting	36 (69.2%)	9 (19.6%)	7 (63.6%)	3 (42.9%)	4 (100%)
Massaging	-	-	1 (9.1%)	-	3 (75%)

leading cause. A study from India found the common causes of NOFP were temporomandibular joint disorders, facial pain, and oral sores.[2,14] The different causes of NOFP as seen in different studies, including the current one can be attributed to the environmental and sociocultural differences in the study population, and design of the studies (retrospective vs prospective, duration, and setting).

In this study, it was illustrated that there was a statistically significant association between the causes of NOFP and the sociodemographic characteristics of the patients. Trauma as the leading cause of the pain was

more common in males and young individuals with stable income. Several local and international studies have reported that males and young individuals are prone to orofacial injuries due to their risk-taking behaviour and tendency of being active and remaining outdoors.[15-21]

Malignant conditions of the orofacial region were the second most common cause of NOFP. Females, elderly individuals aged above 60 years, and those with unstable income were more affected. It was not surprising to find that most patients who suffered from NOFP due to neoplastic conditions had an unstable income, because, most individuals with malignancy were elderly, who could not work, due to their debilitating physical condition caused by their ailment. Though previous epidemiological studies from the setting of the current study pointed out males were more affected with malignant conditions, in this study females reported more with NOFP due to malignant conditions.[22,23] These findings may indicate a shift in the trend of orofacial malignancies or easy accessibility of hospital services to females compared to what was in the past. Another reason can be the mere fact that females have a lower pain threshold compared to males, thus most of the males with malignancy did not complain of pain, therefore, were not included in this study.[24]

In this study, inflammatory conditions included an array of pathologies that affected the; TMJ (TMJ arthritis), salivary glands (sialadenitis), and sinuses (sinusitis). Inflammation is the physiological response of the body to injury which can be either acute or chronic and the NOFP due to inflammatory conditions is often chronic.[11,25] There is evidence in humans that some chronic pain conditions reflect a heightened central excitatory state, resulting from a decrease in central inhibitory control mechanisms.[26] Animal studies have shown that central sensitization is associated with a reduction in descending inhibition and enhancement of descending facilitation from some structures.[26] Due to central sensitization, the neuronal hyperexcitability may not be readily reversible and may become sustained resulting in a chronic state of nociceptive neuronal hyperexcitability.[27]

Pain can either be of sudden onset which begins within a few seconds and steadily increases in severity over the next several minutes, or of gradual onset which begins slowly and becomes more severe only after several hours or even days have elapsed.[28] The findings of the current study depicted that with an exception of neuralgia, the NOFP due to the rest of the causes was of gradual onset. These findings are supported by literature which reports that neuralgia is usually of sudden onset and that pain of gradual onset is commonly associated with neoplasms, trauma, and chronic inflammatory processes.[11,28,29]

In this study, the diverse clinical characteristics of NOFP due to different causes as shown in Table 3 may be partly explained by pain transmitting fibers. It is a fact that the majority of nociceptive impulses from the orofacial region are mainly (but not exclusively) mediated by the trigeminal nerve, which has both the A- and C-fibers.[30] The A-fibers are fast conducting myelinated fibers and result in a short-lasting-sharp-pricking type of pain sensation, while the C-fibers are slow conducting unmyelinated fibers which often results in poor localization and dull pain sensation.[30,31] Chronic inflammatory pain which can be due to malignancy and trauma is typically mediated by C-fibers, while A-fibers mediate neuropathic pain.[30-33]

The intensity of the pain ranged from mild to severe. The malignant conditions had a wide range of intensity from mild to severe. A major concept in the level of pain in malignant lesions is dependent on the stage of presentation of the condition, The more advanced the lesion the more the intensity of the pain.[34] In the current study of the patients with malignant conditions presented to us late with advanced lesions. Thus moderate to severe intensity of pain was not a surprising finding. Most patients with trauma had mild to moderate pain intensity. These findings are concurrent to the report by Dilunga et al.[35] One of the possible explanations for this may be because most patients who were seen in our setting had sustained trauma more than 3 days before reporting to us, as such the acute inflammatory phase had waned off hence less severe pain.

Study limitation

There were certain limitations in the study that should be mentioned. First, the cross-sectional study design cannot infer the causative effect of the variables strongly. Secondly, the study data were collected from a single tertiary hospital as such patients with mild chronic pain in the community might not have come to the particular center for management. However, despite these limitations, the results from the current study are very valuable as they provide very useful information to the clinicians and thus making it easier for them to have a high suspicion index for coming up with a cause of orofacial pain when an odontogenic cause is not found.

Conclusion

The incidence of non-odontogenic orofacial pain is low, however, it is important to take into cognizance, the existence of such pain for adequate diagnosis and management of patients. Trauma and malignant conditions are the leading causes of NOFP. There was a statistically significant association between the cause of NOFP and sociodemographic characteristics of patients. Most of the patients with NOFP have the pain of gradual onset which is of mild to moderate intensity.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, V.A.H. and K.S.S; Methodology, V.A.H., J.R.M., S.S.O. and K.S.S; Software, K.S.S.; Validation, V.A.H., J.R.M., S.S.O. and K.S.S.; Formal Analysis, V.A.H., S.S.O. and K.S.S.; Investigation, V.A.H., J.R.M., and S.S.O.; Resources, V.A.H., J.R.M., and K.S.S.; Data Curation, V.A.H. and K.S.S.; Writing – Original Draft Preparation, V.A.H. and K.S.S.; Writing – Review & Editing, J.R.M., S.S.O. and K.S.S.; Visualization, V.A.H., J.R.M., S.S.O. and K.S.S.; Supervision, J.R.M. and S.S.O.; Project Administration, J.R.M. and S.S.O.

Conflicts of interest

The authors declare that they have no competing interests with regards to authorship and/or publication of this paper.

Data availability

The data from this study is freely available from the corresponding author on reasonable request.

Ethics approval

The study was approved by the Institution Review Board of the Muhimbili University of Health and Allied Sciences (Ref.No.287/298/01A).

References

1. Renton T. Introduction to Pain. *Prim Dent J*. 2019;7(4):17-21.
2. Gbadebo O, Kolude B. Pattern and trend of non-odontogenic orofacial pain at a tertiary health facility in Sub Saharan West Africa. *J Stomatol*. 2015;68(1):59-73.
3. Ravikumar KK, Ramakrishnan K. Pain in the face: An overview of pain of nonodontogenic origin. *Int J Soc Rehabil*. 2018;3(1):1-5.
4. Renton T. Dental (Odontogenic) Pain. *Rev Pain*. 2011;5(1):2-7.
5. Kim S-Y, Kim Y, Yun P, Bae J. Treatment of non-odontogenic orofacial pain using botulinum toxin-A: a retrospective case series study. *Maxillofac Plast Reconstr Surg*. 2018;40(1):21.
6. Oyetola E, Agbelusi G, Dayo A. Pattern of Presentations of Non-odontogenic Pain in oral Medicine Clinic of Lagos University Teaching Hospital (Luth). *Br J Med Med Res*. 2014;4(23):4117-4126.
7. Romero-reyes M, Uyanik JM. Orofacial pain management : current perspectives. *J Pain Res*. 2014;7:99-115.
8. Sajjanhar I, Goel A, Tikku AP, Chandra A. Odontogenic pain of non-odontogenic origin : A review. *Int J Appl Dent Sci*. 2017;3(3):1-4.
9. Shaikh S. Management of Odontogenic and Nonodontogenic Oral Pain. In: *From Conventional to Innovative Approaches for Pain Treatment*. IntechOpen; 2019:13.
10. Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale SF. *Arthritis Care Res (Hoboken)*. 2011;63(S11):S240-52.
11. Renton T. Chronic Pain and Overview or Differential Diagnoses of Non-odontogenic Orofacial Pain. *Prim Dent J*. 2019;7(4):71-86.
12. Gbenga Omitola O, Olabisi Arigbede A. Prevalence and Pattern of Pain Presentation among Patients Attending a Tertiary Dental Center in a Southern Region of Nigeria. *J Dent Res Dent Clin Dent Prospects*. 2010;4(2):42-46.
13. Horst O V, Cunha-Cruz J, Zhou L, Manning W, Mancl L, DeRouen TA. Prevalence of pain in the orofacial regions in patients visiting general dentists in the Northwest Practice-based Research Collaborative in Evidence-based Dentistry research network. *J Am Dent Assoc*. 2015;146(10):721-728.
14. Oberoi SS, Hiremath SS, Yashoda R, Marya C, Rekhi A. Prevalence of Various Orofacial Pain Symptoms and Their Overall Impact on Quality of Life in a Tertiary Care Hospital in India. *J Maxillofac Oral Surg*. 2014;13(4):533-538.
15. Sohal KS, Moshy JR. Etiology, pattern and outcome of management of facial lacerations in Dar es Salaam, Tanzania. *Tanzan J Health Res*. 2019;21(2):1-10.
16. Khanbhai M. LBL. Motorcycle Accident injuries seen at Kakamega Provincial Hospital in Kenya. *East Cent Afr J Surg*. 2012;17(1):43-46.
17. Höfling I, Keinänen P, Kröger H. Injuries caused by motorcycle accidents – a 5-year survey of patients treated in Kuopio University Hospital. *Suom Ortop ja Traumatol*. 2006;29(3):243-247.
18. Lima SM, Santos SE, Kluppel LE, Asprino L, Moreira RWF, De Moraes M. A Comparison of motorcycle and bicycle accidents in oral and maxillofacial trauma. *J Oral Maxillofac Surg*. 2012;70(3):577-583.
19. Ramli R, Abdul Rahman R, Abdul Rahman N, et al. Pattern of maxillofacial injuries in motorcyclists in Malaysia. *J Craniofac Surg*. 2008;19(2):316-321.
20. Oginni FO, Ugboko VI, Ogundipe O, Adegbehingbe BO. Motorcycle-related maxillofacial injuries among nigerian intracity road users. *J Oral Maxillofac Surg*. 2006;64(1):56-62.
21. Owibingire SS, Sohal KS, Kalyanyama BM. Maxillofacial Fractures among Motorcycle Crash Victims Attended at a Tertiary Hospital in Tanzania. *Panam J Trauma, Crit Care Emerg Surg*. 2019;8(3):158-164.
22. Berege GZ, Vuhahula E, Sohal KS, Merkx MW, Simon ENM. Predisposing Factors and Clinico-Pathological Presentation of Malignant Lesions Of the Oro-Facial region in Dar es Salaam , Tanzania. *Med J Zambia*. 2019;46(4):286-296.
23. Sohal K, Moshy J. Six year review of malignant oral and maxillofacial neoplasms attended at Muhimbili National

Hospital, Dar es Salaam, Tanzania. *East Cent Afr Med J*. 2017;3(1):35-38.

24. Bartley EJ, Fillingim RB. Sex differences in pain: A brief review of clinical and experimental findings. *Br J Anaesth*. 2013;111(1):52-58.
25. Hasturk H, Kantarci A, Van Dyke TE. Oral inflammatory diseases and systemic inflammation: Role of the macrophage. *Front Immunol*. 2012;3(MAY):1-17.
26. Sessle BJ. Peripheral and central mechanisms of orofacial inflammatory pain. In: *International Review of Neurobiology*. Vol 97. Elsevier Inc.; 2011:179-206.
27. Sessle BJ. Chronic orofacial pain: Models, mechanisms, and genetic and related environmental influences. *Int J Mol Sci*. 2021;22(13):7112.
28. Sherigar R, Amir KA, Bobba RK, Arsura EL, Srinivas N. Abdominal pain secondary to pylephlebitis: An uncommon disease of the portal venous system, treated with local thrombolytic therapy. *Dig Dis Sci*. 2005;50(5):983-987.
29. Kumar A, Dahiya A, Hooda A, Goel M, Kumar V. Trigeminal Neuralgia : The New Medicinal Treatment Modalities. *J Oral Heal Comm Dent*. 2015;9:149-152.
30. Dasilva AF, Dossantos MF. The role of sensory fiber demography in trigeminal and postherpetic neuralgias. *J Dent Res*. 2012;91(1):17-24.
31. Yam MF, Loh YC, Tan CS, Adam SK, Manan NA, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. *Int J Mol Sci*. 2018;19(8):2164.
32. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860-867.
33. Pierce A, Pittet J. Inflammatory response to trauma: Implications for coagulation and resuscitation. *Curr Opin Anaesthesiol*. 2015;27(2):246-252.
34. Zajączkowska R, Kocot-Kępska M, Leppert W, Wordliczek J. Bone pain in cancer patients: Mechanisms and current treatment. *Int J Mol Sci*. 2019;20(23):65-75.
35. Dilunga GD, Sawe HR, Kulola IB, et al. Pain assessment and management of trauma patients in an emergency department of a tertiary hospital in Tanzania. *Saf Heal*. 2018;4(1):8-13.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.8

http://www.ejcem.ur.edu.pl/en/
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 56–62

ORIGINAL PAPER

Davut Tekyol [1], Ilker Akbas [2], Sinem Dogruyol [1], Abdullah Osman Kocak [3], Zeynep Çakır [3]

Topical lidocaine anesthesia for nasopharyngeal sampling – a double-blind randomized placebo-controlled trial

[1] Department of Emergency Medicine, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey
[2] Department of Emergency Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
[3] Department of Emergency Medicine, Faculty of Medicine, Ataturk University, Erzurum, Turkey

ABSTRACT

Introduction and aim. The aim of this study is to evaluate the effects of topical lidocaine application for nasopharyngeal sampling, on pain perception, the comfort of the patients, and the application difficulty for healthcare staff.

Material and methods. This study conducted with 100 healthy volunteers (50 participants in Lidocaine group and 50 participants in Placebo group). Two ml of a solution containing 10 mg/ml of lidocaine was applied to each nostril of the participants in the Lidocaine group, and the same dose of 0.9% NaCl to the Placebo group. We compared the changes in pain intensity and discomfort intensity using two numerical rating scales, the frequency of undesirable reactions, and the judgment of the practitioner staff.

Results. There were statistically significant decreases in pain and discomfort scores in the Lidocaine group. Similarly, there were statistically significant decreases in the frequency of all undesirable reactions except "grimace", in the second sampling in the Lidocaine group, however, there was a statistically significant decrease only in "holding staff's hand" in second sampling in the Placebo group.

Conclusion. Intranasal lidocaine application reduces the pain that occurs during nasopharyngeal sampling and makes the procedure easier for the patient and the healthcare worker.

Keywords. COVID-19 testing, lidocain, nasopharynx, swab

Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic has had different effects on emergency service.[1-3] Emergency medicine staff take part in the diagnosis and treatment of COVID-19.[4-6] Performing nasal or nasopharyngeal sampling for the polymerase chain reaction (PCR) test in suspected cases presenting to the emergency department is one of the tasks performed by the emergency department staff within this period.[4,5]

Nasopharyngeal sampling is used in the diagnosis of many respiratory diseases. It is also used for the polymerase chain reaction (PCR) test, which is accepted as the current standard procedure of case detection in the COVID-19 pandemic.[7,8] Failure to use an appropriate technique in this diagnostic method for COVID-19 cases may cause false-negative results.[9] Although it is a generally safe diagnostic method, the application of this test, which is performed millions of times a day globally due to the pandemic, without using the correct technique can cause a serious number of complications.[10]

McElfish et al. aimed to investigate the perceived barriers to COVID-19 testing, and they reported that the

Corresponding author: Davut Tekyol, e-mail: dtekyol34@hotmail.com

Received: 04.01.2022 / Accepted: 09.01.2022 / Published: 30.03.2022

Tekyol D, Akbas I, Dogruyol S, Kocak AO, Çakır Z. *Topical lidocaine anesthesia for nasopharyngeal sampling – a double-blind randomized placebo-controlled trial*. Eur J Clin Exp Med. 2022;20(1):56–62. doi: 10.15584/ejcem.2022.1.8.

people's perception of the nasal swab method is irritating and too painful is one of the primary barriers to testing.[11] The general opinion of participants is that this test is painful and uncomfortable, a more "patient-friendly" method can convince more people to take the test, and researchers should find a "new test method" for this purpose.[11] If there is no chance of contactless sampling, the staff being at a distance of less than 2 meters to the case creates a low risk of contamination even if they have sufficient personal protective equipment.[12] The less pain and more compliant the patient is; the easier nasal swab will be applied. This may also reduce the risk of case-to-healthcare worker transmission, because of reducing the potential aerosol-generating characteristic of nasopharyngeal sampling.[13] Lidocaine has been used for local anesthesia of the nasopharynx, and has been found to be effective and safe in different studies for different purposes, but, to the best of our knowledge, not nasopharyngeal swab sampling.[14,15] However, Kanodia et al. studied to evaluate the effects of topical lignocaine application on the patient's comfort in oropharyngeal swab sampling for COVID-19.[16]

Aim

Our study aimed to evaluate the effects of topical lidocaine application for nasopharyngeal sampling, on pain perception, the comfort of the individuals, and the application difficulty for healthcare staff.

Material and methods

Study design and setting

This study is a prospective randomized placebo-controlled study with restricted randomization of an allocation ratio of 1:1. We used Random Allocation Software (RAS) for randomization.[17] The study was conducted following the CONSORT guideline and the tenets of the Declaration of Helsinki at our Emergency Department between 01.09.2020 and 30.09.2020 after obtaining the approval of the Clinical Research Ethics Committee.[18] We have been registered in a clinical trial database (ClinicalTrials.gov Identifier: NCT04885777). Also, the written informed consent of all participants was obtained.

The study population consisted of 100 healthy volunteers, 50 individuals in the Lidocaine group, and 50 individuals in the Placebo group. Initially, we evaluated all participants for eligibility by the inclusion and exclusion criteria. The inclusion criteria of the study were: (1) being 18 years and older, and (2) volunteering to participate in the study. The exclusion criteria of the study were: (1) taking analgesic drugs before admission, (2) pregnancy, (3) lactation, (4) having a bleeding disorder, (5) known allergy to Lidocaine, (6) previous nasal trauma or operation, (7) having respiratory tract infection symptoms (such as fever, headache, runny nose, sore throat, cough, sneeze, breathlessness), and (8) having a chronic disease (diabetes, cancer, heart disease, asthma, COPD, etc.).

Allocation to the study arms was performed using sealed and opaque envelopes to ensure allocation concealment. The participants were allocated using the random allocation sequence list which we generated via RAS software.

Measurements

The sample collection procedure was categorized into four steps, which were; (1) inserting the swab in the nostril, (2) hitting the back of the nasopharyngeal cavity, (3) rotating five times, and (4) removing slowly. To evaluate the placebo effect, we performed two-stage sampling procedure. Initially, a naso pharyngeal swab was performed on each group without any intervention. We waited at least one hour for the second sample collection. If the participant was still in pain or felt discomfort due to the first sampling, the waiting time has been extended. At this stage, one ml of a solution containing 20 mg/ml of lidocaine (Aritmal %2, OSEL İlaç San. ve Tic. A.Ş., Turkey), was applied to each nostril of the participants in the Lidocaine group. Thus, a total of 40 mg of lidocaine, 20 mg for each nostril, was given to the individuals of the Lidocaine group. The Placebo group received only a total of 2 ml of 0.9% NaCl (one ml for each nostril). The second samples were collected after waiting 5 minutes following the administration of the solution. All participants were instructed not to eat or drink for 30 minutes following the sampling procedure to avoid the risk of aspiration. All these procedures were performed by two emergency medicine physicians. We did not send the samples to any laboratory test. All used swabs were disposed of in a medical waste box following the healthcare waste-management policy of our hospital.

To provide double-blinding, all solutions (both with and without Lidocaine) were previously prepared in coated 5 ml syringes without needle (BD Biosciences, USA), and numbered these syringes consecutively by an independent physician. Participants and performer physicians did not know the group number represents which solution until inputting the study data.

Age (year) and sex of the participants were recorded. There are four primary outcomes of the study. The first and second are the changes in the severity of pain and discomfort that were felt during the sampling procedure. These outcomes were measured via a paper questionnaire that had two Numerical Rating Scale (NRS) which the first one is for pain intensity and the second one is for discomfort intensity. The NRSs were 100 mm scales ranging from 0 to 10 (0 as the absence of pain/discomfort and 10 as unbearable pain/discomfort). We gave the questionnaire to the participants and explained how to perform. The scores were recorded two times by the participants after the first and second sampling processes.

The third primary outcome is the frequency of undesirable reactions during sample collection. We recorded head retraction, holding practitioner staff's hand, grimace, cough, and sneeze as the undesirable reaction during the sampling procedure.

The fourth primary outcome is the judgment of the practitioner staff about the sampling procedure. We included the appropriateness and the difficultness of the sample collection procedure, for this outcome. If four steps of the sampling procedure (inserting in the nostril, hitting the back of the nasopharyngeal cavity, rotating five times, and removing) have been completed successfully, practitioner staff defined the sampling as appropriate. If one or more of these steps has not been completed, the staff retried one more time. If still there has been a problem in these steps, the staff defined the procedure as inappropriate. The practitioner staff performed a five-point Likert scale (1-minimally difficult, 2-slightly difficult, 3-moderately difficult, 4-substantially difficult, 5-extremely difficult) for measuring the difficultness of the procedure.

Statistical analysis

We calculated the minimum required sample size using G Power 3.1 software as 41 individuals in each study group (totally of 82 participants with an allocation ratio of 1:1), with a medium effect size of 0.5, type 1 error of 0.05, and a power of 0.80 for Mann-Whitney U Test [19]. Also, we calculated the sample size as 31 individuals (for each group) with the same parameters for Wilcoxon Signed Rank Test. After then, we performed one more calculation for McNemar Test with an Odds ratio of 3, type 1 error of 0.05, a power of 0.80, and a total proportion of expected discordant pairs, and we found the minimum required sample size as 39 for this test. Finally, we approved the minimum required sample size for the study was 82 (41 people in each group).

Statistical analyses were conducted using the SPSS version 20 statistical software (IBM Corp. in Armonk, NY). Descriptive data are presented as mean with standard deviation and median with interquartile range for numerical variables, and the frequency with percentage for categorical variables. Shapiro-Wilk and Kolmogorov – Smirnov tests were used to evaluate the distribution of the numeric data. Independent-Samples Mann–Whitney U test was used for comparing non-normally distributed numeric and ordinal data between Lidocaine and Placebo groups. Related-Samples Wilcoxon Signed Rank Test was used for comparing non-normally distributed numeric and ordinal data among first and second samplings. Pearson chi-square and Fisher's Exact Test were used for comparing categorical variables be-

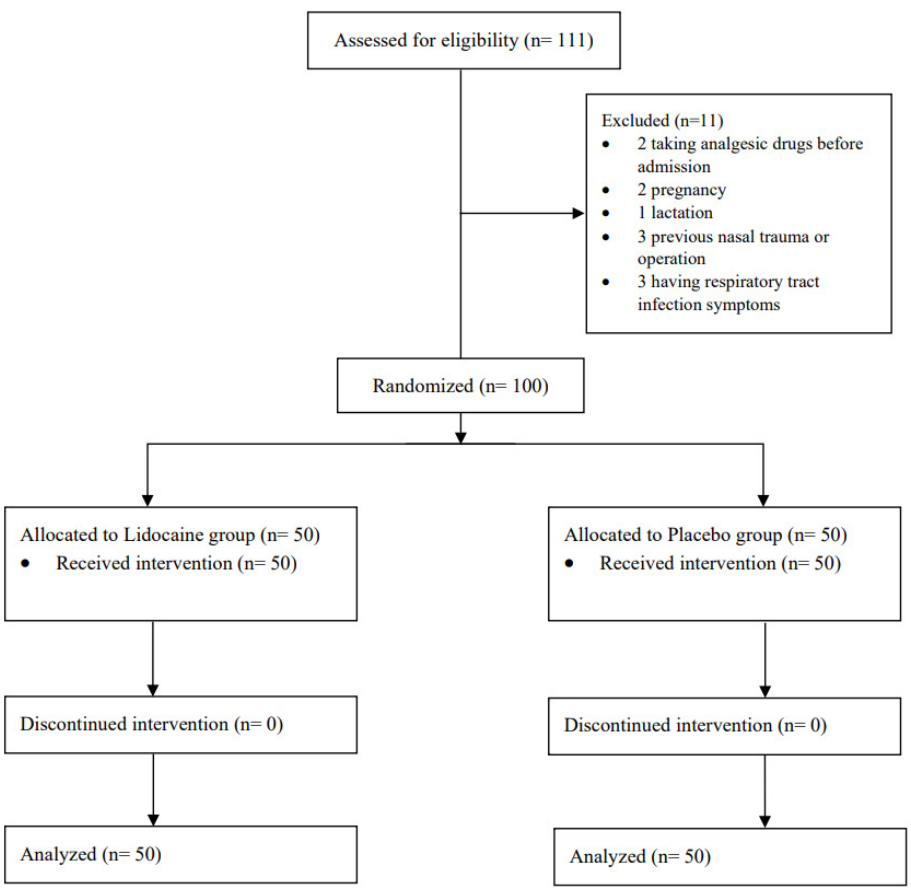

Fig. 1. CONSORT flow diagram of study

tween two study groups. Related-Samples McNemar Test was used for comparing categorical variables between first and second samplings. $p<0.05$ was considered as the statistically significant level.

Results

This study was conducted with healthy volunteers. We assessed the participants for eligibility according to the inclusion and exclusion criteria. After excluding 11 individuals, 100 people were randomized into two study arms half and half. There was no lost to follow-up, and we analyzed all 100 participants' data (Fig. 1).

The mean age of participants was 34.5±9.8 years and 35.4±8.9 years, and the males were 30.0% and 33.0% in Lidocaine and Placebo groups, respectively (Table 1).

Table 1. Demographics of the patients

Demographics	Lidocaine group (n= 50)	Placebo group (n= 50)
Age (years)		
Mean ± SD	34.5±9.8	35.4±8.9
Median (IQR)	32.0 (26.0-42.3)	36.0 (27.8-40.3)
Sex (male), n (%)	30 (60.0)	33 (66.0)

SD – standard deviation, IQR – interquartile range

In the first sampling process, pain and discomfort scores were statistically similar in both Lidocaine and Placebo groups. In the second sampling, there were statistically significant decreases in pain and discomfort scores when compared to the first sampling procedures in the Lidocaine group (p<0.001 and p<0.001, respectively). However, pain and discomfort scores were statistically similar among first and second samplings in the Placebo group (Table 2).

The frequencies of all undesirable reactions were statistically similar among the Lidocaine group and the Placebo group in the first sample collection. There were statistically significant decreases in the frequency of head retraction, holding staff's hand, coughing, and sneezing in second sampling when compared to first sampling in the Lidocaine group (<0.001, <0.001, <0.001, and <0.001, respectively). However, there was a statistically significant decrease only in holding staff's hand in second sampling in the Placebo group (p=0.004). On the other hand, there was no statistically significant difference in grimace between first and second sampling processes both in Lidocaine and Placebo groups (Table 3).

Table 4 presents health staff's judgments on sampling procedure. The appropriateness rate of the first sampling process was statistically similar according to

Table 2. Comparison of pain and discomfort scores

Variables		Placebo group (n= 50)	Lidocaine group (n= 50)	*p**
Pain score in first sampling	Mean ± SD	6.3±2.2	6.1±2.0	0.756
	Median (IQR)	6.0 (5.0-8.0)	6.5 (5.0-8.0)	
Pain score in second sampling	Mean ± SD	5.9±2.2	1.9±1.7	-
	Median (IQR)	6.0 (4.0-8.0)	2.0 (1.0-2.0)	
*p***		0.309	<0.001	
Discomfort score in first sampling	Mean ± SD	7.0±2.3	7.1±1.9	0.813
	Median (IQR)	7.0 (5.0-9.0)	7.0 (6.0-8.3)	
Discomfort score in second sampling	Mean ± SD	6.7±2.2	2.0±1.8	-
	Median (IQR)	7.0 (5.0-9.0)	2.0 (0.0-3.0)	
*p***		0.226	<0.001	

SD – standard deviation, IQR – interquartile range, * independent-samples Mann-Whitney U test was used, ** related-samples Wilcoxon signed rank test was used

Table 3. Comparison of patient reactions

Variables	Placebo group (n= 50)	Lidocaine group (n= 50)	*p*
Head retraction in first sampling, n (%)	36 (72.0)	32 (64.0)	0.391*
Head retraction in second sampling, n (%)	36 (72.0)	5 (10.0)	-
*p***	>0.999	<0.001	
Holding staff's hand in first sampling, n (%)	20 (40.0)	14 (28.0)	0.205*
Holding staff's hand in second sampling, n (%)	8 (16.0)	0 (0.0)	-
*p***	0.004	<0.001	
Grimace in first sampling, n (%)	47 (94.0)	46 (92.0)	>0.999***
Grimace in second sampling, n (%)	48 (96.0)	44 (88.0)	-
*p***	>0.999	0.754	
Cough in first sampling, n (%)	18 (36.0)	25 (50.0)	0.157*
Cough in second sampling, n (%)	13 (26.0)	4 (8.0)	-
*p***	0.063	<0.001	
Sneeze in first sampling, n (%)	12 (24.0)	14 (28.0)	0.648*
Sneeze in second sampling, n (%)	18 (36.0)	0 (0.0)	-
*p***	0.238	<0.001	

* Pearson chi-square test was used, **related-samples McNemar test was used, *** Fisher's exact test was used

health staff among the two study groups. Also, there was no statistically significant difference in the appropriateness between the first and the second samplings both in Placebo and Lidocaine groups.

Table 4. Comparison of staff's judgments

Variables	Placebo group (n= 50)	Lidocaine group (n= 50)	*p*
Appropriateness of first sampling, n (%)	46 (92.0)	45 (90.0)	>0.999*
Appropriateness of second sampling, n (%)	48 (96.0)	49 (98.0)	-
*p***	0.625	0.219	
Difficulty of first sampling, n (%)			0.649***
Minimal	9 (18.0)	7 (14.0)	
Slight	8 (16.0)	11 (22.0)	
Moderate	16 (32.0)	19 (38.0)	
Substantial	10 (20.0)	8 (16.0)	
Extreme	7 (14.0)	5 (10.0)	
Difficulty of second sampling, n (%)			-
Minimal	3 (6.0)	26 (52.0)	
Slight	14 (28.0)	15 (30.0)	
Moderate	15 (30.0)	6 (12.0)	
Substantial	13 (26.0)	3 (6.0)	
Extreme	5 (10.0)	0 (0.0)	
*p****	0.589	<0.001	

* Pearson chi-square test was used, **related-samples McNemar test was used, *** independent-samples Mann-Whitney U test was used, **** related-samples Wilcoxon signed rank test was used

The difficulty of first sample collection was statistically similar among Placebo and Lidocaine groups according to staff opinion. However, practitioner staff expressed that the second sampling process was easier than the first sampling in the Lidocaine group. This difference was statistically significant (<0.001). Besides this, staff found the difficulty of first and second sampling procedures similar in the Placebo group (Table 4).

Discussion

Although we found that the pain and discomfort scores were similar among the two study groups before the intervention, there were statistically significant decrease in pain and discomfort scores only in the Lidocaine group after intervention. Similarly, there were statistically significant decreases in the frequency of all undesirable reactions except grimace, in the second sampling in the Lidocaine group, however, there was a statistically significant decrease only in holding staff's hand in second sampling in the Placebo group. The practitioner staff expressed that the second sampling was easier than the first in the Lidocaine group, but the difficulties of the first and second sampling processes were similar in the Placebo group. Besides, they decided that the appropriateness of the first and second procedures were similar both in the Lidocaine group and Placebo group.

Among the important control approaches to combating the COVID-19 pandemic is increasing the number of tests to detect as many cases as possible.[20] In COVID-19 diagnostic testing, upper respiratory tract (URT) samples such as nasal, nasopharyngeal, and oropharyngeal samples are recommended at the first stage. However, it is recommended to take lower respiratory tract samples in patients with negative results in URT samples and still clinically suspected COVID-19.[21] Nasal (middle turbinate), oropharyngeal, and nasopharyngeal sample collection are acceptable alternative methods for PCR, although nasopharyngeal sample collection is generally recommended by the Center for Disease Prevention and Control and the World Health Organization.[21,22] The nasopharyngeal swab is a widely used specimen worldwide, because of its high-grade sensitivity.[23] However, due to the difficulties in application, methods based on other body fluids or regions are also preferred.[23-25]

In our study, we found that the use of local anesthetic will facilitate nasopharyngeal sampling, both for the patient and for the healthcare personnel. For patients, less pain and more comfort during sampling may be a cause to overcome the prejudgments and barriers to testing.[11] For healthcare workers, sampling performed in a shorter time and without more repetition, patients' resistance and/or undesirable reactions, may reduce the workload and transmission caused by sampling.[7,13]

It may be thought that the application of local anesthetic before sampling may increase the waiting time of the patients for the test. This is important because suspected cases staying together for too long will increase the risk of COVID-19 transmission to potential negatives during testing.[26] However, our findings showed that only a 5-minute wait may provide adequate anesthesia. Also, lidocaine, which we used as the local anesthetic in our study, is easily available, and its intranasal application is a safe option.[15,27] The most important reason for choosing lidocaine in our study is its short-acting effect as well as its sufficient effect during the procedure.[28] Local anesthetics reduce pain by binding voltage-gated sodium channels and blocking the excitation threshold of nociceptive afferent neurons, and inhibit the inflammatory cascade in the dorsal horn of the spinal cord, which sensitizes the free-end nociceptor, thereby reducing excessive excitability.[29] A long-acting nasopharyngeal local anesthetic may increase the risk of adverse effects such as aspiration pneumonia due to ingestion of drug downward from the nasopharynx, and the risk of other systemic side effects.[29,30] This may create significant controversy in particular for COVID-19, the most important clinical presentation of which is pneumonia.

Limitations

This study has several limitations. The first is that the study is a single-center study with relatively small sample size. This negatively affects the external validity of the study. On the other hand, because the study was conducted with healthy volunteers, we cannot say exactly that lidocaine will provide similar effects in the "real" patients. In placebo-controlled studies, "the placebo effect" may be an essential confounder. In our study, we repeated the nasopharyngeal sampling twice, before and after the intervention, to get ahead of this potential bias. The results show us that there is no significant placebo effect in our study design. On the other hand, we did not evaluate the effect of lidocaine or isotonic saline application on the result of the test, such as PCR. However, the aim of our study is only to examine the convenience of lidocaine in the application of nasopharyngeal swab, Kanodia et al.[16] reported that oropharyngeal lignocaine application for oropharyngeal swab sampling did not change the SARS-CoV-2 viral load in RT-PCR test of the COVID-19 patients.

Conclusion

Considering that its effect on diagnostic test such as PCR has not been evaluated, intranasal lidocaine application reduces the pain that occurs during nasopharyngeal sampling and makes the procedure easier for the patient and the healthcare worker. Studies with broad participation in which the effects of the use of local anesthetics before sampling on diagnostic tests such as PCR are also investigated, may pave the way for developments in this area.

Declarations

Funding

The authors declared that this study has received no financial support or any funding.

Author contributions

Conceptualization, K.A.O. and C.Z.; Methodology, D.S. and T.D.; Software, D.S.; Validation, T.D., C.Z.; Formal Analysis, D.S.; Investigation, K.A.O.; Resources, I.A.; Data Curation, D.S.; Writing – Original Draft Preparation, T.D.; Writing – Review& Editing, I.A. and T.D.; Visualization, C.Z.; Supervision, C.Z.; Project Administration, K.A.O.

Conflicts of interest

The author(s) declare no competing interests.

Data availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was conducted following the CONSORT guideline and the tenets of the Declaration of Helsinki at our Emergency Department between 01.09.2020 and 30.09.2020 after obtaining the approval of the Ataturk University Clinical Research Ethics Committee. We have been registered in a clinical trial database (ClinicalTrials.gov Identifier: NCT04885777). Also, the written informed consent of all participants was obtained.

References

1. Niforatos JD, Chaitoff A, Zheutlin AR, Feinstein MM, Raja AS. Barriers to emergency department usage during the COVID-19 pandemic. *J Am Coll Emerg Physicians Open*. 2020;1:1261-1268.
2. Pines JM, Zocchi MS, Black BS, et al. The effect of the COVID-19 pandemic on emergency department visits for serious cardiovascular conditions. *Am J Emerg Med*. 2021;47:42-51.
3. Nourazari S, Davis SR, Granovsky R, et al. Decreased hospital admissions through emergency departments during the COVID-19 pandemic. *Am J Emerg Med.* 2021;42:203-210.
4. Carpenter CR, Mudd PA, West CP, Wilber E, Wilber ST. Diagnosing COVID-19 in the Emergency Department: A Scoping Review of Clinical Examinations, Laboratory Tests, Imaging Accuracy, and Biases. *Acad Emerg Med.* 2020;27:653-670.
5. Tolia VM, Chan TC, Castillo EM. Preliminary Results of Initial Testing for Coronavirus (COVID-19) in the Emergency Department. *West J Emerg Med.* 2020;21:503-506.
6. Mehta N, Mazer-Amirshahi M, Alkindi N, Pourmand A. Pharmacotherapy in COVID-19; A narrative review for emergency providers. *Am J Emerg Med.* 2020;38:1488-1493.
7. Petruzzi G, De Virgilio A, Pichi B, et al. COVID-19: Nasal and oropharyngeal swab. *Head Neck.* 2020;42:1303-1304.
8. Tsujimoto Y, Terada J, Kimura M, et al. Diagnostic accuracy of nasopharyngeal swab, nasal swab and saliva swab samples for the detection of SARS-CoV-2 using RT-PCR. *Infect Dis (Lond).* 2021:1-9.
9. Zedtwitz-Liebenstein K. Correctly performed nasal swabs. *Infection.* 2021:1-2.
10. Föh B, Borsche M, Balck A, et al. Complications of nasal and pharyngeal swabs - a relevant challenge of the COVID-19 pandemic? *Eur Respir J.* 2021;57(4):2004004.
11. McElfish PA, Purvis R, James LP, Willis DE, Andersen JA. Perceived Barriers to COVID-19 Testing. *Int J Environ Res Public Health.* 2021;18(5):2278.
12. Brandt MP, Jäger W, Epple S, Haferkamp A, Schröder A. SARS-CoV-2 outbreak in medical employees in a large urologic department: Spread, containment and outcome. *Am J Infect Control.* 2021;49:674-677.
13. Pasomsub E, Watcharananan SP, Boonyawat K, et al. Saliva sample as a non-invasive specimen for the diagnosis

of coronavirus disease 2019: a cross-sectional study. *Clin Microbiol Infect*. 2021;27:285.e1-.e4.
14. Yu X, Wang J, Huang L, Yu X, He Z. Efficacy and safety of bupivacaine versus lidocaine in local anesthesia of the nasopharynx: A meta-analysis. *Am J Rhinol Allergy.* 2016;30:176-180.
15. Özkiriş M, Aydin R, Gencer ZK, Saydam L. Comparison of topical anesthetic effects of lidocaine, prilocaine, ropivacaine, and bupivacaine in transnasal fiberoptic nasopharyngolaryngoscopy. *Am J Rhinol Allergy.* 2014;28:e141-e143.
16. Kanodia A, Srigyan D, Sikka K, et al. Topical lignocaine anaesthesia for oropharyngeal sampling for COVID-19. *Eur Arch Oto-Rhino-Laryngol.* 2021;278:1669-1673.
17. Saghaei M. Random allocation software for parallel group randomized trials. *BMC Med Res Methodol.* 2004;4:26.
18. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332.
19. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* 2007;39:175-191
20. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. *Nat Rev Microbiol.* 2021;19:171-183.
21. World Health Organization. Diagnostic testing for SARS-CoV-2: interim guidance, 11 September 2020. World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/334254/WHO-2019-nCoV-laboratory-2020.6-eng.pdf. Accessed 20 March 2021.
22. Centers for Disease Control and Prevention. Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html. Accessed 20 March 2021.
23. Bwire GM, Majigo MV, Njiro BJ, Mawazo A. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: A systematic review and meta-analysis. *J Med Virol.* 2021;93:719-725.
24. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med.* 2020;382:1177-1179.
25. Butler-Laporte G, Lawandi A, Schiller I, et al. Comparison of Saliva and Nasopharyngeal Swab Nucleic Acid Amplification Testing for Detection of SARS-CoV-2: A Systematic Review and Meta-analysis. *JAMA Intern Med.* 2021;181:353-360.
26. Chu DK, Akl EA, Duda S, Solo K, Yaacoub S, Schünemann HJ. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. *Lancet.* 2020;395:1973-1987.
27. Chi PW, Hsieh KY, Chen KY, et al. Intranasal lidocaine for acute migraine: A meta-analysis of randomized controlled trials. *PLoS One*. 2019;14:e0224285.
28. Covino BG, Giddon DB. Pharmacology of local anesthetic agents. *J Dent Res.* 1981;60:14549.
29. Dickerson DM, Apfelbaum JL. Local Anesthetic Systemic Toxicity. *Aesthet Surg J.* 2014;34:1111-1119.
30. Neal JM, Barrington MJ, Brull R, et al. The Second ASRA Practice Advisory on Neurologic Complications Associated With Regional Anesthesia and Pain Medicine: Executive Summary 2015. *Reg Anesth Pain Med.* 2015;40:401-430.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.9

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 63–69

ORIGINAL PAPER

Adesola Ojo Ojoawo [1], Blessing Ige [1], Kanode Kunnuji [2]

Muscle energy technique and static stretching in patients with mechanical neck pain – a randomized study

[1] Department of Medical Rehabilitation, Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile Ife, Nigeria
[2] Department of Physiotherapy, Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife Nigeria

ABSTRACT

Introduction and aim. Neck pain is becoming increasingly common throughout the world with a considerable impact on individuals. This study compared the effects of muscle energy techniques (MET) and static stretching (SS) on pain intensity and functional disability of patient with mechanical neck pain.

Material and methods. Fifty subjects with mechanical neck pain recruited were randomly allocated into MET and SS groups equally. Subjects in MET received MET protocol, and SS groups were treated with SS; both groups had treatment twice a week for six weeks. Pain intensity and functional disability at baseline, 3rd and 6th week of treatment were measured. Descriptive and Inferential statistics were used to analyze the data. Alpha level was set at <0.05.

Results. There were 12 males and 13 females for MET with age ranged between 31–53 years mean was 42.41± 7.35 years and 11 males and 14 females in SS group with age range 22–60 years and mean age of 42.91±10.44 years. There was a significant reduction in pain intensity and disability in MET's and SS group ($p<0.05$) when pre-treatment, 3rd week and 6th week treatment were compared. Pain intensity was lower at SS than MET while functional disability was lower in MET than SS $p<0.05$ at 6th week

Conclusion. MET reduces ND more than SS and SS reduces pain intensity better MET.

Keywords. muscle energy techniques, neck disability index, neck pain, static stretching, visual analogue scale

Introduction

Neck pain is becoming increasingly common throughout the world with a considerable impact on individuals, communities, health-care systems and businesses.[1] Neck is the most common site of non-traumatic musculoskeletal pain.[2] Roughly two-thirds of the general population have neck pain at some time in their lives and the prevalence is highest in middle age.[3] Prevalence of neck pain has an increasing trend up to 50 years followed by a decline and it has been found to be more in females.[4] With up to 37% of individuals developing persistent symptoms, neck pain is a condition that places a large economic burden on the health care system.[5]

Mechanical neck pain is a generalized neck pain with mechanical characteristics, including symptoms provoked by maintained back postures, neck movement, or by palpation of the cervical muscles.[6] Causes of neck pain are poor posture, muscle tension and strain, injury, osteoporosis, fibromyalgia, disc herniation and protrusion, spinal stenosis, meningitis.[7] The neck is particularly vulnerable to injury, especially in falls, car accidents, and sports where the muscle and ligaments of the neck are forced to move

Corresponding author: Adesola Ojo Ojoawo, e-mail: aoojoawo@oauife.edu.ng

Clinical trial registered with www.clinicaltrials.gov (NCT04350918)
Received: 16.10.2021 / Revised: 7.12.2021 / Accepted: 11.12.2021 / Published: 30.03.2022

Ojoawo AO, Ige B, Kunnuji K. *Muscle energy technique and static stretching in patients with mechanical neck pain – a randomized study.* Eur J Clin Exp Med. 2022;20(1):63–69. doi: 10.15584/ejcem.2022.1.9

outside of their normal range, neck injury due to sudden jerking of the head is commonly called 'Whiplash'.[8] In an estimated 50-80% of cases involving back or neck pain, an underlying pathology cannot be definitively determined.[9]

Combined manual therapy and exercise has resulted in improved patient outcomes or satisfaction levels when compared to spinal manipulation or exercise alone.[10] Muscle Energy Technique (MET) is a manual medicine procedure that has been described as a gentle form of manipulative therapy effective for treating movement restrictions of both the spine and extremities.[11] MET is a method of treatment that involves the voluntary contraction of a subject's muscle(s) in a precisely controlled direction, against a counterforce provided by the operator. Muscle energy techniques may be used to decrease pain, stretch tight muscles and fascia, reduce muscle tonus, improve local circulation, strengthen weak musculature and mobilizes joint restrictions. MET was reported to be effective in the management of long term neck pain, it increases joint range of motion, chronic lateral epicondylitis and is useful to increase range of motion when there is limitation in function. This technique can also strengthen physiologically weakened muscles and reduce localized edema through muscle pump action.[12] Muscle energy technique has been demonstrated to be effective in increasing the restricted range of trunk rotation and ameliorating rotational asymmetry in asymptomatic subjects.[13]

Stretching involves the application of manual or mechanical force to elongate structures that have adaptively shortened and are hypo-mobile.[14] Stretching is believed to provide many physical benefits including improved flexibility, injury prevention, improved muscle or athletic performance, improved running economy, promotion of healing and possibly decreased of set of muscle soreness.[15] Static stretching involves stretching a muscle to a point of discomfort and holding the stretch for a length of time, followed by a return to normal resting muscle length.[16]

Both MET and stretching are widely used techniques in the field of physiotherapy.[17] Studies using these two techniques individually in symptomatic as well as in asymptomatic population have been shown improvement, but very few studies have compared these techniques in a symptomatic population, where conflicting results are seen.[18,19]

Neck pain is a common problem within our society affecting individual's physical and social functioning considerably and interfering with the patient's daily activities. A wide variety of treatment protocols for mechanical neck pain are available. However, the most effective management remains an area of debate.

Aim

Therefore, this study was done to compare effect of MET with static stretching in the management of pain and functional disability in patients with mechanical neck pain. The intension was to be able to pick the best effective protocol for the management of neck pain.

Material and methods

Subjects

Subjects for this study were patients diagnosed of mechanical neck pain in Physiotherapy Outpatient Department of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria

The following categories of individuals were considered for this study;

a. Male and female patients with history of mechanical neck pain of more than 3 months,

Exclusion criteria

a. Patients with acute neck pain
b. Subjects were excluded if they have neck pain associated with an underlying pathology such as fracture of the cervical spine, neck pain radiating into the arms or upper extremity or associated with headaches or facial pain, malignancy, infections, inflammatory disorders, osteoporosis or cases of disc prolapse.
c. Patients with history of surgery of the cervical spine during the previous 12months

Sample Size Determination

$$N = \frac{4\sigma^2 (Zcrit + Zpwr)^2}{D^2}$$

Where,

N is the total sample size

σ is the assumed SD of each group (assumed to be equal for the groups),[20] this is assumed to be 16.63 from a previous study of Ojoawo et al., [21]

Zcrit is the standard normal deviate corresponding to the selected significant criterion, i.e. .05 (95% = 1,960)

Zpwr is the standard normal deviate corresponding to the selected statistical power (i.e. 0.80 = 0.842)

D is the minimum expected difference among the three means and D = 18 from a previous study of Ojoawo et al. [21]

Therefore, $N = \frac{4x(16.3)^2 (1.96 + 0.842)^2}{18^2}$

Thus, N = 41.22

10% which is 4 of the sample size was added to make making 46, but the sample was rounded up to 50 to accommodate for non-response and attrition. Fortunately, all the 50 completed the study

Research design

This was a quasi-experimental study

Sampling technique

Purposive sampling technique was used to recruit patients with mechanical neck pain.

Randomization

Individuals who meet the inclusion criteria were randomly allocated to MET Group and SS Group using the simple random assignment method without replacement (Fish Bowl) Fig 1

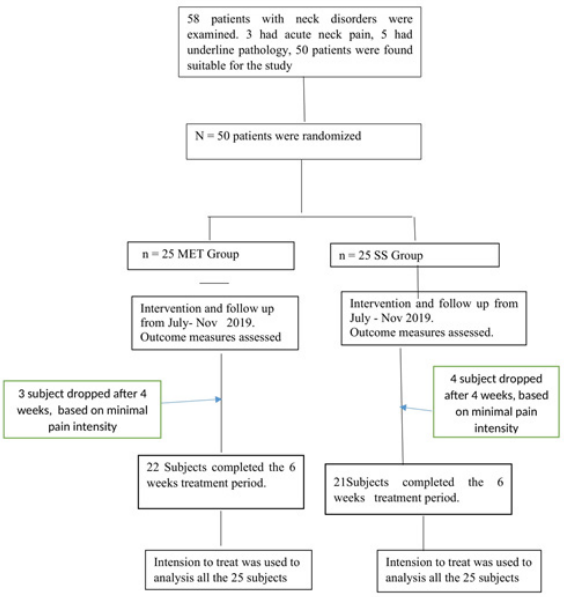

Fig. 1. Flow chart for the Randomized Control Trial (RCT)

Instrumentation

Neck disability index (NDI)

The neck disability index (NDI) was used for patients with neck pain and assess two domains (pain and disability) perceived in the last three months. The NDI for measuring disability in patients with neck pain has a pivotal role in research and clinical settings and is interpreted to have a good reliability.[22] The NDI is strongly correlated (r=0.70) to a number of similar indices and moderately related to both physical and mental aspect of general health.[23]

The NDI can be scored as a raw score or doubled and expressed as a percent.[24] Each session is scored on a 0 to 5 rating scale, in which zero means 'No Pain' and 5 means 'Worst Imaginable Pain'. All the points can be summarized as a total score. The test can be interpreted as a raw score with a maximum score of 50 or as a percentage.

Some benchmark has been found in literature but methodologically, they were not described and their validity and reliability are questionable. Vernon and Moir presented the following interpretations: 0-4 points (0-8%): No disability, 5-14 points (10-28%): Mild disability, 15-24 points (30-48): Moderate disability, 25-34 points (50-64%): Severe disability 35-50 points (70-100%): Complete disability.[24]

Visual analogue scale

The visual analogue scale (VAS) is a frequently used method for the assessment of variations in intensity of pain. In clinical practice, the percentage of pain intensity assessed by VAS, is often considered as a measure of the efficacy of treatment. VAS is considered to be one of the best methods available for the estimation of the intensity of pain.[25] High correlations have been reported between VAS and verbal and numerical rating scales.[26] Visual analogue scale and NRS were found reliable in the assessment of pain at the without an appreciable differences between them.[27] Based on the distribution of pain VAS scores in post-surgical patients (knee replacement, hysterectomy, or laparoscopic myomectomy) who described their postoperative pain intensity as none, mild, moderate, or severe, the following cut points on the pain VAS have been recommended: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm).[28]

Procedure

Application of muscle energy techniques (MET)

Patient was positioned at sitting position on a chair with a pillow to support the back, the neck range of motion was free from all obstructions. Muscles energy technique (MET) with Post Facilitation Stretch protocol was applied to the patients according to Nagrale et al.[5] There was a set of 5 repetitions per session, and 2 sessions per week for six weeks according to Sadria et al. each stretching was held for 10 seconds.[29] Kneading massage was administered for the patient with methyl salicylate ointment for 5 minutes. Subjects in MET Group received 12 treatment sessions of MET two times a week.[5]

Application of static stretching (SS)

Subjects in SS Group received 12 treatment sessions of static stretching according to Reid et al., two times a week.[31] The neck was placed in a side flexion position first to the right side, the shoulder on the contralateral side was stabilized with one hand and the other hand was used to stretch the neck towards the side of flexion. This was held for 20 seconds and ten repetition per a section.[30]

Kneading massage was applied to the patients with methyl salicylate ointment for 5 minutes according to Weerapong.[32] Kneading massage was administered as follows: muscles of the neck were held, lifted up, rolled and squeezed in a compressive action using methyl salicylate gel as coupling medium. The techniques was applied to the muscles of the posterior region of the neck as well, the underline muscles were well compressed with deep pressure.[31]

Pain intensity and functional disability were assessed at the baseline, after 3rd week and at the end of 6 weeks intervention. The application of methyl salicylate was considered as base line for all the subject as adjunct.

Ethical approval

Approval (no.:IRB/IEC/0004553; NERC/27/02/2009a) of the Health Research and Ethical Committee, Obafemi

Awolowo University Teaching Hospitals Complex Ile-Ife was obtained before the commencement of data collection. The study procedure and rationale were explained to the subjects and their informed consent to participate was obtained. They were assured that all information provided by them on the questionnaire would be treated with utmost confidentiality.

Data analysis

The data collected were analyzed using Statistical package foe social sciences International Business machine IBM 21 ((SPSS Inc., Chicago, IL, USA). Descriptive statistics and Mixed Model ANOVA was used to compare the mean values of outcome measures within and across the group. Alpha level was set at 0.05

Results

Physical characteristics of subjects

Shown in Table 1 is the physical characteristics of the subjects in the two groups. The age range for MET group is 31-53 years and the mean of age, height, weight and BMI were 42.92±10.45/yrs., 1.65±0.1/m, 67.50±13.36/kg, and 24.55±3/kg/m^2 respectively while the age range for SS group was 22 -60 years, the mean age, height, weight and BMI for the Static Stretching group were 42.42±7.35/yrs.,1.66±0.05/m, 70.67±10.59/kg and 25.65±3.76/kg/m^2 respectively. There was no significant difference (p>0.05) when the physical characteristics of the two groups were compared. There were 12 males and 13 females for MET and 11 males and 14 females in SS group.

Effect of muscle energy technique on pain intensity and functional disability

Shown in Figure 2 is the line graph of mixed method ANOVA comparing the pre-treatment, 3rd week and 6th week treatments of the subjects in MET group. The results revealed that there was a significant difference in the VAS as well as ND of pre-treatment, 3rd week and 6th week treatment (p<0.001). The mean values were stated in table 1.

Effects of static stretching technique on pain intensity and functional disability

Shown in Figure 3 is line graph from mixed model ANOVA comparing the mean values of the pre-treatment, 3rd week and 6th week treatments of the subjects in static stretching group. The results revealed that there was a significant difference in the PI and ND when the pre-treatment, 3rd week and 6th week treatment p<0.001). The actual mean values could be found in table 1.

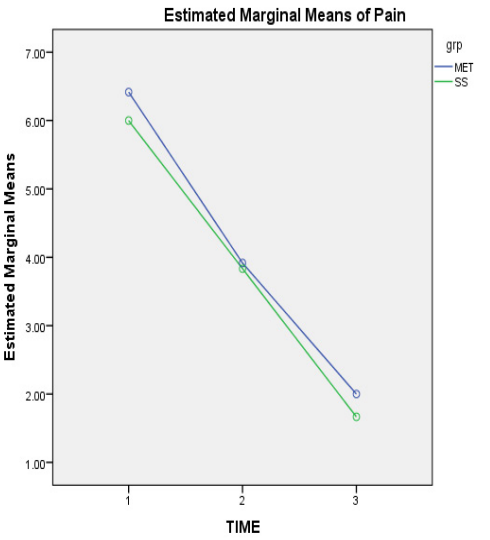

Fig. 2. Effect of muscle energy techniques on pain intensity and disability

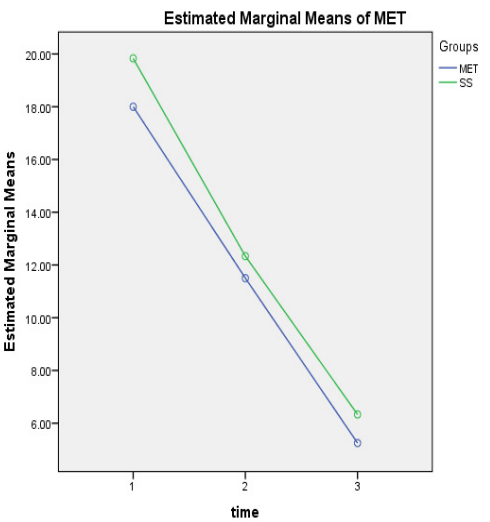

Fig. 3. Effect of static stretching techniques on pain intensity and disability

Table 1. Physical characteristics of the subjects (N= 50)*

Variables	MET	SS	Total	t	p
	Mean±SD, n=25	Mean±SD, n=25	Mean±SD, N=50		
Age (yrs.)	42.92±10.45	42.42±7.35	42.67±8.84	-0.136	0.893
Height (m)	1.65±0.1	1.66±0.05	1.66±0.08	0.643	0.527
Weight (kg)	67.50±13.36	70.67±10.59	69.08±11.9	0.263	0.795
BMI (kg/m^2)	24.55±3	25.65±3.76	25.10±3.38	0.795	0.435

* MET – muscle energy techniques SS – static stretch, BMI – body mass index

Comparisons between the effect of muscle energy technique and static stretching on pain intensity and functional disability

Shown in table 2 is the Mixed Model ANOVA comparing the mean values of the pre-treatment, 3rd week and 6th week of PI and ND of subjects in MET and SS Groups. There was a significant difference in both 3rd and 6th week PI (P= 0.001) and ND (P= 0.001) when the MET and Static Stretching groups were compared. The means value of PI and ND in MET (2.00±1.92; 5.25 ±2.52) at the 6th week was also found to be more than that of SS (1.66±0.98; 6.33±3.33). This implied that SS may be more effective in pain management while MET may be more effective in ND management.

Discussion

The specific objectives of this study were to evaluate the effectiveness of MET, SS and compare their effects on pain intensity and functional disability of patients with mechanical neck pain.

In this study, a significant reduction among the pre-treatment, 3rd and 6th post-treatment pain intensity and functional disability of subjects that received MET and SS was observed. Study revealed that perception of pain intensity was ameliorated by MET due to raise in stretch tolerance of the patient. In every muscle and joints, there are mechanoreceptors and proprioceptors; these organs are stimulated whenever a group of muscle contract isometrically and stretched.[11] The effects of these will consequently lead to drop in discomfort, the muscle is easier to be stretch and patient has more tolerance The results obtained for pain reduction in the MET group could be similar to the previous studies where pain intensity was reduced following MET over the neck area and also at other areas of the body.[5,32,33] A study by Gupta et al., on effects of post-isometric relaxation versus isometric exercises in nonspecific neck pain also concluded that MET showed significant improvement in pain and functional status.[32] However, the results of our study when compared the two groups indicated that SS relieves pain than MET but MET reduced disability than MET. Results of a study by Sharmila on effects of the MET versus conventional exercises in nonspecific neck pain in secondary school teachers are in accordance with the results for MET Group, which concluded that post-isometric relaxation had better reduction in pain and disability.[34]

The reduction in the pain following static stretching could be due to the inhibitory effects of Golgi tendon organs, which reduces the motor neuronal discharges, thereby causing relaxation of the musculotendinous unit by resetting its resting length and pacinian corpuscle modification. These reflexes will allow relaxation in musculotendinous unit tension and decreased pain perception.[35] Kostopoulos et al. found a significant pain reduction in the group treated with passive stretching of upper trapezius, which is in accordance with this study.[36] The results of this study for the stretching group was in tandem with study conducted by Paolo et al. on effects of global posture re-education and static stretching on pain, range of motion and quality of life in women with chronic neck pain which concluded that stretching showed significant improvement in outcome measures.[37]

There was a statistically significant difference found in ND in the treatment groups. This could be because the NDI assesses different aspects of neck pain which consists of pain intensity, daily activities, suggesting that improvement in the score might be due to the reduction in pain.

However, comparing the mean values of the 6th week of PI and ND of subjects in MET and SS groups in this study, there was a reduction of ND in the MET group than that of SS, indicating that MET may be better than SS in improving functional disability. The higher the values of NDI the more the disability, hence MET seems to have improved neck disability than SS. On the other hand, there was a reduction in the values of pain intensity in the SS group than that of MET. The lower the values of visual analogue scale, the less the pain. This implies that SS seems to have improved pain intensity better than MET. The results of this study was not totally in line with the study of Shady et al., and Apoorva et al., they both VAS and NDI scores showed better improvement in the MET group as compared to the stretching group.[17,38] The work of Apoorva et al. was very similar with our own study however their study was for six days whereas our study was for six weeks.[17] The similar results found in the two separate researches is an affirmation of the authenticity of the findings. However, Hatefi et al. though it was on nonspecific low back pain report-

Table 2. Mixed Method ANOVA comparing the METG and SSG pretreatment, third week and sixth week mean values of pain intensity and neck disability, N= 50 *

Variables	METG				SSG				
	Pre Rx	W3Rx	W6Rx	Change	PreRx	W3Rx	W6Rx	Change	p
PI	6.42 (1.31)[a]	3.92 (0.90)b	2.00 (1.92)c	4.37 (4.40)	6.00 (0.74)d	3.83 (0.94)e	1.66 (0.98)f	4.34 (0.154)	<0.0001
NDI	18.00 (6.42)g	11.50 (4.1)h	5.25 (2.52)i	12.75 (3.4)j	19.83 (6.65)	12.33 (6.38)l	6.33 (3.33)m	13.50 (1.01)	<0.0001

* METG – muscle energy techniques group, SSG – static stretching group, Rx – treatment, W – week, indicate significant at p<0.001. Post-hoc List of Significant Difference: mean mode with the same superscript (a,b,c,d ----------) show no significant difference but mean mode with different superscript shows significant different

ed improvement in pain intensity using static stretching exercises which was in agreement with our study.[39]

Recommendation

There may be an interaction between the treatment effects of conventional exercise program and muscle energy technique/ static stretching. Therefore, the results could demonstrate only the relative effectiveness of the two protocol. To find out whether each program was indeed effective in treating mechanical neck pain, further studies are required.

Limitation

There was no blinding in the study. This may have influence on the assessment of outcome measures. The use of methyl salicylate as massage medium could have effect on the outcome measure especially pain intensity. However, massage was carried out for the two groups. The effect may be generalized.

Conclusion

It can be concluded from the study that both Muscle Energy Technique and Static Stretching are effective in improving pain intensity and functional disability in patients with mechanical neck pain. However, MET reduces both disability more than SS while SS reduces pain more than MET.

Declarations

Funding

The funding for the project was contributions from the authors. The study did not enjoy funding from any organization.

Author contributions

Conceptualization, A.O.O. and I.B.; Methodology, A.O.O. and I.B.; Software, A.O.O; Validation, I.B and K.K.; Formal Analysis, A.O.O.; Investigation, K.K.; Resources, I.B.; Data Curation, I.B.; Writing – Original Draft Preparation, A.O.O. and I.B.; Writing – Review & Editing, A.O.O.; Visualization, K.K.; Supervision, A.O.O.; Project Administration, I.B. and K.K.; Funding Acquisition, I.B. and A.O.O.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

Ethics approval

Approval of the Health Research and Ethical Committee, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife (no.:IRB/IEC/0004553; NERC/27/02/2009a) was obtained for the study

References

1. Hoy DG, Protani M, De R, Buchbinder R. The epidemiology of neck pain. *Best Pract Res Clin Rheumatol.* 2010;24(6):783-792.
2. El-Metwally A, Salminen, JJ, Auvinen A, Macfarlane G, Mikkelsson M. Risk factors for development of non-specific musculoskeletal pain in preteens and early adolescents: a prospective 1-year follow-up study. *BMC Musculoskelet Disord.* 2007;8(1):46.
3. Binder AI. Cervical spondylosis and neck pain. *BMJ.* 2007;334 (7592):527-531.
4. Cagnie B, Danneels L, Van Tiggelen D, De Loose V, Cambier D. Individual and work related risk factors for neck pain among office workers: a cross sectional study. *European Spine Journal.* 2007;16(5):679-686.
5. Nagrale AV, Glynn P, Joshi A, Ramteke G. The efficacy of an integrated neuromuscular inhibition technique on upper trapezius trigger points in subjects with non-specific neck pain: a randomized controlled trial. *J Man Manip Ther.* 2010;18(1):37-43.
6. Heintz MM, Hegedus E J. Multimodal management of mechanical neck pain using a treatment based classification system. *J Man Manip Ther.* 2008;16(4):217-224.
7. Barron ME. Cervical Spondylosis: Diagnosis and Management http:// www.emedicine.com/neuro/topic564.htm Accessed July 10, 2021.
8. Yan Y, Jing H, Fan Li, Hu L. Investigation of the Effect of Neck Muscle Active Force on Whiplash Injury of the Cervical Spine. *Appl Bionics Biomech.* 2008;(20):18.
9. Deokhoon, J Johnston V, McPhail SM, Leary SO. A Longitudinal Evaluation of Risk Factors and Interactions for the Development of Nonspecific Neck Pain in Office Workers in Two Cultures. *Int J Hum Factors Ergon.* 2020;63:4.
10. Evans R, Bronfort G, Nelson B, Goldsmith CH. Two-year follow-up of a randomized clinical trial of spinal manipulation and two types of exercise for patients with chronic neck pain. *Spine* 2002;27(21):2383-2389.
11. Chaitow L, DeLany J. Neuromuscular techniques in orthopedics. *Tech Orthop.* 2003;18(1):74-86.
12. Ewan T, Antonio RC, Diba M, Antonino B, Antonio P. The efficacy of muscle energy techniques in symptomatic and asymptomatic subjects: a systematic review. *Chiropr Man Therap.* 2019;27:35.
13. Lenehan KL, Fryer G, McLaughlin P. The effect of muscle energy technique on. gross trunk range of motion. *Journal of Osteopathic Medicine.* 2003;6(1):13-18.
14. Mahajan R, Kataria C, Bansal K. Comparative effectiveness of muscle energy technique and static stretching for treatment of subacute mechanical neck pain. *Int J Health Rehabil Sci.* 2012;1(1):16-21.
15. Hertling, D, Kessler RM. *Management of common musculoskeletal disorders: physical therapy principles and methods.* Lippincott Williams & Wilkins. 2006.
16. Andrews JR, Harrelson GL, Wilk KE. P*hysical Rehabilitation of the Injured Athlete: Expert Consult-Online and Print.* 2012; Elsevier Health Sciences.

17. Apoorva P, Nilima B, Ashok S, Parag S. Effect of muscle energy technique and static stretching on pain and functional disability in patients with mechanical neck pain: A randomized controlled trial. *Hong Kong Journal of Physiotherapy.* 2016;35:5-11.
18. Narain AJ, Singh, S. Bhowmik To compare the effect of core stability exercises and muscle energy techniques on low back pain patients IOSR. *J Sports Phys Educ.* 2013;1:9-15.
19. Hashim AM, Mohd K. Shveta K. Effect of muscle energy technique and static stretching on hamstring flexibility in healthy male subjects. *Ind J Physiother Occup Ther*. 2010;4:32-36.
20. Eng J. Sample size calculation: How many individuals should be studied? *Radiology.* 2003;227:309-313.
21. Ojoawo AO, Olabode A, Esan O, Badru A , Odejide S, Arilewola B. Therapeutic Efficacy of Cervical Traction in the Management of Cervical Radiculopathy: A Control Trial. *Rwanda J Health Sci.* 2013;2(2):25-29.
22. Trouli, MN, Vernon, HT, Kakavelakis, KN, Antonopoulou, MD, Paganas, AN, Lionis, CD. Translation of the Neck Disability Index and validation of the Greek version in a sample of neck pain patients. *BMC Musculoskeletal Disorders*. 2008;9(1):106.
23. Mac Dermid JC, Walton DM, Avery S, et al. Measurement Properties of the Neck Disability Index: A Systematic Review. *J Orthop Sports Phys Ther.* 2009;39(5):400-417.
24. Vernon H, Mior S. The Neck Disability Index: a study of reliability and validity. *J Manipulative Physiol Ther*. 1991;14(7):409-415.
25. Scott J, Huskisson EC. Graphic representation of pain. *Pain*. 1976;2(2):175-184.
26. Alghadir AH, Anwer S, Iqbal A, Iqbal ZA. Test-retest reliability, validity, and minimum detectable change of visual analog, numerical rating, and verbal rating scales for measurement of osteoarthritic knee pain. *J Pain Res.* 2018;11:851-856.
27. Shafshak, TS, Elnemr, R. The Visual Analogue Scale Versus Numerical Rating Scale in Measuring Pain Severity and Predicting Disability in Low Back Pain, JCR: *J Clin Rheumatol.* 2021;27(7):282-285.
28. Aun, C, Lam YM, Collett B. Evaluation of the use of visual analogue scale in Chinese patients. *Pain*, 1986;25(2):215-221.
29. Sadria G, Hosseini M, Rezasoltani A, Akbarzadeh Bagheban A, Davari A, Seifolahi A. A comparison of the effect of the active release and muscle energy techniques on the latent trigger points of the upper trapezius. *J Body Mov Ther.* 2017;21:920-925.
30. Reid JC, Greene R, Young JD, Hodgson DD, Blazevich, AJ, Behm DG. The effects of different durations of static stretching within a comprehensive warm-up on voluntary and evoked contractile properties. *Eur J Appl Physiol.* 2018;118:1427-1445.
31. Weerapong P, Hume PA, Kolt GS. The Mechanisms of Massage and Effects on Performance, Muscle Recovery and Injury Prevention. *Sports Med.* 2005;35:235-256.
32. Prashant N, Anand H, Subhash K. Comparison of muscle energy technique and positional release therapy in acute low back pain—RCT. *Ind J Physiother Occup Ther.* 2010;4:32-36.
33. Gupta S, Jaiswal P, Chhabra D. A comparative study between postisometric relaxation and isometric exercises in non-specific neck pain. *Journal of Exercise Science and Physiotherapy*. 2008;4(2):88.
34. Sharmila, B. Isometric muscle energy technique and non-specific neck pain in secondary school teachers-results of an experimental study. *Ind J Physiother Occup Ther.* 2014;8(2):58.
35. Frontera, WR. (Ed.) *Rehabilitation of sports injuries: scientific basis*. Maiden, MA: Blackwell Science (2003).
36. Kostopoulos D, Nelson Jr AJ, Ingber RS, Larkin RW. Reduction of spontaneous electrical activity and pain perception of trigger points in the upper trapezius muscle through trigger point compression and passive stretching. *J Musculoskelet Pain.* 2008;16(4):266-278.
37. Pillastrini P, de Lima E, Sá Resende F, et al. Effectiveness of Global Postural Re-education in Patients With Chronic Nonspecific Neck Pain: Randomized Controlled Trial. *Phys Ther*. 2016;96(9):1408-1416.
38. Shady MA, Elnaggar I M, Nader I. El Sayed, Ali S. Comparative study between the effect of passive stretching exercises and post isometric relaxation technique in chronic mechanical neck pain patients. *EJMCM.* 2021;8(03):2271-2276.
39. Hatefi M, Babakhani F, Ashrafizadeh M. The effect of static stretching exercises on hip range of motion, pain, and disability in patients with non-specific low back pain. *J Exp Orthop.* 2021;278(1):55.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.10

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 70–74

ORIGINAL PAPER

Emine Siber Namiduru [1], Mustafa Namiduru [2], İlkay Karaoğlan [2], Kübra Koçak [2]

Oxidative and nitrosative stress in patients with meningitis

[1] Department of Nutrition and Dietetics, Faculty of Health Sciences, University of Gaziantep, Gaziantep, Turkey
[2] Department of Infectious Disease, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey

ABSTRACT

Introduction and aim. Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known as the meninges. In this study, oxidative and nitrosative stress were evaluated in cerebrospinal fluid (CSF) and blood samples that were taken from patients with meningitis. Our goal was to identify a fast and a reliable biomarker using these parameters in order to the early diagnose of bacterial meningitis.

Material and methods. In this study, 37 bacterial meningitis, 30 tuberculous meningitis and 30 viral meningitis cases were included. Serum/CSF total oxidant status (TAS) and total antioxidant status (TOS) were measured by the Erel method. Nitrotyrosine concentrations were quantified by using ELISA in both serum and CSF

Results. Serum nitrotyrosine, CSF TAS and TOS levels were not significantly different in three groups ($p>0.05$). CSF nitrotyrosine levels were significantly higher in bacterial meningitis than tuberculous meningitis group ($p<0.05$). Viral meningitis patients had higher serum TOS and TAS concentrations than tuberculous meningitis group ($p<0.05$).

Conclusion. As a result, we can say that the oxidative and nitrosative stress markers studied are not a rapid and reliable biomarker in bacterial meningitis's diagnosis.

Keywords. bacterial meningitis, oxidative stress, nitrotyrosine, tuberculous meningitis, viral menengitis

Introduction

Meningitis is the most serious infection of the central nervous system (CNS) and affects in the meninges, the membranes that cover the brain and spinal cord. It caused by many microorganisms, including bacteria, viruses, parasites and fungi.[1,2] Meningitis is an important public health problem in developing countries, including Turkey.

The brain is particularly vulnerable to oxidative stress due to its high metabolic requirements and the presence of polyunsaturated fatty acids. Under physiological conditions, the total antioxidative activity of the cerebrospinal fluid (CSF) is only one tenth of plasma. It has been reported that free radicals can cause neuronal cell death.[3] In addition, bacterial and host-derived reactive oxygen and nitrogen species combine to form highly reactive, tissue-damaging intermediates. Therefore, it is assumed that the levels of endogenous antioxidant molecules in the CSF elevated in response to infections that can place severe stress on the human body.[4,5] There are studies that found that free radicals are produced in CNS compartment in cases with meningitis.[2,5]

Studies on children with bacterial meningitis (BM) have shown that antioxidant molecule levels and lipid peroxidation in serum/CSF increase as a result of oxidative stress.[3,5]

Nitrotyrosine (NT) is a widely used marker for the formation of reactive nitrogen species, such as peroxynitrite. It has been found that tyrosine nitration is greatly increased during meningitis. This increase has been

Corresponding author: Emine Siber Namiduru, e-mail: enamiduru@gmail.com

Received: 18.11.2021 / Revised: 27.12.2021 / Accepted: 27.12.2021 / Published: 30.03.2022

Namiduru ES, Namiduru M, Karaoğlan İ, Koçak K. *Oxidative and nitrosative stress in patients with meningitis*. Eur J Clin Exp Med. 2022;20(1):70–74. doi: 10.15584/ejcem.2022.1.10

dedected to be more pronounced in inflammatory cells and blood vessels in the subarachnoid space. Therefore, it has been reported that reactive nitrogen species (RNS) may contribute to oxidative brain damage during meningitis.[6,7]

Aim

Our aim was to investigate oxidative and nitrosative stress in blood and CSF samples of bacterial, viral and tuberculous meningitis (TM) cases. According to our knowledge, this is the first study.

Material and methods

Study setting and population

We conducted this study on CSF and blood samples of patients who applied to Gaziantep University Faculty of Medicine Infectious Diseases Clinic and received a pre-diagnosis of meningitis between January 2018 and June 2020. Thirty-seven BM, 30 TM and 30 viral meningitis (VM) cases were included in the study. Paired serum and CSF samples were collected on admission, before the empirical antimicrobial and supportive therapy had started.

The protocol was approved by the Clinical Research Ethics Committee of Gaziantep University with the protocol number (No: 2015 /363) and the research was conducted in compliance with the Declaration of Helsinki (version 2008).

The purpose and procedures of the study were explained and written informed consent was received from each participant or their guardians prior to participation.

Exclusion criteria: Cases who were hospitalized with a prediagnosis of meningitis, cerebrovascular event, malignant infiltration of the menix, immunocompromised patients for any reason, pregnant women, and those lacking laboratory data used for routine diagnosis were excluded from the study.

Diagnosis of meningitis

The diagnosis was made according to the patient's clinics and CSF examination criteria. Lumbar puncture (LP) was performed if clinical signs and symptoms suggest meningitis. LP can be safely performed in the absence of increased intracranial pressure, focal neurological findings and/or papillary edema. The diagnosis of bacterial meningitis is based on a course of clinical history and laboratory experiments. Clinical features were such as the acute onset of headache, fever, and signs of meningeal irritation. Laboratory diagnosis of acute bacterial meningitis (ABM) was made by CSF examination. Positive CSF findings were pleocytosis (≥5/mm^3, mainly neutrophilic), elevated protein concentration (≥45 mg/dL), a reduced ratio of CSF glucose to serum glucose (≤0.60) in additionally a positive CSF culture, smear, or PCR for bacterial pathogens or a good specific response to antibacterial therapy.

Viral meningitis cases had such as clinical features, the acute onset of headache, fever, and signs of meningeal irritation. In addition, there were no signs of cortical involvement such as altered consciousness, aphasia, or seizures. Viral meningitis CSF findings were also pleocytosis (≥5/mm^3, mainly lymphocytic), a negative CSF stain, culture, or PCR for bacteria, mycobacteria, fungi and a positive PCR for viral pathogens or full recovery without any specific treatments including antibacterial or antituberculosis therapy.

Diagnosis of TM clinical symptom, CSF criteria, cerebral imaging criteria and It was placed on the basis of other evidence of tuberculosis. If mycobacterium tuberculosis was detected in CSF of cases a definite diagnosis of TM was made.

Measurements

Blood and CSF taken for analysis before starting treatment. The serum was separated with standard centrifugation procedure and immediately divided in portions which were kept tightly closed at −70°C until analysis

Measurement of TAS

TAS measurement was performed using an Aeroset 2.0 analyzer and a total antioxidant status kit (Rel Assay Diagnostic, Turkey). In this kit the reduced ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)) molecule is oxidized to ABTS•+, using hydrogen peroxide alone in an acidic medium (acetate buffer 30 mmol/L; pH 3.6). The concentrated ABTS•+ molecules (deep green) remain more stable for a long time in the acetate buffer solution. The color is spontaneously and slowly bleached when it is diluted with a more concentrated acetate buffer solution at high pH (acetate buffer 0.4 mol/L; pH 5.8). In the sample's antioxidants accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectrophotometrically and the bleaching rate is inversely related to the total antioxidant capacity (TAC) of the sample. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed as mmol Trolox equivalent/L.[8]

Measurement of TOS

TOS measurement was performed using an Aeroset 2.0 analyzer and a TOS kit (Rel Assay Diagnostic, Turkey). In the sample's oxidants oxidize the ferrous ion–o-dianisidine complex to ferric ions in this kit. In the reaction medium glycerol molecules enhance the oxidation reaction. The ferric ions make a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is

related to the total quantity of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed as the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L).[9]

Nitrotyrosine Analysis

The NT levels were detected in plasma samples using the Bioxytech sandwich ELISA immunoassay (OxisResearch, USA) according to the manufacturer's instructions.

Statistical Analyses

All statistical analyses were performed using PASW, version 18.0 (SPSS Inc., Chicago, IL, USA) for Windows. Continuous variables are presented as mean ± SEM and categorical variables are presented as *n* of patients (%). The Kolmogorov–Smirnov test was used to test the normality of the distribution of continuous variables. Statistical analysis of data between two groups was performed using unpaired *t*-test for parametric data and Mann–Whitney *U*-test for nonparametric data. A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

Comparison of demographic and laboratory profiles of cases in CSF and serum are illustrated in Table 1.

CSF bacterial culture is known as the gold standard method for confirming acute bacterial meningitis, but most cases cannot be confirmed by culture. In this study, of 37 patients with BM, 11 had positive CSF cultures, smears, or PCR for bacterial pathogens, including 2 with Streptococcus species, 7 with Staphylococcus species, and 2 with other species.

It was determined that the oxidative/nitrosative stress parameters analyzed did not correlate with demographic data such as age and gender.

Among the analyzed markers, a weak positive correlation was found between only serum TAS and laboratory profile parameters CSF glucose (r: 0.267; p: 0.036).

Cerebrospinal fluid and serum NT, TAS and TOS concentrations of the subjects are given in Table 2.

Discussion

Activated phagocytic cells (neutrophil eosinophil and macrophages of all types) produce $O_2\bullet^-$ by NADPH oxidase. This event, called oxidative burst (burst), is important in clearing phagocytosed bacteria. Meanwhile, O_2 consumption in phagocytic cells increases 4 to 100 times. Reactive oxygen (ROS) and nitrogen species are produced by the human immune system in response to infection such as meningitis. According to in the results of cases with bacterial meningitis different animal, and several human studies it has been determined that ROS/RNS are produced in activated PMNs during the inflammatory response of the host when bacteria reach the subarachnoid space.[1,10-13] The mechanisms of central nervous system damage during meningitis are not

Table 1. Comparison of demographic and laboratory profiles of cases with bacterial, tuberculosis and viral meningitis

Parameters	Bacterial meningitis (n=37)	Tuberculosis meningitis (n=30)	Viral meningitis (n=30)	p
Demographic Profile				
Men, n (%)	18 (48.6)	17 (56.7)	19 (63.3)	>0.05
Age*, years	40.19±2.73	42.60±3.31	45.40±2.56	>0.05
Blood Profile WBC†x10^6/L	9.42 (2.35-26.21)	8.80 (3.61-17.82)	7.91 (4.60-20.80)	>0.05
Neutrophil† (%)	75.50 (46.0-91.0)	76.0 (46.0 -90.0)	75.0 (51.0-92.0)	>0.05
ESH† (mm/hour)	35.0 (2-103)	56.0 (2-120)	29.0 (2-96)	>0.05b <0.05a,c
CRP† (mg/L)	55.0 (0.5-328.0)	55.0 (2-250)	24.0 (0.78-145.0)	>0.05a <0.05b, c
CSF Profile				
WBC†/mm^3	450 (70-1100)	250 (40-850)	90 (20-450)	>0.05a <0.05b, c
Lymphocyte percentage†	40 (10-75)	87.5 (20-90)	90 (60-100)	<0.05a,b >0.05c
Protein† (mg/dl)	153 (26-1361)	647 (34-1640)	90 (34-215)	<0.05
Glucose ratio *(CSF/Blood)	0.41±0.06	0.30± 0.04	0.59±0.03	<0.05a,c >0.05b
Clinical Profile				
Systolic Blood Pressure† (mmHg)	120 (90-171)	120 (100-206)	119 (90-168)	>0.05
Diastolic Blood Pressure† (mmHg)	70 (50-96)	70 (47-99)	70 (60-86)	>0.05
Death, n (%)	10 (27)	4 (10)	1 (3.3)	<0.05
Sequelae, n (%)	5 (13.5)	2 (6.7)	1 (3.3)	<0.05

† Median (interquartile range)*Mean±SEM a: BM vs. TM; b: BM vs. VM; c: TM vs. VM

CRP: C-reactive protein, CSF: cerebrospinal fluid, WBC: white blood cell, ESH: erythrocyte sedimentation rate; CRP: C-reactive protein; BM: Bacterial meningitis; TM: Tuberculous meningitis; VM: Viral meningitis

fully elucidated, but a wealth of evidence suggests that reactive oxygen and nitrogen species may contribute to brain damage.

Some studies have shown that ROS may have an important role in various pathological processes such as vascular damage, cerebral edema formation and cerebrospinal fluid pleocytosis in BM.[14,15] It has been suggested that oxidative stress has a role in the pathophysiology of TM-associated seizures.[16]

It has been determined that the nitration of tyrosine is increased at the cellular level, especially in cerebral vessels and inflammatory cells in BM. In these cells, 4-hydroxynonenal compound, a lipid peroxidation marker, was detected suggesting a role for RNS in oxidative brain injury. In addition, high concentrations of NT in CSF were associated with worsening of the disease.[6]

Previous studies found significant increases in CSF nitrite and NT levels in children with BM. In this study in cases of BM serum and CSF NT levels were found higher than TM and VM groups.[12,17,18] However, only NT levels in CSF samples of cases with BM were statistically significant compared to the levels of TM cases ($p<0.05$).

These results indicate that ROS/RNS production is more increased in CSF and serum of cases with BM and that oxidative damage may contributes to the pathophysiology of such cases. CSF and blood TOS, TAS, oxidative stress index and S-100B levels were found to vary in pediatric cases with BM. This change was found to be parallel to inflammation.[19]

In our study TAS and TOS levels in CSF samples were not statistically different from each other in all 3 meningitis groups ($p>0.05$). In serum samples, TAS and TOS values showed statistically significant difference only between TM and VM groups ($p<0.05$)

In BM, ROS and RNS are considered to mediate the disruption of the blood brain barrier. Moreover, it has been found that treatment with antioxidants prevents the deterioration of the blood brain barrier.[1,20]

This may be because the inflammation that occurs is due to the proliferation of bacteria, the release of excessive amounts of oxidants produced in phagocytes to destroy bacteria, and the use of antioxidants to neutralize them.

The CSF/plasma glucose ratio in healthy adults is about 0.6 and therefore an abnormal level is less than that, usually 0.5 or less.[21] In our study, this ratio was determined as BM (Mean±SEM: 0.41±0.06) TM (0.3±0.04) and VM (0.59±0.03). The CSF/plasma glucose ratio of the TM group is statistically significantly lower than the other two groups ($p<0.05$).

In our study, the percentage of sequelae (13.5%) in cases with BM was found to be higher than in cases with other meningitis (TM: 6.7% and VM: 3.3% ; $p<0.05$). ROS and RNS have been associated with cognitive sequelae, especially since they cause cellular damage.[22]

In addition, the mortality rate was found to be statistically significantly higher in cases with BM than in TM and VM cases (BM: 27%, TM: 10%, VM: 3.3% ; $p<0.05$).

Conclusion

As a result, we can say that the oxidative and nitrosative stress markers studied are not a rapid and reliable biomarker in BM's diagnosis, but we might conclude that oxidative stress contributes at least in part to the severe neurological dysfunction found in meningitis especially bacterial meningitis. However, in parallel with the inflammation in bacterial meningitis, changes had been occured in CSF and blood TOS, TAS and NT levels, and these changes should be examined in larger case groups in future studies.

Table 2. Cerebrospinal fluid and serum NT, TAS and TOS concentrations of the subjects

	Bacterial Meningitis (n:37)	Tuberculous Meningitis (n:30)	Viral Meningitis (n:30)	p value
Serum				
NT (ng/ml)	393.48±73.4	268.74±31.1	237.14±44.0	>0.05[a,b,c]
TAS (mmol/L)	1.38±0.1	1.22±0.1	1.43±0.1	>0.05[a,b] <0.05c
TOS (µmol/L)	12.16±0.9	9.41±1.1	13.4±1.1	>0.05[a,b] <0.05c
CSF				
NT (ng/ml)	183.39±13.4	129.29±10.8	156.08±14.7	<0.05[a] >0.05b,c
TAS (mmol/L)	0.95±0.1	0.91±0.1	1.09±0.1	>0.05[a,b,c]
TOS (µmol/L)	8.07±0.9	6.97±0.9	9.06±0.9	>0.05[a,b,c]

BM: Bacterial meningitis; TM: Tuberculous meningitis; VM: Viral meningitis CSF: cerebrospinal fluid, Data are given as mean ± standard error, a: BM vs TM; b: BM vs VM; c:TM vs VM

Declarations

Funding

This study was financially supported by the Scientific Research Projects Governing Unit of Gaziantep University (Protocol number: SBF.17.03)

Author contributions

Conceptualization, E.S.N. and M.N.; Methodology, E.S.N; M.N. and I.K.; Software, E.S.N. and M.N; Validation, E.S.N., M.N. and K.K.; Formal Analysis, E.S.N.; Investigation, M.N. and I.K; Resources, E.S.N and M.N; Data Curation, M.N. and K.K; Writing – Original Draft Preparation, E.S.N.; Writing – Review & Editing, E.S.N. and M.N; Visualization, E.S.N.; Supervision, E.S.N., M.N. and I.K; Project Administration, E.S.N.; Funding Acquisition, E.S.N.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

All data generated or analyzed during this study are included in this article [and/or] its supplementary material files. Further enquiries can be directed to the corresponding author.

Ethics approval

The protocol was approved by the Clinical Research Ethics Committee of Gaziantep University with the protocol number (No: 2015 /363)

References

1. Barichello T, Generoso JS, Simões LR, Elias SG, Quevedo J. Role of oxidative stress in the pathophysiology of pneumococcal meningitis. *Oxid Med Cell Longev*. 2013;2013:371465.
2. de Menezes CC, Dorneles AG, Sperotto RL, Duarte MM, Schetinger MR, Loro VL. Oxidative stress in cerebrospinal fluid of patients with aseptic and bacterial meningitis. *Neurochem Res*. 2009;34(7):1255-1260.
3. Miric D, Katanic R, Kisic B, et al. Oxidative stress and myeloperoxidase activity during bacterial meningitis: effects of febrile episodes and the BBB permeability. *Clin Biochem*. 2010;43(3):246-252.
4. Hoffman O, Weber RJ. Pathophysiology and treatment of bacterial meningitis. *The Adv Neurol Disord*. 2009;2(6):401-412.
5. Kawakami Y, Monobe M, Kuwabara K, et al. A comparative study of nitric oxide, glutathione, and glutathione peroxidase activities in cerebrospinal fluid from children with convulsive diseases/children with aseptic meningitis. *Brain Dev*. 2006;28(4):243-246.
6. Kastenbauer S, Koedel U, Becker BF, Pfister HW. Oxidative stress in bacterial meningitis in humans. *Neurology*. 2002;58(2):186-191.
7. Rugemalira E, Roine I, Kuligowski J, et al. Protein Oxidation Biomarkers and Myeloperoxidase Activation in Cerebrospinal Fluid in Childhood Bacterial Meningitis. *Antioxidants (Basel)*. 2019;8(10):441.
8. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*. 2004;37:277-285.
9. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*. 2005;38:1103-1111.
10. Liechti FD, Grandgirard D, Leib SL. Bacterial meningitis: Insights into pathogenesis and evaluation of new treatment options: A perspective from experimental studies. *Future Microbiol*. 2015;10:1195-1213.
11. Srivastava R, Lohokare R, Prasad R. Oxidative stress in children with bacterial meningitis. *J Trop Pediatr*. 2013;59:305-308.
12. Tsukahara H, Haruta T, Todoroki Y, et al. Oxidant and antioxidant activities in childhood meningitis. *Life Sci*. 2002;71:2797-2780.
13. Barichello T, Generoso JS, Collodel A, Moreir AP, de Almeida SM. Pathophysiology of acute meningitis caused by Streptococcus pneumoniae and adjunctive therapy approaches. *Arq Neuropsiquiatr*. 2012;70(5):366-372.
14. Aycicek A, Iscan A, Erel O, Akcali M, Selek S. Total antioxidant/oxidant status in meningism and meningitis. *Pediatr Neurol*. 2006;35:382-386.
15. Tsukahara H, Haruta T, Hata I, Mayumi M. Nitric oxide inseptic and aseptic meningitis in children. *Scand J Clin Lab Invest*. 1998;58:73-79.
16. Misra UK, Kalita J, Tripathi A, Kumar M. Oxidative and endoplasmic reticulum stress in tuberculous meningitis related seizures. *Epilepsy Res*. 2019;156:106160.
17. van Furth AM, Seijmonsbergen EM, Groeneveld PH, van Furth R, Langermans JA. Levels of nitric oxide correlate with high levels of tumor necrosis factor alpha in cerebrospinal fluid samples from children with bacterial meningitis. *Clin Infect Dis*. 1996;22(5):876-888.
18. Kornelisse RF, Hoekman K, Visser JJ, et al. The role of nitric oxide in bacterial meningitis in children. *J Infect Dis*. 1996;174:120-126.
19. Abuhandan M, Çalık M, Almaz V, et al. Bakteriyel Menenjitli Çocuklarda Tedavi Öncesi ve Sonrası S-100B ve Oksidatif Durumun Değerlendirilmesi. *Klimik Dergisi*. 2012;25(2):67-70.
20. Mayhan WG. Nitric oxide donor-induced increase in permeability of the blood-brain barrier. *Brain Research*. 2000;866(1):101-108.
21. Benninger F, Steiner I . CSF in acute and chronic infectious diseases. *Handb Clin Neurol*. 2017;146:187-206.
22. Harris RA, Amor S. Sweet and sour—oxidative and carbonyl stress in neurological disorders. *CNS Neurol Disord Drug Targets*. 2011;10(1):82-107.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.11

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 75–79

ORIGINAL PAPER

Chukwubuike Kevin Emeka [1], Eze Thaddeus Chikaodili [2]

Assessment of the effect of size of the umbilical ring on the risk of umbilical hernia complication in children

[1] Pediatric Surgery Unit, Department of Surgery, Enugu State University Teaching Hospital, Enugu, Nigeria
[2] Department of Surgery, Enugu State University Teaching Hospital, Enugu, Nigeria

ABSTRACT

Introduction and aim. Assessment of risk of complications in umbilical hernia is important. The aim of this study was to evaluate the effect of size of the umbilical ring on the risk of complication occurring in umbilical hernia.

Material and methods. This was a prospective study of children who had umbilical hernia repair for symptomatic umbilical hernia. Using Vernier caliper, the umbilical ring diameter (URD) was measured at surgery and the patients were divided into 2 groups. Group A had URD of less than of 15 millimeter (mm) and group B patients had URD of 15 mm and above. The 2 groups were compared.

Results. Thirty two cases were evaluated. Their mean age was 42 months. All the patients had umbilical pain. Twenty six (81.3%) patients had URD of less than 15 mm (group A) whereas 6 (18.7%) patients had URD of greater or equal to 15 mm (group B). Group A patients had a mean URD of 12.1±3.4 mm whereas group B patients had a mean URD of 30.5±5.0 mm (p=0.001).

Conclusion. Children who have URD of less than 15 mm are at a higher risk of developing umbilical hernia complications.

Keywords. complications, diameter, risk

Introduction

Umbilical hernia is a ventral hernia located at the umbilicus and is very common in blacks, both in Africa and rest of the world.[1,2] Umbilical hernia has been reported to occur in 15% of Caucasian children and 85% of black children; it usually closes spontaneously during the early years of life.[1] It is speculated that the increased incidence of umbilical hernia in blacks may be due to inherited physiologic characteristics. Umbilical hernia results from imperfect closure or inherent weakness of the umbilical ring after separation of the umbilical cord.[3,4] Complications occurring in umbilical hernia could be incarceration, obstruction or strangulation of the abdominal viscera, though these complications are rare.[5,6] The content of the umbilical hernia could be preperitoneal fat, omentum or intestine. In developing countries, the most common indication for umbilical hernia repair is complicated umbilical hernia. This in contrast to what is obtainable in developed countries where cosmetic reason is a common indication.[7,8] Surgery for umbilical hernia is umbilical herniorrhaphy with some form of umbilicoplasty. The umbilical ring forms the fascial boundaries of the umbilical hernia and is at this level constriction of the viscera occurs. However, the extent of the umbilical skin protrusion is not indicative of the size of the fascia defect.

Some previous studies have suggested that the size of the umbilical ring affects the risk of complication occurring in umbilical hernia, while some of the studies have not reported any difference in this risk measure.

Corresponding author: Chukwubuike Kevin Emeka, e-mail: chukwubuikeonline@yahoo.com

Received: 25.10.2021 / Revised: 27.11.2021 / Accepted: 30.11.2021 / Published: 30.03.2022

Emeka CK, Chikaodili ET. *Assessment of the effect of size of the umbilical ring on the risk of umbilical hernia complication in children.* Eur J Clin Exp Med. 2022;20(1):75–79. doi: 10.15584/ejcem.2022.1.11

Aim

The aim of this study was to evaluate the effect of size of the umbilical ring on the risk of complications occurring in umbilical hernia.

Material and methods

This was a prospective study of pediatric patients who presented to the pediatric surgery unit of Enugu State University Teaching Hospital (ESUTH), Enugu with symptomatic umbilical hernia between January 2009 and December 2018. ESUTH is a tertiary hospital located in Enugu, South East Nigeria. The hospital serves the whole of Enugu State, which according to the 2016 estimates of the National Population Commission and Nigerian National Bureau of Statistics, has a population of about 4 million people and a population density of 616.0/km^2. The hospital also receives referrals from its neighboring states. Ethical approval was obtained from the ethics and research committee of ESUTH (ESUTH/CMAC/RA/034/VOL.2/07). Patients who have recurrent umbilical hernia and those who are above 15 years of age were excluded from this study. Patients whose caregivers refused to participate in the study were also excluded.

Pre-operative protocol

Consecutive children, less than 15 years, who presented with symptomatic umbilical hernia were recruited into the study. Patients operated on both electively and emergently were evaluated. On presentation, the patients were clinically evaluated and appropriate investigations done to ascertain fitness for surgery. The procedure is explained to the parents/caregiver and informed consent obtained. At induction of anesthesia, preoperative ceftriaxone was given.

Intra-operative protocol

Access was through a subumbilical curvilinear incision which was deepened to the umbilical fascial ring. The diameter of the umbilical ring was measured using Vernier caliper (made of stainless steel and produced by ESAL medicals) which was sterilized before the surgery. The diameter of the umbilical ring was measured transversely, longitudinally and diagonally. The widest diameter was taken and the measurements were documented in millimeters (mm). At least, two measurements of the diameter of the umbilical ring were made and the average taken. This minimized observer variations. The patients were divided into two groups: Group A represented those whose umbilical ring diameter (URD) was less than 15 mm and group B represented those whose URD was greater or equal to 15 mm. Group A patients were considered to have narrow umbilical ring while Group B patients were considered to have wide umbilical ring. Simple primary suture repair of the umbilical defect was done and the wound closed in layers. Firm dressing was subsequently applied.

Post-operative protocol and follow up

The wound was examined on the seventh post-operative day on out-patient basis. The cases were performed as day cases except children with co-morbidities and those who experienced delayed recovery from anesthesia. The follow up period was for 6 months. The follow-up was done physically and the patients were seen monthly during the follow-up period.

Data collection

The following data were collected: Age of the patient, gender, duration of symptoms before presentation, time interval between presentation and surgery, diameter of the umbilical ring in mm, complications of treatment, duration of hospital stay and outcome of treatment.

Data analysis

Statistical Package for Social Science (SPSS) version 23 (manufactured by IBM Cooperation, Chicago Illinois, USA) was used for data entry and analysis. Data were expressed as percentages, means and standard deviation. Chi square test or student's T test was used to test for significance. P value < 0.05 was considered statistically significant.

Results

Patients' demography

Thirty-five cases of umbilical hernia were repaired during the study period but only 32 patients had complete case records and formed the basis of this report. There were 20 (62.5%) males and 12 (37.5%) females which correspond to a male female ratio of 1.6:1. The ages of the patients ranged from 12 months to 96 months with a mean of 42 months. Seventy five percent of the patients were less than 48 months of age. Details are depicted in Table 1.

Table 1. Demographic profiles of the patients

Parameter	Value
Gender	
Male, n (%)	20 (62.5)
Female, n (%)	12 (37.5)
Mean age of the patients	42 months (range: 12-96)
Mean duration of symptoms	2.1 days (range: 1-4)
Interval from presentation to surgery (irreducible hernias)	1.4 days (range: 1-3)
Mean duration of hospital stay (non-day cases)	1.5 days (range: 1-3)

Clinical and operative findings

All the patients (100%) had abdominal pain at the umbilical area. In addition to pain, 18 (56.2%) patients had vomiting, 10 (31.3%) had constipation and abdominal distension was present in 4 (12.5%) patients. All the pa-

tients were symptomatic. Fifteen (46.9%) patients had reducible symptomatic umbilical hernia; another 17 (53.1%) umbilical hernias were irreducible. Among the irreducible hernias, 2 were strangulated and the omentum was involved. Non-viable omentum was resected. There was no bowel resection.

Umbilical ring diameter (URD)

Twenty six (81.3%) patients had URD of less than 15 mm (group A) whereas 6 (18.7%) patients had URD of greater or equal to 15 mm (group B). Table 2 shows details.

Table 2. Umbilical ring diameters, means, standard deviations and p value

	Group A			Group B	
	Mean	SD[a]	p value	Mean	SD
URDb	12.1 mm	3.4	0.001*	30.5 mm	5.0
Number (%)	26 (81.3%)			6 (18.7%)	

[a]SD=Standard deviation; bURD=Umbilical ring diameter, *statistically significant

Post-operative complications

Complications in the 2 groups of patients are shown in Table 3, p value 0.636.

Table 3. Post-operative complications

Complications	Group A	Group B
None	20	4
Surgical site infection	2	1
Wound breakdown	2	1
Hypertrophied scar	2	0
Total	26	6

Outcome of treatment

Overall, 30 (93.8%) patients did well and were discharged. Two (6.2%) patients signed out, in post-operative period, against medical advice. There was no mortality.

Discussion

Umbilical linea alba is formed by the umbilical aperture through which umbilical herniation occurs. Umbilical hernia sac protrudes through a defect in the umbilical ring due to failure of complete obliteration at the site where the fetal umbilical vessels are joined to the placenta during gestation.[9] Natural history of umbilical hernia is spontaneous closure.[10] However, when umbilical hernia fails to close or becomes symptomatic, there is need for surgical repair.[11] There is a report of omental content of an umbilical hernia as a determinant of the risk of a patient developing complicated umbilical hernia; this is due to adherent nature of the omentum which prevents complete reduction.[12] In the present study, we assessed the risk of umbilical hernia complication with respect to the diameter of the umbilical ring.

The male predominance recorded in the present study is consistent with the report of other series on umbilical hernia.[2,13,14] However, a study done in Jos, Nigeria reported female predominance.[10] The reason for this gender difference is not known. The mean age of our patients tally with the report of some studies but is at variance with the result of others.[5,10,14-16] The differences in sizes of the umbilical ring may explain the discrepancies in the mean ages. Late presentation of our patients is manifested in the 2-day lag period. Poverty and ignorance may be responsible for the late presentation. Mean duration of hospital stay of our patients is unsupported by other studies where umbilical hernia repairs were performed as day cases.[17,18] The length of time a patient stays in the hospital may depend on the extent of the procedure and post-operative status.

The symptoms present in our patients is in line with findings of other studies; pain in the umbilical region been the most consistent.[14,16] The symptoms manifested by the patients at presentation may be related to the time of presentation to the hospital. Our protocol for managing symptomatic umbilical hernia is immediate surgery (emergency) for irreducible umbilical hernia and day case surgery for reducible hernia. This may explain the mean interval of 1.4 days between presentation and surgery for irreducible umbilical hernia.

A statistically significant number of our patients who had surgery for symptomatic umbilical hernia had umbilical ring diameter of less than 15 mm. A study also reported that patients with small umbilical fascial defects (5 to 15 mm in diameter) are more prone to incarceration.[9] One study performed at Mayo clinic, Rochester, USA reported that complications in umbilical hernias are more likely in smaller defects.[15] However, other studies on umbilical hernia reported increased incidence of incarceration in children whose umbilical ring diameter were more than 15 mm.[5,10,14] The reason for the increased incidence of umbilical hernia complications in narrow URD may be explained by the mechanism of hernia incarceration: When intra-abdominal pressure increases, like when the child cries, contents of the hernia are squeezed through a narrow hernia neck into the hernia sac. The subsequent recoil of the hernia neck entraps the contents of the hernia preventing the contents from returning into the abdomen and thus incarceration occurs.[19] The above mechanism of incarceration may not happen when the neck of the hernia is wide. A study done in Senegal reported that the main factors contributing to incarceration of umbilical hernia was age of the child and size of the umbilical defect.[20] The exact reason for the differences in the findings of the different studies is not known.

The post-operative complications recorded in the present study is comparable to the reports of other studies.[4,5] However, the post-operative complications were not statistically significant between the 2 groups of patients in the present study.

During the 6 months follow up period, 2 patients in group A developed an abnormal scar that required plastic surgical review. Komlatse et al in their series also reported abnormal scar following umbilical hernia repair.[21] One study reported hematoma and seroma formation as post-operative complications following umbilical hernia repair.[15] Firm dressing which helps in obliterating dead space assists in preventing hematoma and seroma formation. None of our patients had umbilical hernia recurrence.

Most of our patients did well and were discharged home. Other studies also documented good outcomes following repair of the umbilical hernia.[4,5,21]

Recommendation and limitations of the study

Umbilical hernias with smaller diameters should be repaired early to avoid complications.

Although this was a prospective study, it was limited by the small number of cases. A larger number of cases would have availed better analysis. This study was a single institution experience which may not be generalizable to other institutions and other countries.

Conclusion

Umbilical hernia is a common condition particularly in black children. Children who have URD of less than 15 mm are at a higher risk of developing umbilical hernia complications. Early repair of umbilical hernia with narrow URD is therefore recommended.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, C.K.E, ETC; Methodology, C.K.E, ETC; Software, C.K.E.; Validation, C.K.E.; Formal Analysis, C.K.E.; Investigation, C.K, E, ETC.; Resources, C.K.E, ETC.; Data Curation, C.K.E, ETC; Writing – Original Draft Preparation, C.K.E.; Writing – Review & Editing, C.K.E, ETC.; Visualization, C.K.E.; Supervision, C.K.E.; Project Administration, C.K.E, ETC.; Funding Acquisition, C.K.E.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data is available and can be provided on proper request.

Ethics approval

Ethical approval was obtained from the ethics and research committee of ESUTH (ESUTH/CMAC/RA/034/VOL.2/07)

References

1. Meier DE, Olaolorun DA, Omodele RA, et al. Incidence of umbilical hernia in African children: redefinition of normal and reevaluation of indication for repair. *World J Surg*. 2001;25:645-648.
2. Uba AF, Igun GO, Kidmas AT, et al. Prevalence of umbilical hernia in a private school admission-seeking Nigerian children. *Niger Postgrad Med J*. 2004;11:255-257.
3. Armstrong O. Umbilical hernia. *Rev Prat*. 2003;53:1671-1676.
4. Fall I, Sanou A, Ngom G, et al. Strangulated umbilical hernia in children. *Pediatr Surg Int*. 2006;22:233-235.
5. Ameh EA, Chirdan LB, Nmadu PT, Yusufu LM. Complicated umbilical hernia in children. *Pediatr Surg Int*. 2003;19:280-283.
6. Papagrigoriadis S, Browse DJ, Howard ER. Incarceration of Umbilical Hernias in Children: A Rare but Important Complication. *Pediatr Surg Int*. 1998;14:231-232.
7. Sankale AA, Ngom G, Fall I, et al. Umbilical reconstruction in children. Prospective report of 77 cases. *Ann Chir Plast Esthet*. 2004;49:17-23.
8. Abanese CT, Rengal S, Bermudez D. A novel laparoscopic technique for the repair of pediatric umbilical and epigastric hernias. *J Pediatr Surg*. 2006;41:859-862.
9. Weber TR. *Umbilical and other abdominal wall Hernias*. In: Holcomb GW, Murphy JP, editors. Ashcraft Pediatric Surgery. 5th edition. Philadelphia: Saunders Elsevier; 2010:637-640.
10. Chirdan LB, Uba AF, Kidmas AT. Incarcerated Umbilical Hernia in Children. *Eur J Pediatr Surg*. 2006;16:45-48.
11. Arslan S, Korkut E. The Intra-umbilical approach in umbilical hernia. *Eurasian J Med*. 2014;46:32-35.
12. Sinopidis X, Panagidis A, Alexopoulos V, et al. Omentum in the Pediatric Umbilical hernia: Is It a Potential Alarm for the Appearance of Complications? *Case Rep Pediatr*. 2012;2012:463628.
13. Osifo OD, Ovueni EM. The Prevalence, Spontaneous Resolution, and Parent's Attitudes towards Umbilical Hernia Coexisting in Nigerian Children Presenting with Other Surgical Pathology. *Annals of Pediatric Surgery*. 2009;5:16-20.
14. Ezomike UO, Ituen MA, Ekpemo SC, Eke CB. Profile of Paediatric Umbilical Hernias Managed at Federal Medical Centre Umuahia. *Niger J Med*. 2012,21:350-352.
15. Zendejas B, Kuchena A, Onkendi EO, et al. Fifty three year experience with pediatric umbilical hernia repairs. *J Pediatr Surg*. 2011;46:2151-2156.
16. Brown RA. Complicated umbilical hernia in childhood. *S Afr J Surg*. 2006;44:136-137.
17. Bowling K, Hart N, Cox P, Srinivas G. Management of paediatric hernia. *BMJ*. 2017;359:j4484.
18. Elebute OA, Ademuyiwa AO, Bode CO, Idiodi-Thomas HOI. Pediatric Day Case Surgical Practice at a Tertiary Hospital in Lagos: How Have We Faired? *Ann Med Health Sci Res*. 2014;4: 559-561.

19. Yang XF, Liu JL. Acute incarcerated external abdominal hernia. *Ann Transl Med*. 2014;2:110.
20. Ngom G, Gassama F, Mohamed AS, Ndour O, Umbilical hernia in children in developing country: Does the season have an effect on the occurrence of strangulation? *SAMJ*. 2016;106:744.
21. Komlatse AN, Anani MA, Azanledji BM, et al. Umbilicoplasty in children with huge umbilical hernia. *Afr J Paediatr Surg*. 2014;11;256-260.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.12

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 80–92

ORIGINAL PAPER

Florence N. Uchendu [1], Oyediran E. Oyewole [2]

Contribution of bread and biscuits to vitamin A daily requirement of preschool children in Lagos State, Nigeria

[1] Department of Public Health, Faculty of Health Sciences, National Open University of Nigeria, Jabi, Abuja, Nigeria
[2] Department of Health Promotion and Education, Faculty of Public Health, University of Ibadan, Nigeria

ABSTRACT

Introduction and aim. Vitamin-A-deficiency is a public health problem among preschool children of Nigeria. Study determined the contribution of bread and biscuits to vitamin A-daily-requirement of preschool children in Lagos-State, Nigeria.

Material and methods. A community-based-study using a cross-sectional-design with analytical component was carried out from 2013-2015. Multi-stage-sampling-technique was used to select mothers of preschool-children (n=1599) in 5 Local-Government-Areas of Lagos. Respondents' socio-demographic information and samples consumption-pattern were collected using validated, food-frequency-questionnaire/dietary recall. Retinyl palmitate content of randomly selected commonly-consumed brands of oven-fresh-bread stored for 5-days and biscuits (30- to 60-days) at prevailing outdoor-market-temperatures were analysed using High-Performance-Liquid-Chromatography. Contribution to preschool children's vitamin A-daily-requirements were determined. Data were analysed using Student's t-test and ANOVA at $p<0.05$.

Results. Mean age of preschool children was 31.44±5.28 months. Mean intakes of samples were bread (117.6 ±15.9 g/d) and biscuits (59.8±27.9 g/d). Range of contribution to vitamin-A-daily-requirement of preschool-children was 0-178.4 %. Samples contribution to vitamin A-daily-requirement of pre-school-children were oven-fresh bread (68.3 %); 5 days bread (20.7%); 30-days biscuits (25.0%) and 60-days biscuits (6.8%). Overall contribution to vitamin A-daily-requirement were bread (51.4%) and biscuits (22.4%). Statistically significant difference existed between samples contribution and vitamin-A-daily-requirement of preschool children.

Conclusion. Bread and biscuits samples contributed significantly to the vitamin-A-daily-requirement of preschool children.

Keywords. biscuits, bread, vitamin A daily requirement

Introduction

Vitamin A deficiency (VAD) is a global public health problem. The global prevalence of VAD is 29% and this is similar to that of Nigeria (29.5%).[1-3] In 2013, vitamin A deficiency affected approximately one third of preschool children (24 to 59 months), with the highest rate in Sub-Saharan African countries (48%) and South Asia (44%).[4] The main demographic groups most vulnerable to VAD are preschool children (0-59 months), pregnant and lactating mothers.

Consequences of VAD include preventable childhood night-blindness in children, increased risk of diseases (diarrhoea and measles), stunting, anaemia and premature death from severe infections.[1,4,5] A common cause of vitamin A deficiency might be a shift in the local diet to imported, processed and ready-to-eat foods. The high prevalence of VAD in Nigeria might be as a result of low dietary intake of vitamin A rich foods because Nigerian food staple is dominantly carbohydrate-rich foods which are very low sources of vitamin

Corresponding author: Florence N. Uchendu, e-mail: florenceuchendu@gmail.com

Received: 26.07.2021 / Revised: 23.12.2021 / Accepted: 31.12.2021 / Published: 30.03.2022

Uchendu FN, Oyewole OE. *Contribution of bread and biscuits to vitamin A daily requirement of preschool children in Lagos State, Nigeria*. Eur J Clin Exp Med. 2022;20(1):80–92. doi: 10.15584/ejcem.2022.1.12

A except the biofortified crops which are rich in beta-carotene.[6] Nigeria is biofortifying cassava and sweet potatoes with beta-carotene (a pre-cursor of vitamin A) but the availability and accessibility of these biofortified crops to consumers in the local markets is limited.

Food fortification is recognised as a long-term strategy, most reliable, safe, and cost-effective means of eradicating and controlling micronutrient deficiencies and delivering nutrients to the population at large.[7,8] While vitamin A supplementation reduces child mortality by 23-35%, vitamin A fortification of commonly consumed processed foods reduces deaths among infants and preschool children by 11-45%.[9] However, an Indonesian study reported that some fortified products do not contribute substantially to the total vitamin A intake of the poorest segments of the society.[10] To make good judgments on the benefits of current fortification practices and the opportunities for fortification in the future, it is important to assess how fortified foods contribute to nutrient intakes across the population.[11] Fortified foods are foods to which important nutrients that are not naturally contained in them are added in order to prevent or eradicate some demonstrated deficiencies of those nutrients in the at-risk groups. These fortified foods thereby serve as food vehicles for delivering the nutrients to the vulnerable groups. Example is vitamin A. Wheat flour has been fortified with vitamin A in Nigeria since 2004 at 30,000IU/Kg as a long-term strategy to eradicate vitamin A deficiency in the country and it is a major raw material for the production of bread and biscuits. This fortification level has been recently reviewed.[12] Low vitamin A stability has been reported in vitamin A fortified wheat flour, bread and biscuits in Nigeria.[13-16]

In Ghana, the average consumption weight of flour products was 56 g/day.[17] Lagos State has the highest consumption of flour products (8.89%) and household expenditure pattern in the consumption of baked/processed products (5.1%) in Nigeria (share in food expenditure).[18,19] However, the contribution of these vitamin A fortified wheat flour products to the daily nutrient requirement of preschool children has not been determined. The vitamin A Recommended Daily Allowance (RDA) for preschool children (1-6 years) has been given as 400 µg retinol/day (1,333 IU/day).[20] Generally, provision of 15-50% of the RDA can meet both nutritional and safety goals of vitamin A.[21-22] Vitamin levels used in food fortification are normally within the safe range of 15-25% of the RDA per serving or at least one-third (10-15%) of the children's vitamin A RDA.[23] Some countries have assessed the RDA contribution of their vitamin A fortified foods in their preschool children and reported thus: 50% South Africa; 35-55% from Chapattis in rural Bangladesh; 15-30% in Brazil and Vietnam and 33% in baked *Pandesal* in Philippines.[9,22-27] Fortified foods contributed one-half of recommendation in vitamin A intake in poor urban Guatemalan Toddlers. [28]

Vitamin A contents and stability in flour, bread and biscuits and their compliance level have been studied in Nigeria.[13-16,29] But the contribution of bread and biscuits to vitamin A daily requirement of preschool children in Nigeria has not been jointly determined. It cannot be assumed that just because bread and biscuits were made with 'vitamin A fortified wheat flour' that they will contribute adequately to the vitamin A daily requirement of preschool children given that low vitamin A stability has also been reported in these products.

Aim

The aim of this study was to determine the contribution of bread and biscuits to vitamin A daily requirement of preschool children in Lagos State, Nigeria.

Material and methods

Ethical considerations

The study was approved by the University of Ibadan/University College Hospital (UI/UCH) Health Research Ethics Committee, Institute of Advanced Medical Research and Training (IMRAT) (UI/IRC/07/0095). On-the-spot written voluntary informed consent was obtained from mothers willing to participate in the survey. All the bakers that agreed to participate supplied free bread samples.

Study design/Area

A cross-sectional design with analytical component was used to carry out a study in 5 out of 20 Local Government Areas (LGAs) in Lagos State namely: Agege, Mushin, Oshodi/Isolo, Lagos Island, and Ikorodu LGAs representing the poor-urban communities in the three senatorial districts in the State. The population of the LGAs were Oshodi-Isolo (602,159), Agege (459,939), Mushin (633,009), Lagos Island (317,720) and Ikorodu (535,619).[30]

Study population

The study population comprised of preschool children who were children from the ages of two to less than 6 years (24-59 months) and their mothers. These group of children are just beginning to go to school and those who were enrolled in school earlier than the enrollment age are in nursery and primary one classes. In Nigeria, parents enroll their children in schools earlier than the age of enrollment probably because some of them are working class mothers. These group of children are among the at-risk groups for VAD. The respondent was the mother since preschool children cannot remember their food intake.[31] Until children have reached the developmental stage when they are able to give account of

their food intake and can begin to conceptualize (approximately 7-8 years), the onus for dietary reporting is on the parents.[32] In a dietary recall studies, which compared the results of direct observation of children's food intake with 24-hour recalls by parents, the evidence suggests that parents can be reliable reporters of their children's food intake in the home setting.[32]

Study sample products

The study sample products were breads and biscuits baked with wheat flour fortified with vitamin A (30,000 IU/Kg). After the study population, it became necessary to sample biscuits and breads which were the food products under study from bakeries and shops.

Determination of sample size (n)

The sample size for the study was estimated based on the prevalence of household consumption of flour-based products (5.1%) considering 95% confidence interval, a relative precision of 5% and a design effect of 3.[19,33] This gave a total sample size (n) of 320 preschool children per LGA.

$$n = DEFF * Z^2pq / d^2$$

where:

n = Minimum sample size

DEFF = Design Effect = 3[33]

Z^2 = Standard score corresponding to a given confidence level. Example, at 95% confidence level or 5% level of significance (α=0.05), Z=1.96.

P = 5.1% = prevalence of household consumption of flour-based products (5.1%)[18]

q = (1 – p) or percentage of failure which is 100 – 5.1% = 94.9%

d = Precision limit or proportion of sampling error (standard error) was 5% confidence limit.

$$n = DEFF + Z^2P (1-p) / d^2$$

— $$n = 3 * \frac{(1.96)^2 \times 0.051 \times 0.949}{(0.05)^2} = 222$$

Adjustment of sample size for individual non-response: 10 % non-response (90 % response)

— n = 222/0.9 = 246

Adjustment of sample size for household non-response: 23 % non-response (77 % response)

— n = 246/0.77 = 319 .5 households

The number was rounded up to 320 households/LGA.

Total Sample size for the 5 LGAs = 320 households * 5 LGAs = 1600 households

Sample and sampling technique

Eligibility criteria

Inclusion criteria: Households were included in the study if the households/children (24-59 months) consume bread and biscuits.

Study location was selected based on the State that had the highest consumption of bread and biscuits. Lagos State was chosen for this study because it had the highest consumption of bread and similar foods (8.89 %) in Nigeria.[18]

Exclusion criteria: Households were excluded from the study if the households/children (24-59 months) does not consume bread and biscuits.

A three-stage stratified systematic random sampling technique was used to select LGAs, wards and households using probability proportionate to size technique. Lagos State is made up of 3 Senatorial Zones and 20 LGAs. The state was stratified into three senatorial zones namely Lagos Central, Lagos East and Lagos West Zones. Five (5) LGAs were selected out of the twenty (20 LGAs) as a representative sample. The number of LGAs that was randomly selected from each of the senatorial zones was proportionately calculated to get Lagos Central (1 LGA); Lagos East (1 LGA) and Lagos West (3 LGAs). Probability proportionate to size technique was also used to selected 22 wards out of 107 wards as follows: Oshodi/Isolo LGA (4 wards); Ikorodu LGA (7 wards); Mushin LGA (4 wards); Lagos Island (4 wards) and Agege LGA (3 wards). This technique was also used to select the number of children across the wards to make up the sample size per LGA. A pair of mother and child between 24 -59 months old was selected from the identified households to make up the 320 preschool children selected from each of the five LGAs. All eligible pairs in the selected households were included in the study.

Data collection

Data on socio-demographic information, food consumption pattern and repeated 24-hour dietary recall were collected using a pre-tested, structured and semi-quantitative interviewer-administered food frequency questionnaire (FFQ).[34,35] After the pre-test, leading, ambiguous, repetitive and irrelevant questions were eliminated and double barrel questions were rewritten as separate questions based on literature and expert advice. The FFQ were administered to mothers with the help of 15 trained research assistants. In the 3-days dietary recall including one weekend day 24-hour dietary recall, respondents were asked 'Since this time yesterday, has the child eaten bread/biscuits? If the answer was yes, they were probed more for brand names and quantities in packets, prices (N) and slices within the three days similar to the study done by Brand.[36] Names and quantities in packets, prices and slices enabled the researchers to source and calculate the actual grammes of samples consumed by the preschool children for the estimation of the contribution (vitamin A intake IU/g) of the samples to the daily nutrient requirement of the children. Sample conversion from price to

grammes weight, slice to grammes weight and packet to grammes weight were determined afterwards to get gramme consumptions per child.[37] Children that consumed same grammes of bread/biscuits were grouped together for each LGA and mean number of children calculated. These portion sizes and number of children were then used to calculate the vitamin A (IU) content of samples per gramme, mean, total vitamin A in samples consumed and their contribution to the daily requirement of the children (% RDA). A 'narrow interval' was used for bread consumption (g)/child to enable the researchers to calculate the actual vitamin A intake per child. One 3-day including one weekend day 24-hour recall each was done during school and holiday periods to accommodate the effects of seasonal variation.

Samples for vitamin A analysis were sourced from retail outlets (biscuits) and bakeries (bread) rather than directly from factories and milling companies because the study was seen as an evaluation of the levels of vitamin A actually present in samples as finally consumed by the public rather than levels present at the point of manufacture as done by[38,] The most commonly consumed bread samples were collected by researcher from 15 bread hawkers and non-hawkers and 8 brands of biscuit samples were purchased in cartons from major markets in the study LGAs (Ejigbo, Mushin and Oshodi markets). Samples consumed by at least 5% of the total population per LGA were included and regarded as commonly consumed.[35]

Laboratory analysis of vitamin A contents of bread and biscuits

Data on pre-storage (at collection (day 1)) and post-storage (5-days storage (bread) and biscuits (30- and 60-days storage)) vitamin A (retinyl palmitate) contents of samples were obtained by direct laboratory analysis using HPLC as cited in sources.[14,15,16]

Calculation of contribution of samples to the vitamin A daily requirement of U-5

The contribution of bread and biscuits to vitamin A daily nutrient requirement of pre-school children was esti-

Table 1. Socio-demographic characteristics of children and their mothers (N=1599)

	Local Government Areas				
Variables	**Agege**	**Ikorodu**	**Lagos Island**	**Mushin**	**Oshodi/Isolo**
Gender of children					
Male, n (%)	141 (44.1)	146 (45.6)	177 (55.5)	183 (57.2	159 (49.7)
Female, n (%)	179 (55.9)	174 (54.4)	142 (44.5)	137 (42.8)	161 (50.3)
Age of children (months)					
23-35, n (%)	196 (61.3)	160 (50)	274 (85.9)	138 (43.1)	102 (31.9)
35-47, n (%)	74 (23.1)	100 (31.2)	29 (9.1)	81 (25.3)	127 (39.7)
47-59, n (%)	50 (15.6)	60 (18.8)	16 (5)	101 (31.6)	91 (28.4)
Mean age (months)	**31.44±5.28**				
Child's education					
None, n (%)	74 (23.1)	134 (41.9)	39 (12.2)	50 (15.6)	60 (18.8)
Nursery, n (%)	230 (71.9)	150 (46.9)	241 (75.6)	170 (53.1)	246 (76.8)
Primary 1, n (%)	16 (5)	36 (11.2)	39 (12.2)	100 (31.3)	14 (4.4)
Age of mothers (Years)					
18-29, n (%)	62 (19.4)	134 (41.9)	153 (48)	16 (5)	205 (64.1)
30-39, n (%)	206 (64.3)	150 (46.9)	135 (42.3)	228 (71.3)	115 (35.9)
40+, n (%)	52 (16.3)	36 (11.2)	31 (9.7)	76 (23.7)	0 (0)
Mean age (years)	**36.11±4.22**				
Maternal education					
No formal education, n (%)	2 (0.6)	10 (3.1)	21 (6.6)	39 (12.2)	16 (5)
Primary School, n (%)	34 (10.6)	48 (15)	18 (5.6)	34 (10.6)	68 (21.3)
Secondary School, n (%)	121 (37.8)	136 (42.5)	154 (48.3)	58 (18.1)	134 (41.8)
Tertiary Institution, n (%)	142 (44.4)	110 (34.4)	91 (28.5)	189 (59.1)	102 (31.9)
Dropped out, n (%)	21 (6.6)	16 (5)	35 (11)	0 (0)	0 (0)
Maternal occupation					
Full house wife, n (%)	57 (17.8)	50 (15.6)	31 (9.7)	48 (15)	66 (20.6)
Artisan, n (%)	46 (14.4)	40 (12.5)	15 (4.7)	42 (13.1)	57 (17.8)
Skilled worker, n (%)	122 (38.1)	110 (34.4)	73 (22.9)	157 (49.1)	66 (20.6)
Trading, n (%)	95 (29.7)	120 (37.5)	159 (49.8)	73 (22.8)	115 (36)
Unemployed, n (%)	0 (0)	0 (0)	41 (12.9)	0 (0)	16 (5)

mated as a proportion of vitamin A contents of samples and mean consumption grammes of commonly consumed samples per child per sample to the recommended dietary allowance (RDA) using data from the 3-days 24-hour dietary recall. This estimation was based on the vitamin A RDA (400 µg retinol/day (1,333 IU/day)) for pre-school children.[20] Calculation was done based on grammes weight and number of packets eaten by specific number of children. Percentage DNR was calculated for day 1, day 2, one weekend day, and the mean value taken. Samples that had zero vitamin A values in the 2 storage periods were excluded from the study during data calculation and analysis. The percentage contribution of each sample to vitamin A DNR was calculated using the following formulae:

$$\text{Mean vitamin A intake per child per sample} = \frac{\text{Total vitamin A intake per sample (IU)}}{\text{Total number of children}}$$

$$\%\ \text{vitamin A daily requirement/child} = \frac{\text{Sample mean vitamin A intake/child (IU)}}{\text{RDA of age group (1,333 IU/day)}} \times 100$$

Statistical analysis

Data were entered into Excel sheet, cleaned and presented in tables, mean and percentages. Student's t-test and ANOVA were used to analyse the statistically significant differences between samples using the Statistical Package for the Social Sciences (SPSS) 15.0 version 15 at $p<0.05$. Out of the 1,600 questionnaires collected, one was disqualified during data entry because consumption of bread and biscuits by the child were not reported thus bringing the total questionnaires used to n = 1,599. Response rate was 99.94 %.

Results

Table 1 shows the socio-demographic characteristics of the children and their mothers who were the respondents for the study (N=1599). The mean age of children across the LGAs was 31.44±5.28 months.

Table 2 shows that the bread brands most commonly consumed by pre-school children across LGAs were *Agege* bread (70.6 %), Premium HarvestPlus sliced bread (8.9 %) and Queensmeal sliced bread (6.9 %). The unbranded *Agege* bread was the most frequently consumed white bread across the LGAs 1130 (70.6 %).

Table 3 shows the eight (8) commonly consumed biscuit brands: wafers (6.9%), coaster (16.87%), crackers (12.73%), butter bread (15.2%), spicy fish (6.3%), digestive (8.9%), richtea (10.5%) and noreos (15.8%). These were the brands of biscuits normally consumed by the preschool children.

Tables 4 shows that a statistically significant difference existed between commonly consumed brands of bread assessed for vitamin A content and other brands of bread

Table 2. Brands of bread consumed by pre-school children across the LGAs

	Local Government Areas					
	Oshodi/Isolo	**Agege**	**Mushin**	**Lagos Island**	**Ikorodu**	**Total**
Bread Brand	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
None	19 (5.9)	16 (5)	16 (5)	15 (4.7)	10 (3.1)	76 (4.8)
Agege	210 (65.7)	250 (78.1)	236 (73.8)	180 (65.8)	254 (79.4)	1130 (70.6)
HarvestPlus	32 (10)	10 (3.1)	58 (18.1)	11 (3.5)	12 (3.8)	123 (7.7)
Queens meal	27 (8.4)	13 (4.1)	0 (0)	60 (18.8)	10 (3.1)	10 (6.9)
Fresh bake	11 (3.4)	7 (2.2)	0 (0)	0 (0)	10 (3.1)	28 (1.8)
Family bake	5 (1.6)	10 (3.1)	0 (0)	15 (4.7)	4 (3.1)	34 (2.1)
Today's bread	0 (0)	0 (0)	0 (0)	14 (4.4)	7 (2.2)	21 (1.3)
UTC	6 (1.9)	4 (1.3)	0 (0)	5 (1.6)	0 (0)	15 (0.9)
Val-U	5 (1.6)	0 (0)	0 (0)	9 (2.8)	8 (2.5)	22 (1.4)
Ok Special	-	-	2 (0.6)	-	2 (0.6)	4 (0.3)
Butter Field	-	1 (0.3)	-	2 (0.6)	1 (0.3)	4 (0.3)
Tea Mate	-	1 (0.3)	2 (0.6)	1 (0.3)	-	4 (0.3)
Quin Tea	1 (0.3)	-	1 (0.3)	-	-	2 (0.1)
DonitPremium	-	1 (0.3)	-	1 (0.3)		2 (0.1)
Good Luck	-	1 (0.3)	-	1 (0.3)	2 (0.6)	4 (0.3)
Flourish	-	-	1 (0.3)	-	-	1 (0.3)
Premier Special	-	3 (0.9)	-	2 (0.6)	-	5 (0.3)
Malta Chocolate	-	2 (0.6)	-	1 (0.3)	-	3 (0.2)
Nouvelle	-	-	2 (0.6)	1 (0.3)	-	3 (0.2)
Ifelodun	4 (1.3)	1 (0.3)	2(0.6)	1 (0.3)	-	8 (0.5)
Total	**320**	**320**	**320**	**319**	**320**	**1599**

consumed by preschool children (F =107.163, p=<.001). The Post hoc test conducted also shows that the consumption of *Agege* bread brand was significantly higher than the consumption of other bread samples (p<0.05).

Table 5 also shows that there was a statistically significant difference between commonly consumed brands of biscuits used for vitamin A content analysis and other brands of biscuits consumed by preschool children (F=3.605, p<.001).

Table 6 shows the vitamin A contents of bread and biscuit samples according to their storage periods. Mean vitamin A content of oven-fresh bread was 7,571.6 IU/Kg and at the 5th day (1,460.6 IU/Kg). Mean vitamin A content of 1- and 2-months stored biscuits were 5,164.7±4,851.7 IU/Kg and 739.9 ±1361.5 IU/Kg respectively. Mean calculation excluded samples that had zero vitamin A values in the 2 storage periods in order to get the actual vitamin A value in the samples that retained vitamin A to avoid 'watering down effect'. For those samples that had zero vitamin A, it could be that there was zero vitamin A fortification compliance or very poor premix was used in the fortification of their wheat flour

Table 7 presents the mean quantity of bread consumed by the children in the repeated 3-days 24- hour recall including one weekend day 24-hour dietary recall across LGAs. The highest weight (in gramme) of bread and the children (in %) that consumed the biscuits across the LGAs were Oshodi/Isolo LGA 88.3 g (12.6 %), Agege LGA 120.2 g (15.9 %), Mushin LGA 87.7 g (2.8 %), Lagos Island LGA 76.8 g (6.6 %), and Ikorodu LGA 113.9 g (12 %). Mean consumption of bread was 117.6±15.9 gramme per day per child.

Table 8 shows the quantities of biscuits (packets) and number of preschool children that actually con-

Table 3. Brands of biscuits normally consumed by Preschool children in LGAs

	Local Government Areas					
	Oshodi/Isolo	**Agege**	**Mushin**	**Lagos Island**	**Ikorodu**	**Total**
Biscuit Brand	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
None	62 (19.4)	171 (53.4)	205 (64)	167 (52)	170 (53.1)	775 (48.5)
Wafers	22 (6.9)	9 (2.8)	25 (7.8)	16 (5)	10 (3.1)	82 (5.1)
Coaster	43 (13.5)	43 (13.5)	10 (3.1)	65 (20.3)	10 (3.1)	171 (10.7)
Crackers	38 (11.9)	41 (12.9)	10 (3.1)	37 (11.6)	12 (3.8)	138 (8.6)
Butter bread	46 (14.4)	3 (0.9)	4 (1.3)	1 (0.3)	9 (2.8)	63 (3.9)
Spicy fish	19 (5.9)	2 (0.6)	15 (4.7)	4 (1.3)	12 (3.7)	52 (3.3)
Mini cookies	7 (2.2)	3 (0.9)	10 (3.1)	3 (0.9)	6 (1.9)	29 (1.8)
Digestive	27 (8.5)	4 (1.3)	5 (1.6)	2 (0.6)	8 (2.5)	46 (2.9)
Richtea	32 (10)	3 (0.9)	4 (1.3)	1 (0.3)	5 (1.6)	45 (2.8)
Parle-G Glucose	7 (2.2)	1 (0.3)	2 (0.6)	4 (1.3)	10 (3.1)	24 (1.5)
Pepper Snacks	1 (0.3)	2 (0.6)	0 (0)	0 (0)	0 (0)	3 (0.2)
Pearl	1 (0.3)	2 (0.6)	2 (0.6)	2 (0.6)	0 (0)	7 (0.4)
Cabin	2 (0.6)	11 (3.4)	0 (0)	8 (2.5)	0 (0)	21 (1.3)
Football	2 (0.6)	2 (0.6)	14 (4.4)	1 (0.3)	8 (2.5)	27 (1.7)
Twist	2 (0.6)	1 (0.3)	5 (1.6)	2 (0.6)	0 (0)	10 (0.6)
Beloxxi	2 (0.6)	3 (0.9)	1 (0.3)	3 (0.9)	7 (2.2)	16 (1)
Chic-choc	1 (0.3)	7 (2.3)	6 (1.9)	1 (0.3)	5 (1.6)	20 (1.4)
Noreos	3 (0.9)	8 (2.5)	2 (0.6)	2 (0.6)	48 (15)	63 (3.9)
Short cake	3 (0.9)	4 (1.3)	0 (0)	0 (0)	0 (0)	7 (0.4)
Total	**320 (100)**	**320 (100)**	**320 (100)**	**319 (100)**	**320 (100)**	**1599 (100)**

*Numbers in brackets are in percentages; Total of children who did not consume biscuit 775 (48.5%).

Table 4. ANOVA table showing differences in consumption of commonly consumed brands of bread in Lagos State

Brands of bread	**Mean***	**Standard Deviation**	**F-ratio**	**p-value**
Agege	226.0[a]	30.9	107.163	<0.001
HarvestPlus	28.6b	23.6		
Sliced bread A	22.0b	23.3		
Fresh bake	8.6d	5.6		
Family bake	8.8d	5.4		
Today's bread	4.2c	6.2		
UTC	2.0c	2.8		
Val-U	4.4c	4.3		

*Means with the same letters along the same column are not significantly different

sumed biscuits in the repeated 24-hour recall across the LGAs. Most of the children consumed 1 packet of biscuit per day. Mean gramme consumption of biscuits was 59.8±27.9 gramme per day. It was observed that in the 2h-hr dietary recall, the number of children that actually consumed biscuits were lower than that reported by their mothers in Table 3. This might be expected because biscuits were eaten as snacks and the preschool children use them to go to school. So their consumption in the dietary recall might have been affected by family meals and weekend days when mothers were at home to prepare meals for their children who are also at home.

Table 9 shows the mean biscuit consumption by children across the LGAs in the repeated 3-day including one weekend day 24-hr dietary recall. Approximately 59.8 % of the children did not consume biscuits and

Table 5. ANOVA table showing differences in consumption of commonly consumed biscuit brands*

	Mean*	Std. Deviation	F-value	p-value
Wafers	16.4000[a]	7.09225	3.605	<0.001
Coaster	34.2000[b]	23.84743		
Crackers	27.6000[b]	15.24139		
Butter bread	12.6000[c]	18.90238		
Spicy fish	10.4000[c]	7.23187		
Mini cookies	5.8000[d]	2.94958		
Digestive	9.2000[d]	10.18332		
Richtea	9.0000[d]	12.94218		
Parle G	4.8000[e]	3.70135		
Pepper Snacks	1.4000[f]	0.89443		
Pearl	5.4000[d]	5.54977		
Cabin	2.0000[f]	1.87083		
Football	3.2000[f]	2.28035		
Twist	4.0000[d]	2.82843		
Beloxxi	12.6000[c]	19.94492		
Chic-choc	1.4000[f]	1.94936		
Noreos	3.8000[f]	3.03315		
Short cake	3.8000[f]	3.63318		

*Means with the same letters along the same column are not significantly different

Table 6. Vitamin A contents of bread and biscuit samples according to their storage periods

	Bread samplesb	Vitamin A content (IU/Kg)			Vitamin A content (IU/kg)	
S/N[a]	LGAs	Oven-fresh	5 (days)	Biscuit brands	1 month	2 months
	***Agege* bread (A)**					
1	*Agege*	3,434	0	Spicy fish	0	0
2	*Agege*	12,597	0	Coaster	1,847.7	0
3	*Agege*	0	0	Digestive	6,797.6	0
4	*Agege*	18,239	6,344	Richtea	12,873	3,136
5	*Agege*	0	0	Wafers	0	0
6	*Agege*	17,472	0	Cream Cracker	0	0
7	*Agege*	5,615	0	Butter bread	3,361.5	0
8	*Agege*	998.7	678.8	Noreos	943.8	563.6
9	*Agege*	3,438.1	2,221.8	[a]**Mean**	**5,164.7**	**739.9**
10	*Agege*	4,826.8	3,031.2		**±4,851.**7	**±1361.**5
11	Sliced bread B	0	0			
12	Sliced bread B	0	0			
13	Sliced bread C	0	0			
14	Sliced bread C	0	0			
15	Sliced bread C (Mixed flour)	1,524	869.7			
	cMean	**7,571.6**	**1,460.6**			
		±6,730.6	**±2,132.7**			

[a]1-2=Agege LGA; 3-4, 13-15=Mushin; 5-6=Lagos Island, 7-8=Oshodi/Isolo LGA; 9-10= Ikorodu LGA; 11-12 Ojo LGA; bBread samples; cExcluding samples that had zero vitamin A values in the 2 storage periods.

Table 7. Mean bread consumption quantities by preschool children in repeated 24-hour recall across the LGAs

	Local Government Areas (LGAs)				
Bread consumption (g)/child	**Oshodi/Isolo (%)**	**Agege (%)**	**Mushin (%)**	**Lagos Island (%)**	**Ikorodu (%)**
0	213.5±13.4 (65.0)	214±14.1 (66.9)	286±2.8 (89.4)	251.5±6.4 (78.8)	246.5±0.7 (77)
38.4	3.5±2.1 (1.3)	-	-	9±1.4 (2.8)	-
65.1	-	-	-	21±1.4 (6.6)	12.5±3.5 (3.9)
76.8	2 (0.7)	4.5±3.5 (1.4)	4.0±1.4 (1.3)	4.5±3.5 (1.4)	-
80.1	-	16±8.5 (5.0)	8±1.4 (2.5)	1±1.4 (0.3)	-
87.7	-	1±1.4 (0.3)	9±1.4 (2.8)	14±1.4 (4.4)	-
88.3	38.5±4.9 (12.6)	3±1.4 (0.9)	-	-	-
97.7	-	-	-	-	18.5±5.0 (5.8)
113.9	-	-	-	-	38.5±2.1 (12)
115.3	7.0± 0.0 (2.3)	51.0±12.7 (15.9)	3.0±1.4 (0.9)	3.5±4.9 (1.1)	-
120.2	-	0±1.4 (0.9)	6.5±2.1 (2.0)	2. (0.6)	-
130.2	-	-	-	-	4.0±5.7 (1.3)
132.4	28.5±2.1 (9.2)	-	-	-	-
147.8	-	1±1.4 (0.3)	1±1.4 (0.3)	4±1.4 (1.4)	-
153.7	0.5± 0.7 (0.3)	17.5±12 (5.5)	1.0±1.4 (0.3)	4.0±2.8 (1.4)	-
160.2	-	7±4.2 (2.2)	1.5±0.7 (0.5)	3±1.4 (0.9)	-
176.5	26.5±7.8 (8.6)	-	-	-	-
240.3	-	2 (0.6)	-	1±2.1 (0.3)	-
Total	**320(100)**	**320(100)**	**320.0 (100)**	**319.0(100)**	**320(100)**

[a]Figures in parenthesis are in percentages; Mean total zero (0.0) consumption was 1211.5 (75.76 %)

Table 8. Mean number of children and quantity of biscuit brands commonly consumed in packets in 24-hr dietary recall

	Number of biscuit packets per child/LGA																			
Biscuit brands	**Oshodi/Isolo LGA**				**Agege LGA**				**Lagos Island LGA**				**Mushin LGA**				**Ikorodu LGA**			
	1 pkt	**2 pkts**	**3 pkts**	**4 pkts**	**1 pkt**	**2 pkts**	**3 pkts**	**4 pkts**	**1 pkt**	**2 pkts**	**3 pkts**	**4 pkts**	**1 pkt**	**2 pkts**	**3 pkts**	**4 pkts**	**1 pkt**	**2 pkts**	**3 pkts**	**4 pkts**
Wafers	10	14	7	7	5	4	0	0	3	0	0	0	8	2	0	0	7	3	0	0
Coaster	19	13	10	1	24	16	2	1	47	20	1	0	20	5	0	0	8	2	0	0
Crackers	16	14	8	0	22	18	1	0	17	13	7	0	10	0	0	0	10	2	0	0
Butter bread	25	14	11	2	2	1	0	0	0	0	0	0	4	0	0	0	8	1	0	0
Spicy fish	8	7	3	1	0	0	0	0	0	0	0	0	13	2	0	0	12	0	0	0
Digestive	12	10	4	1	0	0	0	0	2	0	0	0	5	0	0	0	5	0	0	0
Richtea	17	10	2	1	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
Neroes	0	0	0	0	8	0	0	0	2	0	0	0	2	0	0	0	40	6	0	0
Total	**107**	**82**	**45**	**13**	**61**	**39**	**3**	**1**	**71**	**33**	**8**	**0**	**62**	**9**	**0**	**0**	**95**	**14**	**0**	**0**

Pkts = packets

Table 9. Mean biscuit consumption in 3-days including one weekend day 24-hr dietary recall by children across the LGAs

Biscuit consumption (Packets)	Oshodi/Isolo	Agege	Lagos Island	Mushin	Ikorodu	Mean Total
0	73 (22.8)	216 (67.5)	207 (64.9)	249 (77.8)	211 (65.9)	956 (59.7)
1	107 (33.4)	61 (19.1)	71 (22.3)	62 (19.4)	95 (29.7)	396 (24.8)
2	82 (25.6)	39 (12.2)	33 (10.3)	9 (2.8)	14 (4.4)	177 (11.1)
3	45 (14.1)	3 (0.9)	8 (2.5)	0 (0)	0 (0)	56 (3.5)
4	13 (14.1)	1 (0.3)	0 (0)	0 (0)	0 (0)	14 (0.9)
Total	**320 (100)**	**320 (100)**	**319 (100)**	**320 (100)**	**320 (100)**	**1599 (100)**

Table 10. Contribution of bread to vitamin A daily requirement of preschool children

Bread Sample[a]	Vitamin A content IU/g (µgRE/g)	Portion size (g)	Total vitamin A IU (µgRE) /day	% Contribution to RDAb
1 *Agege bread*		128.2±11.5		
Oven-fresh	3.4 (1.0)		447.5±44.5 (134.4±13.4)	33.6±3.3
2 *Agege bread*		128.2±11.5		
Oven-fresh	12.6 (3.8)		1614.7±145.2 (484.9±43.6)	121.1±10.89
3 *Agege bread*		100.0±0.3		
Oven-fresh	18.2 (5.5)		1821.4±2.4 (547±0.7)	136.6±0.2
5 days	6.3 (1.9)		635.0±1.2 (190.7±0.4)	47.6±0.1
4 *Agege bread*		131.7±27.9		
Oven-fresh	17.5 (5.3)		2377.6±377.6 (714±113.4)	178.4±28.4
5 *Agege bread*		131.7±27.9		
Oven-fresh	5.6 (1.7)		764.08±121.37 (229.45±36.45)	57.3±9.1
6 *Agege bread*		126.5±2.3		
Oven-fresh	1.0 (0.3)		126.4±2.3 (38±0.7)	9.5±0.1
5 days	0.7 (0.2)		85.9±1.6 (25.8±0.5)	6.45±0.1
7 *Agege bread*		126.5±2.3		
Oven-fresh	3.4 (1.0)		435.7 ±7.8 (130.8±2.3)	32.7±.6
5 days	2.2 (0.7)		281.2±5.0 (84.4±1.5)	21.1±.4
8 *Agege bread*		102.4±4.6		
Oven-fresh	4.8		494.7±22.2 (148.4±6.7)	37.1±1.7
5 days	3.0		296.8±5.2 (89.1±26.7)	22.3±0.4
9 Sliced bread (C)		93.9±1.4		
Oven-fresh	1.5 (0.5)		138.8±18.3 (41.68±5.5)	10.5±1.3
5 days	0.9 (0.3)		79.2±10.4 (23.78±3.1)	6.0±0.8
Total	**5.8±6.0**	**117.6±15.9**	**685.5±725.1 (205.7)**	**51.4±54.4**

RDA= Recommended Dietary Allowance; [a] Samples with zero vitamin A contents were excluded; bRDA = 1333 IU/ 400 µgRE/ day for 24-59 months old children[19]; 1 RE = 3.33 IU for fortified foods or vitamin A activity from retinol; 1 I.U = 0.3 µgRE

this was considered high even though not unexpected since biscuits are snacks and not the main meals eaten by the preschool children. The number of children that actually consumed biscuits were 643 (40.2 %). Approximately 25% consumed 1 packet of biscuit.

Table 10 shows the vitamin A contribution of oven-fresh and 5-days bread to vitamin A daily requirement of preschool children. Mean daily consumption of bread was 117.6±15.9 g. The contribution of bread to preschool children's vitamin A nutriture ranged from 6 to 178 % excluding six (6) samples that had zero vitamin A content. Mean vitamin A contribution was 51.4 %±54.4.

Table 11 presents mean contribution of commonly consumed biscuit brands to vitamin A daily requirement of preschool children. The mean grammes intake of biscuits per day was 59.8±27.9g while the mean total vitamin A intake of biscuits per day was 298.8±354.1 IU/day (98.3 µgRE/day) and this contributed 22.6±26.5 percent to vitamin A daily requirement of the children.

Figure 1 shows the vitamin A contents of bread and biscuits derived after vitamin A analysis at different storage periods. These values were used to estimate the contribution of the samples to the vitamin A daily requirement of the preschool children in IU per day and RDA percentages.

Table 11. Mean contribution of biscuits to vitamin A daily requirement of U-5 children

Biscuit	N	Vitamin A content	Portion size	Total vitamin A	% Contribution
		IU/g	(g)	IU/g	to RDA
Coaster					
Oshodi/Isolo	43	1.9	49.9	92.3	6.9
Agege	43		41.7	77.1	5.8
Lagos Island	68		36.0	66.5	5.0
Mushin	25		32.6	60.3	4.5
Mean	**45**		**40.1±7.6**	**74.0±14.0**	**5.6±1.1**
Digestive					
Oshodi/Isolo	32	6.8	142.0	965.5	72.4
Lagos Island	2		85.8	582.9	43.7
Mushin	4		85.8	582.9	43.8
Mean	**13**		**104.5±32.5**	**710.4±220.9**	**53.3±16.6**
Richtea					
1 (day)*	30	12.9	82.6	1063.1	79.8
30 (days)*	30	3.1	51.7	162.1	12.2
Mean			**67.1±21.9**	**612.6±637.1**	**46.0±47.8**
Butter bread					
Oshodi/Isolo	52	3.4	73.5	224.4	16.8
Agege	3	3.4	47.7	160.3	12.0
Mushin	4	3.4	35.8	120.2	9.0
Mean			**52.31±19.29**	**175.6±65**	**13.2±4.9**
Nereos					
Ikorodu					
1 (day)*	46	1.0	34.5	32.5	2.4
30 (days)*	46	0.6	35.3	19.4	1.5
Mean			**34.9±0.6**	**26.0±9.3**	**2.0±0.7**
Total	**31±21**	**4.0±3.3**	**59.8±27.9**	**300.7±352.7**	**22.6±26.5**

RE=Retinol equivalent; 1 µgRE = 3.33 IU for fortified foods or vitamin A activity from retinol; RDA, Recommended Daily Allowance; RDA = 1333 I.U or 400 µgRE/day[19]; 1 I.U = 0.3 µgRE; N = Number; *storage period

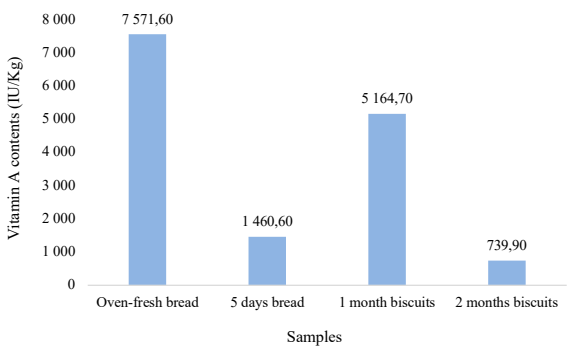

Fig. 1. Vitamin A contents of bread and biscuit samples at different storage periods

Figure 2 shows the mean contribution of bread and biscuits to the vitamin A daily requirement in International Units per day (IU/day) of preschool children across the 5 LGAs in Lagos State. The mean vitamin A intake (IU/day) for bread and biscuits were 685.5±725.1 IU/day and 300.7±352.7 IU/day respectively.

Figure 3 shows the mean percentage contribution of bread and biscuits to the vitamin A daily requirement of pre-school children across the 5 LGAs in Lagos State. The mean vitamin A contribution of bread was 51.4±54.4% per day while that of biscuits were 22.6±26.5% per day.

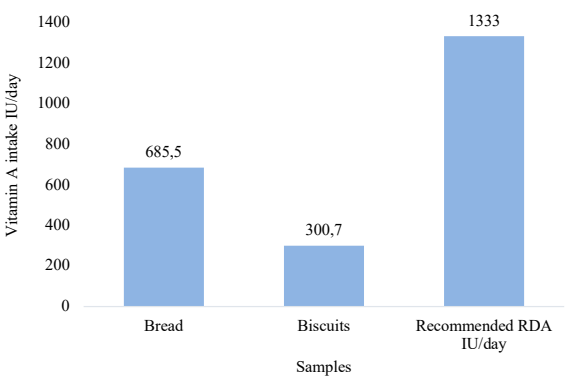

Fig. 2. Mean contributions of bread and biscuits to the vitamin A daily requirements of preschool children in International Units per day (IU/day).

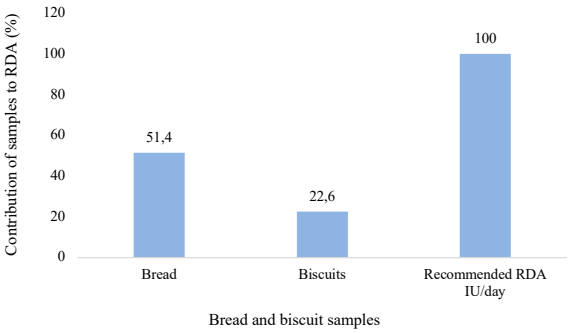

Fig. 3. Mean percentage contributions of bread and biscuits to the vitamin A daily requirement of preschool children

Discussion

The mean consumption gramme of bread by the preschool children was approximately 118 g per day while the mean total vitamin A intake was approximately 686 IU (206 µg RE). This vitamin A intake was more than half of the level recommended by the Indian Council of Medical Research (ICMR) (1333 IU/day) for preschool children.[20] Oven-fresh bread contributed significantly to the vitamin A daily requirement (68.3%) of preschool children than the five days bread (20.7%) ($p<.05$). This contribution of bread ranged from 0 -178 % inclusive of samples that had zero vitamin A contents. The mean total contribution of bread to vitamin A requirement of the children was 51%. Bread therefore contributed one-half of recommended vitamin A daily requirement in poor-urban Lagosian pre-school children. This result indicated that bread made from vitamin A fortified wheat flour can make an important contribution to the vitamin A daily requirement of preschool children if adequately fortified and consumed. This contradicts the concern that some fortified products do not contribute substantially to the total vitamin A intake of the poorest segments of the society.[10] The contribution of bread to vitamin A requirement of the children in this study was similar to and even higher than the values obtained in Bangladesh chapattis, 35-55% and Philippine pandesal, 33%.[21,24] Preschool children are one of the at-risk groups for VAD. The aim of vitamin A fortification of wheat flour is to use it as one of the long-term sustainable strategies to eradicate VAD among vulnerable groups.

Nevertheless, a closer look at those three bread samples that contributed above 100 % vitamin A to this study group might spark a little worry for consumers from affluent homes who have access to animal sources of vitamin A and also consume bread regularly. They might stand a risk of over-consumption of vitamin A. This is due to the unusually high level of vitamin A fortification of wheat flour in Nigeria. Nigeria started fortifying wheat flour with vitamin A with 30,000 IU/kg (9 mg/kg) in 2004. Even though, most of the bread samples did not have vitamin A content, some of those that had, had excess and contributed as high as 178%. A study suggested that there may be no risk of pre-disposition of consumers to hyper-vitaminosis as previously speculated.[28] But the result of this study has confirmed that the possibility of hyper-vitaminosis is a fact not speculation. This result helps to justify the revised Nigerian vitamin A fortification level which is now less than 30,000 IU/kg.[12] Fortification programs that add preformed vitamin A to foods should carefully adjust the fortification levels to benefit consumers whose diets are most deficient without exposing wealthier segments of society, whose diets might be richer in preformed vitamin A, from consuming excessive amounts. This is because vitamin A if consumed in excess, is hypertoxic. However, vitamin A zero bread samples contributed zero vitamin A to the dietary intake of children that consumed them. To ensure that all the groups of consumers benefit from vitamin A fortification, proper choice of vehicles should be made, use of good premix or vitamin A matrices, lower fortification levels and enforcement of compliance will bridge all these risks.

The average weight consumption of biscuits (59.8 g) was similar to that reported in Ghana (56 g/day of flour products).[17] Biscuits stored for one month contributed a higher vitamin A value (25%) than that stored for 2 months (6.8 %). Biscuits contributed one-fifth (22.4±26.6%) of the vitamin A daily requirement of the children. This result was within the range obtained in Phillipine for 4-6 years old children (10-46% for vitamin A); lower than that obtained in Vietnam (30%) and South Africa (50%).[9,23,25] Conclusively, the result obtained in this study is within the recommendation that wheat flour products fortified at 2.1 µgRAE/g or 7 IU/g at <75 g/day flour intake will contribute approximately 22% of RDA for preschool-aged children vitamin A daily requirement.[21] The Researchers recommend that Nigeria should fortify wheat flour at 7,000 IU/kg using a good vitamin A matrix for the premix. Consequently, Nigerian vitamin A fortification of wheat flour has been revised to 6,000 IU/kg.[12]

While fortified foods contribute one-half of recommendation in vitamin A intake in poor urban Guatemalan Toddlers, bread alone contributed the same amount (one-half) of vitamin A requirement of pre-school-aged children in Lagos state and biscuits contributed one-fifth of the DNR.[27] The classification of fortified foods is the responsibility of the authorities of each country.[26] Vitamin levels used in food fortification are normally within the safe range of 15-25% of the RDA per serving or at least one-third (10-15%) of the children's vitamin A RDA.[23] Nigerian bread and biscuits therefore qualify to be called fortified foods. The mean vitamin A contribution of bread and biscuit samples fell within this safe range 15-25%. According to Food and Drug

Administration (FDA), terms to define a serving of food that has 20% or more of the RDA include: "high", "rich in", or "excellent source of" vitamin A.[26] In Nigeria, a fortified product is declared an excellent source of vitamin A if it contributes at least 30% of Nutrient Reasonable Value (NRV) and a good source if it contributes at least 15% of NRV per 100g.[12] Using the FDA classification, fresh bread and biscuits are excellent sources of vitamin A in poor-urban preschool children in Lagos metropolis. Based on Nigerian standards, bread is an excellent source while biscuits are good sources of vitamin A in the daily nutrient requirements of preschool children in this study.

Conclusion

Fresh samples of bread and biscuits contributed adequately to the daily nutrient requirement of the children even though some of the samples had zero and over contribution. There is a need to encourage retention of vitamin A in flour-products and avoid overdose of the vitamin if the aim and objectives of vitamin A fortification of wheat flour in Nigeria will be met.

Acknowledgments

The authors acknowledge the kind support of Standard Organisation of Nigeria, HoneyWell Flour Mills PLC and BATO Chemicals Ltd for subsidising the cost of the analysis and all the bakers who supplied free bread samples. The Authors also thank Dr. Nino Ozara for his guidance.

Declarations

Funding

The authors declare that there was no external funding.

Author contributions

Conceptualization, F.N. and O.O.; Methodology, F.N. and O.O..; Software, F.N..; Validation, F.N. and O.O..; Formal Analysis, F.N.; Investigation, F.N.; Resources, F.N.; Data Curation, F.N. and O.O.; Writing – Original Draft Preparation, F.N.; Writing – Review & Editing, F.N. and O.O..; Visualization, F.N..; Supervision, O.O.; Project Administration, F.N.; Funding Acquisition, F.N.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data is available as presented.

Ethics approval

The study was approved by the University of Ibadan/ University College Hospital (UI/UCH) Health Research Ethics Committee, Institute of Advanced Medical Research and Training (IMRAT) (UI/IRC/07/0095). On-the-spot written voluntary informed consent was obtained from mothers willing to participate in the survey. All the bakers that agreed to participate supplied free bread samples.

References

1. Hidden Hunger Index Identifies Global Hot Spots of Micronutrient Deficiencies for First Time. For Immediate Release. Sight and Life. AllAfrica Website. https://allafrica.com/stories/201306141442.html. Published 14 June 2013. Accessed October 20, 2021.
2. Maziya-Dixon B, Akinyele IO, Oguntona EB, et al. Nigerian Food Consumption and Nutrition Survey 2001-2003. Summary. *Nigeria, Ibadan: International Institute of Tropical Agriculture (IITA).* 2004:1-75.
3. Stevens GA, Bennett JE, Hennocq Q, et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. *Lancet Glob Health.* 2015;3:e528-36.
4. Vitamin A Deficiency. United Nations International Children's Education Fund. UNICEF Website. https://data.unicef.org/topic/nutrition/vitamin-a-deficiency/. Published October 21 2019. Accessed March 11, 2021.
5. Nigeria releases more cassava with higher pro-vitamin A to fight micronutrient deficiency. International Institute of Tropical Agriculture (IITA) Website. https://www.iita.org/news-item/nigeria-releases-cassava-higher-pro-vitamin-fight- micronutrient-deficiency/. Published July 4 2014. Accessed November 23, 2014.
6. Uchendu FN. The role of biofortification in the reduction of micronutrient food insecurity in developing countries. *African J of Biotechn.* 2013;12(37):5559-5566.
7. Afidra OR. Review of the Public Health Evidence of Flour Fortification Impacting serum folate, neural tube defects, serum ferritin, and hemoglobin. Flour Fortification Initiative website. http://www.ffinework.org/about/calendar/2013/documents/healthimpact.pdf. Published November 2013. Accessed April 25, 2014.
8. Vitamin A fortification of staple foods World Health Organisation Website. http://www.who.int/elena/titles/vitamina_fortification/en/. Updated May 13 2019. Accessed November 18, 2014.
9. Solon FS, Solon M, Nano T, et al. Wheat flour fortification with vitamin A. The Philippine food fortification program. Federal Republic of Philippines Department of Health Website.http://www.2.doh.gov.ph/food/searches.asp? Published 2008. Accessed February 13 2008
10. Melse-Boonstra A, Pee S, Martin E, et al. The potential of various foods to serve as a carrier for micronutrient fortification, data from remote areas in Indonesia. *European J Clin Nutr.* 2000; 54:822-887.
11. Berner LA, Clydesdale FM, Douglass JS. Fortification Contributed Greatly to Vitamin and Mineral Intakes in the United States, 1989–1991. *J Nutr.* 2001;131:2177-2183.

12. Food fortification Regulations 2019. Levels of mandatorily fortified foods with vitamin A, Schedule 111. National Agency Food and Drug Administrative and Control Website. https://www.nafdac.gov.ng/wpcontent/uploads/Files/Resources/Regulations/All_Regulations/Food-Fortification-Regulations-2019.pdf. Published 2019. Accessed November 23, 2021.
13. Uchendu FN, Atinmo T, Oyewole O. Stability of Vitamin A in Selected Nigerian Bread Made from Commercial Fortified Wheat Flour. *Intern J of Applied Sci & Techn*. 2012;2(3):93-98.
14. Uchendu FN, Atinmo T. Vitamin A stability in Nigerian wheat flour and fortification compliance level. *African J of Food Sci.* 2016;10(3):33-40.
15. Uchendu FN, Oyewole EO. Stability of Vitamin A in Nigerian Retailed Biscuits. *J Food Research.* 2016;5(5):94-104.
16. Uchendu FN, Atinmo T. Baking and storage stability of vitamin A in retailed bread consumed in Lagos state, Nigeria. *Nigerian J Nutr Scis.* 2019;40(2):31-40.
17. Nyumuah RO, Hoang TC, Amoaful EF, et al. Implementing Large-Scale Food Fortification in Ghana:Lessons learned. *Food Nutr Bull* 2012;33(4):S293-S300.
18. Consumption Pattern in Nigeria 2009/10. Preliminary Report. National Bureau of Statistics Website. http://nigeriastat.gov.ng/pages/download/44. Published March 2012. Accessed April 3, 2014.
19. Consumption Expenditure Pattern in Nigeria 2019. National Bureau of Statistics Website.
20. https://www.nigeriastat.gov.ng. Published May 2020. Accessed August 20, 2021.
21. Revised recommended dietary allowances for Indians. A report of the expert group of the Indian Council of the Medical Research. National Institute of Nutrition India Council of Medical Research. 2010. India Council of Medical Research (IMCR) Website. Hyderabad – 500 007. https://www.enacnetwork.com/files/pdf/ICMR_RDA_BOOK_2010.pdf. Published 2010. Accessed June 14, 2021.
22. Klemm RDW, West KP Jr, Palmer AC, et al. Vitamin A fortification of wheat flour: Considerations and current recommendations. *Food Nutr. Bull.* 2010;31(1):S47-S61.
23. Technical Specifications for fortified wheat flour. Version:4.0. World Food Programme (WFP) Website. http://documents.wfp.org/stellent/groups/public/documents/manual_guide. Published May 23, 2011. Accessed April 20, 2021.
24. Dary O, Mora JO. Food Fortification to Reduce Vitamin A Deficiency: International Vitamin A Consultative Group Recommendation. *American J Nutr.* 2002;132:29275-29335.
25. Miller M, Humphrey J, Johnson E, et al. Why do children become vitamin A deficient? Proceedings of the XX International Vitamin A Consultative Group Meeting. *Am Soc J Nutr Sci.* 2002;28675-28805.
26. Rahman AS, Wahed MA, Alam MS, et al. Randomized double-blind controlled trial of wheat flour (Chapatti) fortified with vitamin A and iron in improving vitamin A and iron status in health, school aged children in rural Bangladesh. *Matern Child Nutr.* 2015;11(4):120-131.
27. Frigg M. Biscuits in Vietnam. *Sight and Life Newsletter*. 2003;3:5.
28. Liberato S, Pinheiro-Sant'Ana H. Fortification of industrialized foods with vitamins. *Rev. Nutr. Revista de Nutrição.* 2006;19(2):1-23.
29. Krause VM, Delisle H, Solomons NW. Fortified Foods contribute one and half of recommended vitamin A intake in poor urban Guatemalan Toddlers. *J Nutr.* 1998;128(5):860-864.
30. Ogunmoyela OA, Adekoyeni O, Aminu F. A Critical Evaluation of Survey Results on Vitamin a and Fe Levels in the mandatory fortified Vehicles and Some Selected Processed Foods in Nigeria. *Nigerian Food J*. 2013;31(2):52-62.
31. Federal Republic of Nigeria 2006 Population Census. National Bureau of Statistics Website. 2007. http://www.nigerianstat.gov.ng/nbsapps/Connections/Pop2006.pdf. Published 2007. Accessed June 2, 2008.
32. Vepsäläinen H, Nevalainen J, Fogelholm M, et al. Like parent, like child? Dietary resemblance in families. *Int J Behav Nutr Phys Act.* 2018;15:62.
33. Livingstone MBE, Robson PJ. Measurement of dietary intake in children. *Proceedings of the Nutrition Society.* 2000;59:279-293.
34. World Health Organization Vaccination Coverage Cluster Surveys: Reference Manual. World Health Organization Website. https://apps.who.int/iris/handle/10665/272820. Published: June 2018. Updated March 2019. Accessed November, 2021.
35. Blanton CA, Moshfegh AJ, Baer DJ, et al. The USDA automated multiple-pass method accurately estimates group total energy and nutrient intake. *J Nutr* 2006;136(10):2594-2599.
36. Fox MK, Reidy K, Karwe V, et al. Average Portions of Foods Commonly Eaten by Infants and Toddlers in the U.S. *Am J Dietetic Asso*. 2006;106(1):1:66-76.
37. Stephenson K, Amthor R, Mallowa S, et al. Consuming cassava as a staple food places children 2-5 years old at risk for inadequate protein intake, an observational study in Kenya and Nigerian. *Nutr J*. 2010;9(9):1-18.
38. Dos-Passos AME, Carolina CFF, Pacheco MTB, et al. Proximate and mineral composition of industrialized biscuits. *Food Sci.Techn.* 2013;33(2):101-206.
39. Yusufali R, Sunley N, de Hoop M, et al. Flour Fortification in South Africa: Post-implementation survey of micronutrient levels at point of retail. *Food and Nutr. Bull*. 2012;33(4):321-329.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.13

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 93–101

ORIGINAL PAPER

Ashiyat Kehinde Akodu [1], Temiloluwa Grace Oti [1],
Abdulrazzaq Oluwagbemiga Lawal [2]

Breast hypertrophy, forward head posture, neck and shoulder pain-related disabilities and selected anthropometrics variables of female undergraduate students

[1] Department of Physiotherapy, College of Medicine, University of Lagos, Lagos, Nigeria
[2] Department of Surgery, College of Medicine, University of Lagos, Lagos, Nigeria

ABSTRACT

Introduction and aim. Large breast sizes frequently contribute to women presenting with severe pain symptoms. This study determined the association between breast hypertrophy, forward head posture (FHP), neck and shoulder pain related disabilities and selected anthropometric variables of female undergraduate students of College of Medicine, University of Lagos.

Material and methods. A cross-sectional analytical study was conducted among 89 female undergraduate students (mean age = 21.45±1.29 years) with breast hypertrophy (cup size D and above). Breast cup sizes, neck and shoulder pain related disabilities, forward head posture were measured using a measuring tape, neck pain disability scale, shoulder pain disability index and craniovertebral angle (CVA) using photography method.

Results. The prevalence of forward head posture among the participants was 43(48.3%). Twenty-eight (31.3%) participants had a "DD" cup size, twenty-six (29.2%) participants had a "DDD" cup size. Sixty-five (73%) of the participants had neck pain related disabilities and 10 (11.2%) of the participants had shoulder pain related disabilities. There was association among weight, forward head posture (p=0.027) and breast hypertrophy (p=0.016).

Conclusion. Neck, shoulder pain related disabilities, and forward head posture is prevalent among undergraduates with breast hypertrophy and weight has an influence on forward posture and breast hypertrophy.

Keywords. breast hypertrophy, craniovertebral angle, neck pain, shoulder pain

Introduction

Large breast sizes add to many health issues in women, which could include discomfort in the neck, upper limb, back and head, and it has been found that these problems can be so intense and severe to compel females with breast hypertrophy to undergo breast reduction for pain relief.[1] The mass and dimension of the female breast can be different between individuals such as difference in the volume, width, length, projections, shape, and position on the chest wall.[2,3] Research has also shown that hormonal changes influence breast size.[4] Findikcioglu et al., revealed that in females with brassiere size A, B, or C the thoracic kyphosis and lumbar lordosis angle was smaller than in females with brassiere size D and above.[5]

Neck pain is an unpleasant sensory experience in the neck it may present as fatigue, tension or pain that radiates down to the shoulders, upper extremities or head. Fifty percent of the populace will complain of an episode of neck pain in their life.[6] Pain in the neck is associated with a lot of co-morbidities which includes headache, back pain, arthralgias, and depression.[7,8] The prevalence of neck pain is higher in females than in males, and literature is varied as to whether it rises or levels in mid-

Corresponding author: Ashiyat Kehinde Akodu, e-mail: aakodu@unilag.edu.ng, akoduashiyat@gmail.com

Received: 4.12.2021 / Revised: 28.12.2021 / Accepted: 16.01.2022 / Published: 30.03.2022

Akodu AK, Oti TG, Lawal AO. *Breast hypertrophy, forward head posture, neck and shoulder pain-related disabilities and selected anthropometrics variables of female undergraduate students*. Eur J Clin Exp Med. 2022;20(1):93–101. doi: 10.15584/ejcem.2022.1.13

dle age.[7] Guzman et al., reported that neck pain has a pathologic cause that can be identified and treated while others consider neck pain of any form as a primarily non-organic problem with psychosocial roots.[9,10]

The prevalence of shoulder pain in the general population has been said to vary between 7% and 30% it increases with age and has been said to be higher in women than in men.[1,11,12] According to a broad research on conditions of living of the Japanese People conducted in 2010, 13% of women and 6% of men complained of shoulder-neck pain.[13] Tenna et al. investigated the effects of decrease in breast size on posture and also described an improvement using static stabilometry.[14]

Body posture can be defined as the positioning of the body for a particular time, while the state of slightly engaging the musculoskeletal system in maintaining body balance without generating discomfort can be referred to as ideal posture.[15] Forward head posture (FHP) is the projection of the head in the sagittal plane so that the head is placed anterior to the trunk. It can occur because of anterior shift of the head, lower cervical flexion, or both, and it is also claimed to be associated with an increase in upper cervical extension.[16] It is associated with shortening of the posterior cervical extensor muscles, the upper trapezius, the sternocleidomastoid muscle, and levator scapulae muscle.[17] Thus, forward head posture can also contribute to neck and shoulder pain.[18] Craniovertebral angle (CVA) is one of the common tools used in assessing forward head posture.[19,20]

The CVA is defined as the angle between a straight line that passes through the spinous process of the cervical vertebrae number seven and the line connecting the spinous process of cervical vertebrae number seven with tragus of the ear.[19] Chiu et al., found that roughly 60% of individuals with neck pain had FHP.[21] Another research by Griegel-Morris et al., revealed that 66% of forward head posture was observed in the neck region in a group of healthy participants between the ages of 20 and 50 years.[22] A smaller CVA indicates a greater forward head posture.[23] The average normal value of CVA in a pain free population is about 50^0, any value below 50^0 leads to a form of cervical disorder referred to as forward head posture.[19] Akodu et al., reported differences in the FHP of patients with neck pain while Hanten et al., failed to detect such differences in their own study.[8,24]

Aim

Since literature is scarce in the association between breast hypertrophy, CVA, neck and shoulder pain related disabilities, this study was therefore aimed at determining the association between breast hypertrophy, CVA, neck and shoulder pain related disabilities and selected anthropometrics variables among female undergraduate students of College of Medicine, University of Lagos.

Material and methods

Eighty-nine female undergraduate students with breast hypertrophy participated in this cross sectional analytical survey which was conducted between February and August, 2021. The participants were enrolled from different departments of college of medicine, University of Lagos with the sample size derived from the formula by Cochran[25] where Z= standard normal variate (at 5%), type 1 error ($p< 0.050$) is 1.96, using a prevalence of forward head posture in a group of physiotherapy undergraduates (51.51%)[8.] A purposive sampling technique was adopted for this study. Participants were included in the study if they have breast hypertrophy, and participants that have breast hypertrophy with neck and shoulder pain were excluded from the study.

Before starting the study, permission to conduct the study was obtained from health research and ethics committee of college of Medicine, University of Lagos (CMUL) with approval number (CMUL/HREC/12/19/707), the objectives of the study was explained to the participants and they were assured of confidentiality of their information. Written informed consent was obtained from all the participants after explanation of the study objectives. The participant's demographic variables such as age, sex, current level in the university and department were recorded while the weight, height, Body Mass Index and the participants' breast size and CVA were measured and recorded before the distribution of questionnaires; neck and shoulder pain disability index.

Study gadgets

Digital Camera: A Sony 7.2 Mega pixels DSC 5650, made in China was used to take pictures of the participants.

Corel draw X7 software: this software alongside an HP laptop was used for digitalizing process and calculation of craniovertebral angle for each participant.

Camera tripod stand: A Pawaca 3Pcs Camera DSLR Stand tripod extendable 130cm CAM-002 was used to position the camera.

Plumb line: This is a piece of string that is high enough to accommodate the tallest participant. A small weight is attached to the end of the rope to make it straight and align to the lateral anatomical position. Laptop: HP 455 G5 15.6" LED HD, A9-9420, 8GB DDR4, 256GB SSD, WIN10 Pro.

Plumb line stand: this is made of wood that is 7 feet high with a longitudinal side attached to the top. A rope was attached to the edge of the longitudinal side. A small weight will be attached to the end of the rope to make it align to normal lateral anatomical position.

Breast size assessment

To assess the breast size, this was done by using a tape measure: which was recorded to the nearest 0.1 centimetres and then converted to inches. If band size calculates

to an odd number, the number is rounded up or down because bras are available only in even-numbered sizes. Cup size was then calculated by comparing the size of the band to bust circumference, which is the circumference of the chest around the fullest part of the breasts, commonly taken at the level of the nipples with the subject with or without a bra or wearing a pad-less bra. A bust circumference 1inch greater than band size matches with an "A" cup, 2 inches matches with a "B" cup, 3 inches to a "C" cup, and so forth. For example, a woman who has a bust circumference of 39 inches with a band size of 36 would fit a size 36C bra by this formula (39–36=3=C cup).[26]

Assessment of craniovertebral angle

A 2 meters plumb line was placed away from the participant with the tripod stand and camera placed behind it for the assessment of craniovertebral angle, the plumb line was to fall in front or through the tragus of the ear. The participants were instructed to expose their ear, the neck to its base and the shoulder. Adhesive tape was used to mark the lateral surface at the tragus of the ear, spinous process of the seventh cervical vertebrae and the acromion process of the shoulder contrasting the skin. Participants' photographs were taken and imported to Corel draw X7 software version to measure the CVA.[19,20]

Administration of questionnaire

The questionnaires (neck pain disability index and shoulder pain disability index) were self-administered by personally distributing to female students with large breast sizes of college of medicine, University of Lagos after the sizes of the breast was determined using the procedure of Niddam et al. and they were collected afterwards after each participant had fully completed the questionnaire.[26]

Description of questionnaires

Neck pain and disability scale: The Neck Pain and Disability scale (NPAD) includes 20 item questionnaire that was developed for neck pain patients.[27] The questionnaire measures problems with neck movements, neck pain intensity, effect of neck pain on emotion and cognition, and the level of meddling with activities of living. Patients are required to mark along a 10 cm visual analog scale (VAS). The ranges of score of each item is from 0-5 while the total score is a total of the item scores ranging from 0 (no pain) – 100 (maximal pain). NPAD requires less than 5 minutes to complete.[28] It has been found to be a valid, reliable tool available in other languages for assessing disability in chronic neck pain patients. Data has shown that Neck Pain and Disability Scale has better construct validity with Cronbach's alpha of 0.93. Items-total correlations range from 0.45 to 0.73.[29]

Shoulder pain and disability index (SPADI): is a self-report questionnaire for measuring pain and disability of the shoulder. It is a 13 itemed index made up of two subscales: pain (5 items) and disability (8 items); each subscale is summed and transformed to a score out of 100. A mean is taken of the two subscales to give a total score out of 100, higher score indicating greater impairment or disability. For a non-specific population: Test-retest reliability of SPADI total combined subscale scoring ranging from 0.64 to 0.66.[30] Approximately 95% of the pairs of observation did not differ by more than 17 points.[31] ICC for the disability subscale ranged from 0.57 to 0.84.[32] Correlation ranged from -0.55 to -0.80.[30]

Data analysis

Data were analyzed using the Statistical package for Social Sciences (SPSS) windows version 22 (IBM, New York city, New York, USA) and was summarized using descriptive statistics of mean, standard deviation, frequencies and percentages. Inferential statistics of Chi-square was used to find the association between variables. Level of significance was set at $p \leq 0.05$.

Result

Eighty-nine copies of questionnaire were distributed and returned. This gave a response rate of 100%. Therefore, eighty-nine copies of the questionnaire were valid for analysis.

In Table 1 more than half 54 (60.7%) of the participants were within the age range of 21-22 years with mean age of 21.45±1.29 years. All the participants were females.

Table 1. Demographic characteristics of the participants

Variable	Frequency (n=89)	Percentage (%)
Age (years)		
19 – 20	20	22.5
21 – 22	54	60.7
23 – 24	12	13.4
>24	3	3.4
Mean age = 21.45±1.29		
Height (m)		
1.50 – 1.60	22	24.7
1.61 – 1.70	50	56.2
1.71 – 1.80	17	19.1
Mean height = 1.65±0.08		
Weight (kg)		
41 – 62	37	41.6
63 – 82	36	40.4
83 – 102	10	11.2
>102	6	6.7
Mean weight = 68.30±16.39		
Body mass index (kg/m²)		
<18.5	4	4.5
18.5 – 24.9	46	51.7
25 – 29.9	24	27
>=30	15	16.9
Mean BMI = 25.11±5.95		

Thirty-six (40.4%) of the participants' weight was within 63-82kg, and 6 (6.7%) of the participants' weight was greater than 102 kg. The mean weight of the participants was 68.30±16.39 kg. Majority, 50 (56.2%) of the participants had height between 1.61-1.70 m with mean height of 1.65±0.08. Twenty-four (27%) of the participants were overweight with a BMI of 25-29.9 kg/m^2 with a mean BMI of 25.11±5.95 kg/m^2.

Table 2. Prevalence of forward head posture and breast sizes among female undergraduate students of College of Medicine University of Lagos (CMUL)

Variables	Frequency (n=89)	Percent (%)
CVA		
<50	43	48.3
>=50	46	51.7
Total	89	100
Mean CVA= 50.06±5.98		
Cup-size*		
D	27	30.3
DD/E	28	31.3
DDD/F	26	29.2
G	7	7.9
I	1	1.1
Total	89	100

*cup-size (D, DD, DDD, G, I) – breast size classification for large breast sizes in this study, CVA – craniovertebral angle

Prevalence of forward head posture, breast sizes and pain related disabilities of the neck and shoulder among female undergraduate students of College of Medicine University of Lagos

Table 2 shows that prevalence of forward head posture (craniovertebral angle less than 50^0) was 43(48.3%) with mean CVA of 50.06±5.98^0 among the participants. Concerning breast hypertrophy, twenty-eight (31.3%) participants had a "DD" cup-size. Twenty-six (29.2%) participants had a cup-size "DDD" cup-size.

Table 3 shows that sixty-five (73.00%) participants had pain related disability of the neck while 10 (11.20%) participants had pain related disability of the shoulder.

Table 3. The prevalence of pain related disabilities of the neck and shoulder of female undergraduate students with breast hypertrophy in CMUL

Variable	Frequency (n=89)	Percentage (%)
NPAD*		
No pain	24	27
Pain	65	73
Total	89	100.
SPADI*		
No disability	79	88.8
Disability	10	11.2
Total	89	100

*NPAD – neck pain and disability, SPADI – shoulder pain and disability index

Association between breast hypertrophy, forward head posture (CVA less than 50^0), neck - shoulder pain related disabilities, age and selected anthropometric variables

Table 4 shows that there was no significant association between breast hypertrophy and forward head posture (p=0.065), neck pain related disabilities (p=0.545) and shoulder pain related disabilities (p=0.854).

Table 5 shows that there was no significant association between breast hypertrophy, age (p=0.243), height (p=0.243) and BMI (p=0.255). But there was significant association between the weight and breast hypertrophy (p=0.016).

Table 4. Association between breast hypertrophy, forward head posture, neck and shoulder pain related disabilities*

Variables	Cup-size					χ^2	p-value
	D	DD	DDD	G	I		
CVA							
<50	7 (16.3%)	15 (34.9%)	16 (37.2%)	4 (9.3%)	1 (2.3%)		
>=50	20 (43.5%)	13 (28.3%)	10 (21.7%)	3 (6.5%)	0 (0%)	8.84	0.065
NPAD							
No	10 (41.7%)	8 (33.3%)	4 (16.7%)	1 (4.2%)	1 (4.2%)		
Mild	15 (28.3%)	17 (32.1%)	17 (32.1%)	4 (7.5%)	0 (0%)	10.81	0.545
Moderate	1 (12.5%)	2 (25%)	3 (37.5%)	2 (25%)	0 (0%)		
Severe	1 (25%)	1 (25%)	2 (50%)	0 (0%)	0 (0%)		
SPADI							
No	24 (30.4%)	24 (30.4%)	23 (29.1%)	7(8.9%)	1 (1.3%)		
Mild	3 (33.3%)	4 (44.4%)	2 (22.2%)	0 (0%)	0 (0%)		
Moderate	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	4.04	0.854
Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		

*NPAD – neck pain and disability, SPADI – shoulder pain and disability index, NO – no pain related disability, MILD – mild pain related disability, MODERATE – moderate pain related disability, SEVERE – severe pain related disability, CVA – craniovertebral angle, χ^2 – Chi-square value, cup-size (D, DD, DDD, G, I) – breast size classification for large breast sizes in this study

Association between forward head posture, neck-shoulder pain related disabilities, age and selected anthropometric variables

In table 6 there was no significant association between forward head posture, neck pain related disabilities (p=0.434) and shoulder pain related disability (p=0.384).

While table 7 shows that there was no significant association between forward head posture, age (p=0.250), height (p=0.650) and body mass index (p=0.055) but a significant association exist between the weight and forward head posture (p=0.027).

Discussion

The prevalence of forward head posture (FHP) (craniovertebral angle (CVA) less than 50^0) was observed to be 48.3%, among female undergraduates with breast hypertrophy. The mean CVA was 50.06 ± 5.98^0. This corresponds to the findings of the study by Akodu et al., who reported the prevalence of FHP in female undergraduates to be 51.5% and the mean value of craniovertebral angle was 51.83 ± 5.7^0.[8] The study done by Sutantar et al., showed the prevalence of FHP among their study participants to be 73%.[33] Mamania and Anap, revealed a prevalence of 70% of FHP.[34] In a study by Abrish et al., 3.1% of the participants had severe FHP, 40.6% of

Table 5. Association between breast hypertrophy, age and selected anthropometric values*

Variables	Cup-size					χ^2	p-value
	D	DD	DDD	G	I		
Age (years)							
19-20	5 (25%)	5 (25%)	6 (30%)	4 (20%)	0 (0%)		
21-22	17 (31.5%)	20 (37%)	13 (24.1%)	3 (5.6%)	1 (1.9%)	15.178	0.232
23-24	5 (41.7%)	3 (25%)	4 (33.3%)	0 (0%)	0 (0%)		
>24	0 (0%)	0 (0%)	3 (100%)	0 (0%)	0 (0%)		
Height (m)							
1.50 -1.60	2 (50%)	8 (36.4%)	6 (27.3%)	0 (0%)	0 (0%)		
1.61 -1.70	13 (26%)	14 (28%)	18 (36%)	5 (10%)	0 (0%)	10.321	0.243
1.71 -1.80	6 (35.3%)	6 (35.3%)	2 (11.8%)	2 (11.8%)	1 (5.9%)		
Weight (kg)							
41 -62	13 (35.1%)	8 (21.6%)	14 (37.8%)	2 (5.4%)	0 (0%)		
63 – 82	13 (36.1%)	13 (36.1%)	8 (22.2%)	2 (5.6%)	0 (0%)	24.828	0.016
83 – 102	1 (10%)	4 (40%)	3 (30%)	2 (20%)	0 (0%)		
>102	0 (0%)	3 (50%)	1 (16.7%)	1 (16.7%)	1 (16.7%)		
BMI (kg/m²)							
<18.5	2 (50%)	0 (0%)	1 (25%)	1 (25%)	0 (0%)		
18.5 - 24.9	18 (39.1%)	12 (26.1%)	14 (30.4%)	2 (4.3%)	0 (0%)	14.763	0.255
25 – 29.9	5 (20.8%)	10 (41.7%)	6 (25%)	3 (12.5%)	0 (0%)		
>=30	2 (13.3%)	6 (40%)	5 (33.3%)	1 (6.7%)	1 (6.7%)		

*BMI – body mass index, χ^2 – Chi-square value, CUP-SIZE (D, DD, DDD, G, I) – breast size classification for large breast sizes in this study

Table 6. Association between forward head posture and neck-shoulder pain related disabilities*

CVA				
Variable	**<50**	**>=50**	χ^2	**p-value**
NPAD				
No	10 (41.7%)	14 (58.3%)		
Mild	25 (47.2%)	28 (52.8%)	2.738	0.434
Moderate	6 (75%)	2 (25%)		
Severe	2 (50%)	2 (50%)		
SPADI				
No	40 (50.6%)	39 (49.4%)		
Mild	3 (33.3%)	6 (66.7%)	1.914	0.384
Moderate	0 (0%)	1 (100%)		
Severe	0 (0%)	0 (0%)		

*NPAD – neck pain and disability, SPADI – Shoulder Pain and Disability Index, NO – no pain related disability, MILD – mild pain related disability, MODERATE – moderate pain related disability, SEVERE – severe pain related disability, CVA – craniovertebral angle, χ^2 – Chi-square value

the participants had moderate FHP, 50% had mild FHP while 6.3% had normal craniovertebral angle.[35] The lower prevalence in this study could be as a result of the involvement of only female participants with breast hypertrophy.

Table 7. Association between forward head posture, age and selected anthropometric variables*

CVA				
Variables	**<50**	**>=50**	**χ^2**	**p-value**
Age (years)				
19 – 20	11 (55%)	9 (45%)		
21 – 22	24 (44.4%)	30 (55.6%)	4.104	0.25
23 – 24	5 (41.7%)	7 (58.3%)		
>24	3 (100%)	0 (0%)		
Height (m)				
1.50 – 1.6	12 (54.5%)	10 (45.5%)		
1.61 – 1.7	22 (44%)	28 (56%)	0.86	0.65
1.71 – 1.8	9 (52.9%)	8 (47.1%)		
Weight (kg)				
41 – 62	17 (45.9%)	20 (54.1%)		
63 – 82	13 (36.1%)	23 (63.9%)	9.197	0.027
83 – 102	8 (80%)	2 (20%)		
>102	5 (83.3%)	1 (16.7%)		
BMI (kg/m^2)				
<18.5	2 (50%)	2 (50%)		
18.5 – 24.9	20 (43.5%)	26 (56.5%)	7.59	0.055
25 – 29.9	9 (37.5%)	15 (62.5%)		
>=30	12 (80%)	3 (20%)		

*CVA – craniovertebral angle, BMI – body mass index, χ^2 – Chi-square value

Seventy-three (73) percent of the participants in this study presented with pain related disabilities of the neck but in a study carried out by Gharib and Hamid the prevalence of mechanical neck pain among female students was 54%.[36] Findings in the study carried out by Chan et al., on the prevalence of neck pain and associated risk factors among undergraduate students showed the point prevalence of neck pain as 17.5%.[37] In a study done by Fahad and Sana, the results showed a total of 51.8% students had neck pain ranging from mild to severe.[38] The high prevalence rate of pain related disability of the neck in this study could be as a result of large breast sizes that all the participants present with.

The prevalence of shoulder pain related disabilities was observed to be 11.2% but in a study carried out by Luime et al., the results showed a point prevalence of 6.9–26% for shoulder pain.[14] The results of these two studies confirms that shoulder pain related disabilities may not really be a common musculoskeletal disorder that affect individuals with breast hypertrophy.

In this study, there was no significant association between forward head posture and neck pain related disabilities. This means that having forward head posture may not necessarily predispose one to having neck pain and its related disabilities which corresponds to a study carried out by Martínez-Merinero et al., which reported that there was no association between forward head posture, neck pain, disability, and headache.[39] In a study carried out by Mahmoud et al., it was shown that forward head posture was significantly correlated with neck pain in adults and older adults which also concurs with the report of Akodu et al., but there was no association found between forward head posture and neck pain in adolescents.[8,40] Abrish et al., asserted that 50% of the students with complaint of neck pain had slight postural deformity having mild forward head posture (FHP) and fewer students, 3.1% had severe postural deformity.[35] Raoufi et al., revealed a significant reverse correlation between CV angle and neck pain.[41]

In this study, the cup sizes shows that all the participants in this study have breast hypertrophy. This did not corresponds to the result in the study by Dundas et al,[42] who reported a percentage of 48% on measurement and categorization of breast size for radiation therapy who had a cup size of D or E. In the study by Odebiyi et al., the results reported a prevalence of 75% for cup size of D and above.[43]

Literature is scarce in the association between breast hypertrophy and CVA, but in this study the results showed that there was no significant association between breast hypertrophy and craniovertebral angle. Szeto et al., and Moore stated that maintaining the head forward for long periods of time may cause musculoskeletal disorders such as 'upper crossed syndrome, which involves having reduced lordosis of the lower cervical, in conjunction with kyphosis of the upper thoracic vertebrae.[44,45] In a research by Findikcioglu et al., it was reported that women with breast cups size D and above tends to have greater curvatures of the spine than women with smaller breast sizes.[5]

This study shows that there was no significant association between breast hypertrophy and neck and shoulder pain related disabilities. This result corresponds to the findings of the study done by Myint et al., on relationship between brassiere cup size and shoulder-neck pain in women, which showed that there was no significant relationship between shoulder-neck pain and breast size.[46] In a study carried out by Coltman et al., the results showed young women with breast hypertrophy with nipple-to-nipple distance have a higher upper torso musculoskeletal discomfort.[47]

Age and forward head posture were not associated in this study. The reason could be due to the limited margin in the age range of the participants in this study. In a study by Nemmers et al., the data showed an age-related effect with the older women showing a more severe FHP than those that are much younger.[48] Kocur et al., revealed that with more advancement in age, subjects had smaller craniovertebral angle values which in-

dicates increased prevalence of forward heads posture as age increases.[49]

In this study age and breast hypertrophy were associated. This means increase or decrease in age has no effect on the size of the breast and this result corresponds to the findings in a study by Brown et al., the results showed that there was no correlation between age and breast hypertrophy.[50]

There was a significant association between weight and breast hypertrophy of the participants but no significant association between body mass index and breast hypertrophy of the participants in this study. In a study carried out by Brown et al., it was reported that body mass was strongly correlated with breast mass which indicates that heavier women had larger breasts.[50] The results of the study carried out by Coltman et al., showed that breast size was significantly influenced by BMI, with the breast size of overweight and obese women 2 to 3 times bigger than the women with normal BMIs.[51] Steele et al., buttressed that the participants classified as obese had significantly larger breasts sizes.[52] This corresponds with report of this study.

There was a significant association between weight and craniovertebral angle of the participants, but no significant association between BMI and craniovertebral angle of the participants in this study. This means that the weight of an individual with breast hypertrophy has an influence on the craniovertebral angle but body mass index has no specific influence on the craniovertebral angle. A study carried out by Shaghayegh et al.,[53] revealed a negative correlation between the BMI and CVA.

Conclusion

Based on the results of this study, the following conclusions were made: There was high prevalence of neck pain related disabilities, forward head posture among participants with breast hypertrophy in this study, weight as an influence on the prevalence of forward head posture and breast hypertrophy.

Declarations

Funding

This research did not receive any specific grant from funding agencies.

Author contributions

Conceptualization, A.K.; Methodology, A.K. and T.G.; Validation, A.K., T.G. and A.O.; Formal Analysis, A.K.; Investigation, T.G.; Resources, A.K. and T.G.; Data Curation, A.K. and T.G.; Writing – Original Draft Preparation, A.K.; Writing – Review & Editing, A.K., T.G. and A.O.; Visualization, T.G. and A.O.; Supervision, A.K.; Project Administration, A.K. and T.G.; Funding Acquisition, T.G.

Conflicts of interest

The authors declared that there are no conflict of interests.

Data availability

The data used during the current study are available from the corresponding author on request.

Ethics approval

Permission to conduct the study was obtained from health research and ethics committee of college of Medicine, University of Lagos (CMUL) with approval number (CMUL/HREC/12/19/707).

References

1. Greenbaum AR, Heslop T, Morris J, Dunn KW. An investigation of the suitability of bra fit in women referred for reduction mammaplasty. *Br J Plast Surg.* 2003;56:230-236.
2. Gefen A, Dilmoney B. Mechanics of the normal woman's breast. *Technol Health Care*. 2007;15:259-271.
3. Avsar DK, Avgit AC, Benlier E, Top H, Taskinalp O. Anthropometric breast measurement: a study of 385 Turkish female students. *Aesthet Surg J.* 2010;30:44-50.
4. Scheurnhammer ES, Tworoger SS, Eliassen AH, et al. Body shape throughout life and correlations with IGFs and GH. *Endocr Relat Cancer.* 2007;14:721-732.
5. Findikcioglu K, Findikcioglu F, Bulam H, Sezgin B, Ozmen S. The impact of breast reduction surgery on the vertebral column. *Ann Plast Surg.* 2013;70:639-642.
6. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J.* 2006;15(6):834-848.
7. Palacios-Ceña D, Alonso-Blanco C, Hernández-Barrera V, Carrasco-Garrido P, Jiménez-García, R, Fernández-de-las-Peñas C. Prevalence of neck and low back pain in community-dwelling adults in Spain: an updated population-based national study (2009/10–2011/12). *Eur Spine J.* 2014;24(3): 482-492.
8. Akodu AK, Odunsi FA, Giwa SO. Effects of Neck Stabilization Exercise on Pain, Disability, Craniovertebral Angle and Psychological Status in Patient with Non-Specific Chronic Neck Pain. JRCRS. 2018;6(1):10-15.
9. Guzman J, Hurwitz EL, Carroll LJ, et al. A new conceptual model of neck pain. Linking onset, course, and care: the Bone and Joint Decade 2000-2010 Task Force on Neck Pain and Its Associated Disorders. *Spine*. 2008;33(4S):14-23.
10. Ariens GA, Van-Mechelen W, Bongers P, Bouter LM, VanderWal G. Psychosocial risk factors for neck pain: a systematic review. *Am J Ind Med.* 2001;39:180-193.
11. Linsell L, Dawson J, Zondervan K, et al. Prevalence and incidence of adults consulting for shoulder conditions in UK primary care; patterns of diagnosis and referral. *Rheumatology (Oxford).* 2006;45:215-221.
12. Luime J, Koes B, Hendriksen I, et al. Prevalence and incidence of shoulder pain in the general population; a systematic review. *Scand J Rheumatol.* 2004;33(2):73-81.

13. Nakamura M, Toyama Y, Nishiwaki Y, Ushida, T. Prevalence and characteristics of chronic musculoskeletal pain in Japan. *J Orthoped Sci.* 2011;16(4):424-432.
14. Tenna S, Brunetti B, Trivelli M, Salvinelli F, Persichetti P. Postural Variations after Breast Reduction Introduction of a New Technique to Achieve an Objective Analysis. *Ann Plast Surg.* 2011;68:261-264.
15. McEvoy MP, Grimmer K. Reliability of upright posture measurements in primary school children. *BMC Musculoskelet Disord*. 2005;6:35.
16. Silva AG, Punt TD, Sharples P, Vilas-Boas JP, Johnson MI. Head posture and neck pain of chronic non-traumatic origin: a comparison between patients and pain-free persons. *Arch Phys Med Rehabil.* 2009;90:669-674.
17. Lynch SS, Thigpen C A, Mihalik JP, Prentice WE, Padua D. The effects of an exercise intervention on forward head and rounded shoulder postures in elite swimmers. *Br J Sports Med*. 2010; 44(5):376-381.
18. Fares J, Fares Y, Fares M. Musculoskeletal neck pain in children and adolescents: risk factors and complications. *Surg Neurol Int.* 2017;8(1):72.
19. Yip CHT, Chiu TTW, Poon ATK. The relationship between head posture and severity and disability of patients with neck pain. *Man Ther*. 2008;13(2):148-154.
20. Akodu A, Akinfeleye A, Atanda A. Work related musculoskeletal disorders of the upper extremity with reference to working posture of secretaries. *S Afr J Physiother*. 2015;45(3):16-22.
21. Chiu TTW, Ku WY, Lee MH, et al. A study on the prevalence of and risk factors for neck pain among university academic staff in Hong Kong. *J Occup Ther.* 2002;12(2):77-91.
22. Griegel-Morris P, Larson K, Mueller-Klaus K, Oatis CA. Incidence of common postural abnormalities in the cervical, shoulder and thoracic regions and their association with pain in two age groups of healthy participants. *Phys Ther.* 1992;72:425-431.
23. Lau KT, Cheung KY, Chan KB, Chan MH, Lo KY, Chiu TTW. Relationships between sagittal postures of thoracic and cervical spine, presence of neck pain, neck pain severity and disability. *Man Ther*. 2010;15:457-462.
24. Hanten WP, Olson Sl, Russell JL, Lucio RM, Cambel AH. Total head excursion and resting head posture: normal and patients' comparisons. *Arch Phys Med Rehabil.* 2000;81:62-66.
25. Cochrane Cochran WG. *Sampling technique*. 1977; 3rd edition. John Wiley and Sons.
26. Niddam J, Lange F, Meningaud J, Bosc R. Complexity of bra measurement system: implications in plastic surgery. *Eur J Plast Surg.* 2014;37(11):631-632.
27. Scherer M, Blozik E, Himmel W, Laptinskaya D, Kochen MM, Herrmann-Lingen C. Psychometric properties of a German version of the neck pain and disability scale. *Spine*. 2008;17:922-929.
28. Blozik E, Himmel W, Kochen MM, Herrmann-Lingen C, Scherer M. Sensitivity to change of the Neck Pain and Disability Scale. *Spine*. 2011;20:882-889.
29. Jorritsma W, DeVries GE, Dijkstra PU, Dijkstra PU, Reneman MF. Neck Pain and Disability Scale and Neck Disability Index: reproducibility of Dutch language versions. *Eur Spine J.* 2010;19(10): 1695-1701.
30. Roach KE, Budiman-Mak E, Songrsiridej N, Lertratanakul Y. Development of a shoulder pain disability index. *AHPA*. 1991;4:143-149.
31. Tveitå EK, Ekeberg OM, Juel NG, Bautz-Holter E. Responsiveness of the Shoulder Pain and Disability Index in patients with adhesive capsulitis. *BMC Musculoskelet Disord*. 2008;9:161.
32. Bot S, Terwee C, Vanderwindt D, Bouter L, Dekker J, De--Vet H. Clinimetric evaluation of shoulder disability questionnaires. A systematic review of the literature. *Ann Rheum Dis.* 2004;63(4):335-341.
33. Sutantar S, Kavita K, Smriti J. Prevalence of forward head posture and its impact on the activity of daily living among students of Adesh University – A cross-sectional study. *AUJMSR*. 2020;2:1-4.
34. Mamania J, Anap D. Prevalence of Forward Head Posture amongst Physiotherapy Students: A Cross-sectional Study. IJHSR 2019;1(4):125-127.
35. Abrish HA, Aslam M, Ashraf T, Arshad NM. Evaluation of the Forward Head Posture, its association with Neck Pain & Quality of life of Female DPT Students. *JRCRS*. 2016;4(2):59-64.
36. Gharib NM, Hamid NS. Prevalence of Mechanical Neck Pain in Taif University Female Students: A Survey Study. *J Am Sci.* 2013;9:347-354.
37. Chan LLY, Wong AYL, Wang MH, Cheung K, Samartzis D. The prevalence of neck pain and associated risk factors among undergraduate students: A large-scale cross-sectional study. *Int J Ind Ergon.* 2020;76,102934.
38. Fahad T, Sana S. Prevalence of Neck Pain among Doctor of Physical therapy Students of University of Lahore due to bad posture. *Rawal Med J*. 2017;42:172-175.
39. Martínez-Merinero P, Susana N, Alexander A, Fernandez-Matias R, Pecos-Martin D, Gallego-Izquierdo T. Relationship between Forward Head Posture and Tissue Mechanosensitivity: A Cross-Sectional Study. *J Clin Med.* 2020;9:634.
40. Mahmoud NF, Hassan KA, Abdelmajeed SF, Moustafa IM, Silva AG. The Relationship between Forward Head Posture and Neck Pain: a Systematic Review and Meta-Analysis. *Curr Rev Musculoskelet Med*. 2019;12(4):562-577.
41. Raoofi Z, Sarrafzadeh J, Emrani A, Ghorbanpour A. Relationship between Forward Head Posture and Neck Pain as Well as Disability. *J Clin Physiother Res.* 2019;4(1):26-30.
42. Dundas K, Atyeo J, Cox J. What is a large breast? Measuring and categorizing breast size for tangential breast radiation therapy. *Australas Radiol*. 2007;51(6):589–593.
43. Odebiyi DO, Aweto HA, Gbadebo OA, et al. Association between bra use and pectoral girdle myalgia in Nigeria: a cross-sectional study. *AJM*. 2015;9(4):181-188.

44. Szeto GP, Straker L, Raine S. A field comparison of neck and shoulder postures in symptomatic and asymptomatic office workers. *Appl Ergon*. 2002;33:75-84.
45. Moore MK. Upper crossed syndrome and its relationship to cervicogenic headache. *J Manipulative* Physiol Ther. 2004;27:414-420.
46. Myint Oo, Zhuo W, Toshihiko S, Yuichi K. Relationship between Brassiere Cup Size and Shoulder-Neck Pain in Women. *Open J Orthop*. 2012;6(1):140-142.
47. Coltman CE, Steele JR, McGhee DE. Can breast characteristics predict upper torso musculoskeletal pain? *Clin Biomech (Bristol, Avon)*. 2018;53:46–53.
48. Nemmers TM, Miller JW, Hartman MD. Variability of the Forward Head Posture in Healthy Community-dwelling Older Women. *J Geriatr Phys Ther.* 2009;32(1):10-14.
49. Kocur P, Tomczak M, Wiernicka M, Goliwąs M, Lewandowski J, Łochyński D. Relationship between age, BMI, head posture and superficial neck muscle stiffness and elasticity in adult women. *Sci Rep*. 2019;9(1):8515.
50. Brown N, White J, Milligan A, et al. The Relationship between Breast Size and Anthropometric Characteristics. *Am J Hum Biol.* 2012;24(2):158-164.
51. Coltman CE, Steele JR, McGhee DE. Breast volume is affected by body mass index but not age. *Ergonomics.* 2017;60:1576-1585.
52. Steele JR, Coltman CE, McGhee DE. Effects of obesity on breast size, thoracic spine structure and function, upper torso musculoskeletal pain and physical activity in women. *J Sport Health Sci.* 2019;00:1-9.
53. Shagaye S, Fard B, Ahmadi A, Maroufi N, Sarrafzadeh J. Evaluation of forward head posture in sitting and standing positions. *Eur Spine J.* 2016;25(11):3577–3582.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.14

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 102–108

REVIEW PAPER

Klaudia Dynarowicz, David Aebisher

Nanomedicine – a review

Department of Photomedicine and Physical Chemistry, Medical College of The University of Rzeszów, University of Rzeszów, Poland

ABSTRACT

Introduction and aim. Nanomedicine is a discipline of technology and science, the potential of which has recently fascinated scientists in the fields of physics, biotechnology, chemistry and medicine. This department deals with everything on the nano scale, i.e. on the level of individual atoms and molecules.

This work presents the nano scale, i.e. on the level of individual atoms and molecules. Nanotechnology is currently one of the most popular and dynamically developing fields, not only in electronics, but above all in pharmacy and medicine.

Material and methods. In this article a narrative review regarding nanomedicine.

Analysis of the literature. The desire to summarize information about nanomedicine application of singlet oxygen is presented. Nanotechnology is a discipline of technology and science, the potential of which has recently fascinated scientists in the fields of physics, biotechnology, chemistry and medicine.

Conclusion. The use of nanostructures is currently very efficient. The areas in which the potential of nanoparticles is constantly researched and confirmed by numerous articles are: radio- and chemotherapy, cancer diagnostics and imaging medicine (MRI and fluorescence imaging).

Keywords. nanomedicine, nanoscale, nanostructure

Introduction

In order to be able to explain the concept of nanostructures and to present the application of nanotechnology in various fields of science, the prefix nano should be specified and explained. Nano is a unit of measure prefix with the symbol n, which stands for a multiplier of 10^{-9} (one billionth of a part).[1] The name of the nano prefix comes from the Greek language (Greek: nanos, meaning dwarf). It is therefore a very small size that cannot be observed with the human eye.[2]

Nanotechnology is a relatively young technique, the origins of which date back to 1959, when Richard Feynman, an American physicist and Nobel Prize winner in physics, gave a lecture at the American Physical Society congress, entitled There's Plenty of Room at the Bottom.[2] Feynman presented the concept of miniaturization and the possibilities of using technology in the nanometer range. In the following years, numerous studies in the field of semiconductor and lithographic techniques made it possible to distinguish another physical field under the name of nanotechnology. The term was first used by a scientist, Professor Norio Taniguchi from the University of Tokyo during a scientific conference.[3] Since then, this field has been constantly evolving, and the application of nanostructures in electronics, physics and biology, chemistry and medicine is constantly being researched and multiplied.

Aim

The aim of this work is to present the review of nanomedicine.

Corresponding author: Klaudia Dynarowicz, email: kdynarowicz@ur.edu.pl

Received: 30.12.2021 / Revised: 10.02.2022 / Accepted: 13.02.2022 / Published: 30.03.2022

Dynarowicz K, Aebisher D. *Nanomedicine – a review.* Eur J Clin Exp Med. 2022;20(1):102–108. doi: 10.15584/ejcem.2022.1.14

Material and methods

This article is a review done in regards to discuss the role of nanomedicine in current treatment and diagnostics.

Analysis of literature

Currently, there is a division of nanostructures into naturally occurring in nature and made by human. Natural nanoparticles result from the decomposition of plant or animal remains, as a result of the erosion of geological materials, volcanic fumes or as a result of the combustion of mineral fuels.[4] Another type of classification of nanostructures is their chemical composition. This division distinguishes between organic and inorganic nanoparticles. The last classification criterion is division due to the mutual relation of dimensions and their number. According to this criterion, the following structures can be distinguished: three-dimensional, two-dimensional, one-dimensional and zero-dimensional.[5] Table 1 shows the classification of nanostructures.

Table 1. The classification of nanostructures based on three criteria

Due to the way it is received	Due to the chemical composition	Due to the size
Natural	Organic	Three-dimensional (3D)
Human-mad	Inorganic	Two-dimensional (2D)
		One-dimensional (1D)
		Zero-dimensional (0D)

Organic nanostructures include: fullerenes, viruses, dendrimers and carbon nanotubes. On the other hand, inorganic nanostructures are compounds such as: zirconium oxide (ZrO_2), calcium, silver, gold or platinum.[6] Due to the last point of the criterion, the following structures can be distinguished:

- 3D (these are materials made of nanometer-sized monoblocks), fullerenes, colloidal particles, quasi-crystals, nanoporous silicon or semiconductor quantum dots;
- 2D: nanofibers, carbon nanotubes, magnetic and metallic nanowires;
- 1D: nano-layer materials, aluminum nano-dust, nano-grained surface layers or semiconductor quantum studs;
- 0D (these are structures having nanometer dimensions in three directions), an example of which is a quantum dot.

Methods of obtaining nanostructures

Thanks to the research carried out in the field of solid state physics, it is possible to obtain nanostructures by various methods, adapting a given technique to the scope and method of intended use of the compounds produced. The most popular techniques are the "top down" and "bottom up" methods.[7] The first of the top-down methods means obtaining structures by grinding, dividing or disintegrating macroscopic materials. This technique includes: high-energy grinding, lithographic process and standard material processing. High-Energy Ball-Milling HEBM is a common method of obtaining nanomaterials. The starting material is a pre-powdered alloy (<100μm) with a specific chemical composition and a specific crystallographic structure, in contrast to the mechanical synthesis process where high purity metal powders are used. During the process, stresses are induced in the material. After several dozen hours, amorphous material is obtained. The applied heat treatment (crystallization) causes the return to the crystal structure, the so-called output. In turn, the lithographic process is quite commonly used in the electronics industry. It is used mainly for the production of integrated circuits and transistors with a silicon substrate.[8] This technique consists of several steps. The first is to apply a protective and photosensitive layer to the surface of the substrate. In the second step, a pattern in the shape of a negative or a positive of the structure to be created is applied to the protective layer. Then the whole is irradiated and etched. The etching agent acts on places not covered with a protective layer. In this way, the desired layer structure is obtained. In turn, the "bottom up" method is characterized by the formation of nanoparticles from individual atoms, i.e. the creation of structures from scratch. This technique includes methods such as vapor deposition, plasma assisted deposition, and molecular beam epitaxy. Physical vapor deposition (PVD) uses the spraying of solid materials (the so-called target), from which the layer is to be made, eg by means of an electron beam. A properly prepared substrate (the so-called substrate) with a strictly controlled temperature is placed near the sprayed material. The sprayed material settles slowly on the substrate. The thickness of the obtained layer and its structure depend on the deposition time, spray rate and the temperature of the substrate, as well as on the composition of the diluted gas atmosphere. On the other hand, the method of applying thin layers with the use of plasma (Plasma Assisted Chemical Vapor Deposition) enables the deposition of thin layers on electrically conductive and non-conductive materials, using radio frequency and low frequency current discharges. It aims to create hard surface layers and layers with special surface and volume properties. Generally, in this process, chemical reactions take place under the conditions of electrical activation of the gaseous environment. The last example is the Molecular Beam Epitaxy (MBE), which consists in depositing thin semiconductor layers from molecular (atomic) beams in high vacuum (<10-7 Pa). Thanks to the use of various types of techniques, it is possible to obtain the desired nanostructures depending on the destination and application direction.

Biological and medical use of nanostructures

As already mentioned, the potential of nanostructures is enormous. More and more research is being created, and thus scientific publications about the potential and explored possibilities of nanoparticles not only in industry, electronics and physics, but also in the field of chemistry and biology.[9] The term nanomedicine characterizes all branches of medicine in which research and diagnostic activities with a nanometer range are used. It covers such areas as: pharmacy, surgery, diagnostics, oncology and many others.

Nanopharmacology

The main goals of nanopharmacology are target therapy (TT) and the controlled drug delivery system (DDS). The use of nanoparticles in pharmacology enables precise transport of the drug in the appropriate concentration to the neoplastic site, without disturbing the structure of normal (healthy) cells.[10] Currently, there are several types of nanoparticles that enable the transport of various types of drugs. These include: polymer and magnetic nanoparticles, liposomes, polymeric micelles, dendrimers and nanotubes.

Polymer nanoparticles are stable, colloidal structures in the form of nanospheres and nanocapsules. They can come from synthetic and natural polymers. Polymers (from the Greek polymeres - multi-part, made of many parts) are chemical compounds containing a large number of repeating structural elements, called mers. Synthetic polymers include: polycaprolactone, polyacrylamide and poly (methyl methacrylate).[11] On the other hand, natural polymers are mainly gelatin, heparin, chitosan and albumin. Nanoparticles made of polymers improve the efficiency of transporting currently administered drugs or proteins to specific cells, which means that the compounds are no longer so toxic and harmful to healthy, i.e. uninfected cells. The size of the nanostructures allows for increased drug penetration through cell membranes and thus increased stability, which means that the administered drug remains much longer in the patient's circulatory system. The most popular materials in nanopharmacology are biodegradable polymers (CS, PLA, gelatin, HMPA (N- (2-hydroxypropyl) methacrylamide). Table 2 presents a list of selected biodegradable polymers along with the labeling of the product that is formed as a result of the decomposition of a given polymer. due to the fact that these polymers are completely degraded in the human body, and thus can be easily excreted, and they do not stimulate the immune system.

According to numerous articles, the drug, created by combining albumin with paclitaxel (albumin-bound paclitaxel) – in 2005, abraxane was approved for the treatment of breast cancer in the United States. The paclitaxel capsule in albumin molecules makes it unnecessary to administer toxic solvents of this type of drug.[12] Moreover, albumin facilitates the penetration of substances through endothelial cells by means of the albumin gp-60 receptor. There is ongoing research into the use of abraxane to treat other types of cancer. An example would be non-small cell lung cancer.

Table 2. Examples of biodegradable polymers.

Polymer	Product resulting from degradation
Polylactide (PLA)	Lactic acid
Polyglycolide (PGA)	Glycolic acid
Poly (ε-caprolactone) (PCL)	Caproic acid
Polyidioxanone (PDS)	Glyoxylic acid
Poly (β-hydroxybutyrate) (PHB)	Hydroxybutyric acid

In turn, magnetic nanoparticles are compounds that exhibit magnetic properties. In medicine, there are examples of iron-based nanostructures. Iron is the basic building factor of many body structures, including liver, spleen or heart. Additionally, it forms the basis of important biological compounds such as hemoglobin, myoglobin and ferritin. Due to their magnetic properties, iron nanoparticles are used in various fields of medicine. These structures are used at the diagnostic and therapeutic level. They can be used for: cell analysis, biological material purification, and, above all, as MRI (Magnetic Resonance Imaging) imaging agents.[13] However, at the therapeutic level, they can be used as drug carriers and in radiotherapy combined with MRI. In addition, at the level of experimental and experimental research there are also research projects on iron nanostructures based on the core-shell structure. Core-shell nanoparticles are made of a silicon core covered with a thin layer of gold, to which biological ligands can be additionally attached. Due to the high absorption capacity and the possibility of dispersing electromagnetic radiation in the range from visible radiation to near infrared radiation (0.38 μm-5 μm), it is possible to use them in optics and in medical imaging. They are mainly used in targeted therapy in the photodynamic method.[14] Thanks to numerous scientific studies, it has been confirmed that nanomaterials in combination with photosensitizers can increase the efficiency of photodynamic therapy and eliminate its side effects. Photodynamic therapy is a multi-step treatment procedure that uses photosensitive substances that respond to a specific type of light. When exposed, these substances become toxic to cancer cells and other diseased cells. In order to be able to carry out a photodynamic reaction, it is necessary to use three basic components such as: a photosensitizer, which locates in the treated tissue and sensitizes it to light, and a light source of an appropriate wavelength, which is excited by the photosensitizer accumulated in the tumor tissue. Dissolved oxygen in the tissue is also necessary. The condition for initiating the photochem-

ical reaction is the correlation of the emission band of the light source with the dye absorption band. Thanks to the use of nanomaterials, it is also possible to reduce the toxic photosensitizer that can enter healthy tissues. Core-shell nanoparticles were used by the Hirsch team to treat mouse tumors in vivo and in vitro on the SKBR3 cell line. These nanoparticles were injected interstitial into the neoplastic lesion and in the next stage were irradiated with low doses of near-infrared radiation (0.82 μm).[15] After the experiment, strong heating of the inside of cancer cells was noticed, causing their destruction, while maintaining the metabolic balance of healthy cells surrounding the pathological area.

Liposomes (called liposomes) are another group of nanoparticles that improve the transport of drugs to various places in the living organism. They are colloidal structures having a spherical shape. Constructed of a lipid bilayer, they surround the water zone in which the drug is placed.[16] Liposomes are made of synthetic or natural phospholipids with a diameter of approx. 100 nm. Their membrane (sheath) is built in the same way as the cell membranes surrounding cell organelles.

Liposomes occur naturally in living organisms (e.g. as blood lipoproteins) and are produced on a laboratory and industrial scale (e.g. used in the production of drugs). For this reason, there is now a division into artificial and natural liposomes.[17] Artificial liposomes can be divided in terms of size, number of envelope layers and the way they are made. Table 3 shows the generally accepted division of artificial liposomes.

Table 3. Division of artificial liposomes

Liposomes with more than one lipid layer	Single-layer liposomes	Liposomes with lots of vesicles
Multilayer liposomes (MLV) 0.4-10 μm in size	Small unilamellar liposomes (SUV) with a size of 0.02-0.03 μm	Multiple vesicle liposomes (MVV)>1 μm in size
Oligolamellar vesicles (OLV)	Large unilamellar liposomes (LUV) with a size of 0.05-1 μm	
	Giant unilayer liposomes (GUV)	

In turn, the group of natural liposomes includes: lipids (e.g. cholesterol, triglycerides), which are transported in the body's water environment with blood and tissue fluid in the form of lipoprotein particles. They take the form of vesicles or disks surrounded by a double or single lipid layer of the membrane, made of phospholipids (which consist of a hydrophilic "head" and a hydrophobic "tail") and a surrounding chain (apolipoprotein protein).[18] Due to this structure, hydrophilic and hydrophobic substances can be stored in the liposome in various ways. Additionally, there are a number of other advantages of using liposomes as nanocarriers. These benefits include: improved bioavailability of drugs placed inside, a significant reduction in the toxicity of the released substances, controlled release of various types of substances as a result of internal or external factors, and (one of the most important) the possibility of gradual release of substances by liposomes in neoplastic tissues. One of the antibiotics that is transported through the liposomes is the anthracycline antibiotics (daunorubicin and doxorubicin). They are readily available. Daunorubicin (Doxorubicin-Ebewe) is an anti-cancer antibiotic used in cancer chemotherapy. This preparation is mainly used in the treatment of soft tissue sarcomas and sarcomas derived from bone tissues. However, the use of doxorubicin is very limited due to its high cytotoxicity.[19-21]

In turn, polymeric micelles are amphiphilic spherical structures, built of a hydrophobic core and a hydrophilic shell. The core acts as a drug depot, the hydrophilic shell of which stabilizes. Moreover, thanks to its properties, these polymers are water-soluble. The substances can be placed inside the micelles in various ways, which enables the drug to act more effectively. Polymer micelles are used in the treatment of lung, breast, ovary and colon cancer.

Dendrimers and carbon nanotubes

Dendrimers (Greek: dendros, tree) are polymers with a size of approx. 20 nm. They are characterized by a branched, three-dimensional structure. Their shape resembles a sphere. In the structural structure, a multifunctional core is distinguished from which the dendrimer branches depart.[22] These branches are called dendrons, with free functional groups at their end. These groups can be changed by various kinds of substituents while changing the properties of the dendrimers. In general, there are two generations of dendrimers, half and complete. Half dendrimers are terminated with a carboxyl group (-COOH), while complete dendrimers have, among others, amino ($-NH_2$) and hydroxyl (-OH) groups. Due to the chemical structure of the dendrimer, its shape and thus the level of activity are determined. Thanks to the vacancies in the construction site, the so-called cavities, dendrimers are a kind of reservoir of various molecules, which enables the delivery of drugs directly to the affected areas. On the basis of numerous studies and publications, dendrimers are used primarily as a transport of drugs such as cisplatin and doxorubicin.

Carbon nanotubes (carbon nanotubes) are graphene planes rolled into thin tubes with a diameter of approx. 1 nm and a length ranging from a few nanometers to several millimeters. Due to the number of layers, nanotubes are divided into mono- and polyhedral. They are formed in the process of slow condensation of hot vapors of carbon atoms.[23] They are characterized by high mechanical strength and high thermal conductivity. Due to

their structure, nanotubes are very difficult to dissolve in water, and therefore functionalisation is carried out. This process is based on the deposition of various functional groups on their surface. As a consequence, these nanoparticles can be used, for example, in as drug carriers and biosensors.

Application of nanoparticles – quantum dots in the marking of neoplastic cells

According to the latest statistical research, cancer is one of the most dynamically developing diseases on a global scale. The number of actions taken in the fight against various types of cancer is constantly expanded with more and more modern solutions. An example is the use of nanoparticles in anti-cancer therapy, mainly in aspects of radiation therapy.[24] An example of the use of nanomaterials for imaging and combating various types of cancer cells are quantum dots as a representative of a zero-dimension system. A quantum dot is a small volume completely limited by a semiconductor with a larger band gap, constituting a barrier. The principle of the material idea is based on the knowledge in the field of quantum mechanics concerning the motion of a particle closed in a potential well. Regardless of whether a particle is confined in an infinitely high potential well or in a finite height well, its energy and momentum are quantized. This means that they only accept strictly selected permitted values. More importantly - the values of allowable energy and momentum depend on the width of the L potential well. In such systems, the processes of absorption and emission of electromagnetic radiation energy are important. Absorption, i.e. absorption of the photon's energy, occurs when the charge carrier at a certain energy level E_i absorbs the radiation quantum hν, whose energy strictly corresponds to the distance between a given level of E_i and some higher E_j. The emission process is the reverse process and is accompanied by the release of previously absorbed energy ΔE, while the charge carrier moves from a higher energy level E_j to a lower energy level E_i:

$$\Delta E = E_{higher} - E_{lower}$$

In the material system which is a quantum dot, the movement of the carrier is quantized in all three directions, there is no free movement. Most importantly, the allowed energy of the carrier in such a system is fully quantized, it depends on the size of the system with three components L_x, L_y, L_z and the effective mass of the carrier. Similarly, all the components of the carrier's momentum are also quantized. The most important methods for producing such thin films are Molecular Beam Epitaxy (MBE) and Chemical Vapor Deposition (CVD). The most important for the processes of light emission in such systems is the form of the density function of the energy states of the charge carriers of the system, because the intensity of the optical transition depends on its value. In addition, in electron transport, the density of states determines the number of states available for carrier movement, and their scattering time depends on the number of states into which they can dissipate. In the processes of light emission and absorption, two principles must be met: the principle of conservation of energy and momentum. Since the momentum of the carrier has 3 components for the 3D system, in order for the energy to be emitted at the transition of e.g. an electron from a higher energy level (in the conduction band) to a lower one (in the valence band), such a state of the carrier must be additionally available (e.g. holes in the conduction band). valence), which has identical components px, py pz as in the higher state. In other words, the components px, py from the recombining electron and the holes must be identical. Along with the reduction of the size of the system, the number of momentum coordinates that must be adjusted to each other decreases, thus the number of available carriers for the emission process increases. In the 0D system, we no longer need to reconcile the components, so each excited electron can recombine with the hole at a lower energy level. So the light emission due to the 0D recombination of electrons and holes is huge. From the point of view of the huge possible applications of such systems, the most important thing is bioimaging used in today's science in biology and medicine. Because there is the possibility of precise introduction of quantum dots into specific places of living organisms, e.g. into the nucleus of a cell by injection through a nano-needle, and observation of the expansion of the material along with the growing cell (e.g. cancerous). Moreover, by light emission, it is possible to obtain a precise image of the examined cell structure fragment. It should be emphasized that the possibility of maneuvering the wavelength of light emitted by a quantum dot means that its application potential in the field of physics, chemistry and biology is constantly expanded.

The main purpose of quantum dots is to label diseased cells. A quantum dot in combination with a properly selected atom or molecule (ligand) and precisely placed inside a tissue or cell is a very good marker of cell surface and intracellular structures in in vitro and in vivo tests. Due to the properties of quantum dots, long-term monitoring of labeled cells is essential. Such possibilities are guaranteed by the use of a fluorescent probe based on nanocrystals. The process that is determined by fluorescent probes is called bioconjugation or bioconjugation.[25] Molecules that can be attached to the surface of quantum dots include nucleic acids, various types of peptides and antibodies. In order to make the so-called coupling, cross-linkers and biomolecules are used, which are attached to nano-

structures by covalent bonds or electrostatic interactions directly to the material made of nanostructures. In this method, linker molecules connect given quantum dot functional groups with conjugated biomolecules. In this way, a covalent bond is created between the linkers and groups such as: amino, carboxyl or thiol groups. Proteins and antibodies are attached directly to the surface of quantum dots using a polyhistidine tag to zinc or other metal atoms on the surface of a given nanocrystal. conjugated polypeptide.[26] The attachment of peptides to the surface of the nanocrystal increases the solubility of quantum dots in aqueous solutions, and also introduces functional groups to the surface. So they can be used for further transformations. The bioconjugation method based on the principle of interaction of oppositely charged molecules is also known.[27] At the time of ligand exchange, the quantum dot has a negatively charged surface, which means that a protein with a positive charge is attached to its surface. For example, such a protein can be and is avidin. Avidin is an animal protein consisting of four polypeptide chains, weighing approx. 70 kD.[28]

Most importantly, avidin exhibits one of the most powerful unconventional interactions observed in nature. Therefore, the resulting strong binding of avidin with e.g. biotin enables various functional molecules, e.g. antibodies, to be attached to the surface of quantum dots. Due to the emerging difficulties in the preparation of fluorescent markers, the bioconjugation of molecules with quantum dots is largely limited. One of the main barriers is the inability to control the direction and orientation of attached molecules to the surface of the quantum dot. As a result, the molecule does not sufficiently fulfill its function. In addition, molecules can uncontrollably attach to the surface of nanocrystals, which means that the fluorescent probe may not accurately count the number of particles attached.

On the other hand, the use of quantum dots as a material acting as a contrast agent in in vivo research is also associated with various types of problems. It is known from the general principles of physics that tissues located in deeper states absorb only a part of the fluorescent radiation emitted by quantum dots, which makes the obtained quantum data not fully reliable. The ideal solution is to mark quantum dots with β^+ particle-emitting radionuclides, thanks to which they can be used in combined imaging methods, in biology and chemistry as bimodal agents. Thanks to this, they can function in a similar way to iron oxide nanoparticles.

Another example of the use of quantum dots is the transport and observation of drug carriers to specific locations in the cell. Based on the structure and functioning of quantum dots, systems are created that deliver chemical compounds (including e.g. drugs) with the simultaneous observation of distribution in the body. According to the research of numerous research centers, one of the main examples of the use of quantum dot materials as drug carriers are experiments on the delivery of doxorubicin to prostate cancer cells. Additionally, on the basis of numerous publications, quantum dots are also used as one of the elements of the system that monitors the extinction of gene expression with small interfering RNA.[29-31]

Conclusion

The fundamental element of medical diagnostics is in vivo imaging of cells, tissues or pathological organs. The combination of a conventional imaging method with optical imaging with the use of fluorescent markers based on the quantum dot model is a great hope in further research and an invaluable tool in the treatment of pathological cells.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, K.D. and D.A.; Writing – Original Draft Preparation, K.D. and D.A.; Writing – Review & Editing, K.D. and D.A.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein J Nanotechnol.* 2018;9:1050-1074.
2. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The History of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. *Molecules*, 2019;25(1):112.
3. Mehnath S, Kumar Das A, Kumar Verma S, Jeyaraj M. Chapter 9-Biosynthesized/green-synthesized nanomaterials as potential vehicles for delivery of antibiotics/drugs. *Compr Anal Chem.* 2021;94:363-432.
4. Griffin S, Masood MI, Nasim MJ, et al. Natural nanoparticles: a particular matter inspired. *Antioxidant (Basel).* 2017;7(1):3.
5. Boverhof DR, Bramante CM, Butala JH, et al. Comparative assessment of nanomaterial definitions and safety evaluation considerations. *Regul Toxicol Pharmacol.* 2015;73(1):137-150.

6. Kumar P, Saini M, Dehiya BS, et al Comprehensive survey on nanobiomaterials for bone tissue engineering applications. *Nanomaterials (Basel)*. 2020;10(10):2019.
7. Mabrouk M, Das DB, Salem ZA, Beherei HH. Nanomaterials for biomedical applications: production, characterisations, recent trends and difficulties. *Molecules*. 2021;26(4):1077.
8. Salah N, Habib SS, Khan ZH, et al. High-energy ball milling technique for ZnO nanoparticles as antibacterial material. *Int J Nanomedicine*. 2011;6:863-869.
9. Thiruvengadam M, Rajakumar G, Chung IM. Nanotechnology: current uses and future applications in the food industry. *3 Biotech*. 2018;8(1):74.
10. Sanna V, Pala N, Sechi M. Targeted therapy using nanotechnology: focus on cancer. *Int J Nanomedicine*. 2014;9:467-483.
11. Zielińska A, Carreiró F, Oliveira AM, et al. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. *Molecules*, 2020;25(16):3731.
12. Miele E, Spinelli GP, Miele E, Tomao F, Tomao S. Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. *Int J Nanomedicine*. 2009;4:99-105.
13. Bao Y, Wen T, Samia AC, Khandhar A, Krishnan KM. Magnetic nanoparticles: material engineering and emerging applications in lithography and biomedicine. *J Mater Sci*. 2016;51(1):513-553.
14. Sowa P, Rutkowska-Talipska J, Rutkowski K, Kosztyła-Hojna B. Optical radiation in modern medicine. *Postepy Dermatol Alergol*. 2013;30(4):246-251.
15. Hirsh LR, Stafford RJ, Bankson JA, et al. Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc Natl Acad Sci USA*. 2003;100(23):13549-13554.
16. Bozzuto G, Molinari A. Liposomes as nanomedical devices. *Int J Nanomedicine*. 2015;10:975-999.
17. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: classification, preparation, and applications. *Nanoscale Res Lett*. 2013;8(1):102.
18. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. *Pharmaceutics*. 2017;9(2):12.
19. Petre CE, Dittmer DP. Liposomal daunorubicin as treatment for Kaposi's sarcoma. *Int J Nanomedicine*. 2007;2(3):277-288.
20. Luk BT, Fang RH, Zhang L. Lipid- and Polymer-Based Nanostructures for Cancer Theranostics. *Theranostics* 2012;2(12):1117-1126.
21. Rzeszutek J, Matysiak M, Czajka M, et al. Application of nanoparticles and nanomaterials in medicine. *Hygeia Public Health* 2014;49(3):449-457.
22. Douloudi M, Nikoli E, Katsika T, Vardavoulias M, Arkas M. Dendric polymers as promising additives for the manufacturing of hybrid organoceramic nanocomposites with ameliorated properties suitable for an extensive diversity of applications. *Nanomaterials (Basel)* 2020;11(1):19.
23. Luong JH, Male KB, Mahmoud KA, Sheu FS. Purification, functionalization, and bioconjugation of carbon nanotubes. *Methods Mol Biol*. 2011;751:505-532.
24. Zhao CY, Cheng R, Yang Z, Tian ZM. Nanotechnology for cancer therapy based on chemotherapy. *Molecules* 2018;23(4):826.
25. Barroso MM. Quantum dots in cell biology. *J Histochem Cytochem*. 2011;59(3):237-251.
26. Sapsford KE, Pons T, Medintz IL, Mattoussi H. Biosensing with luminescent semiconductor quantum dots. *Sensors* 2006;6:925-953.
27. Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM. Biological applications of quantum dots. *Biomaterials* 2007;28:4717-4732.
28. Gao XH, Yezhelyev MV, Qi L, O'Regan RM, Nie SM. Proton-sponge-coated quantum dots for siRNA delivery and imaging. *J Am Chem Soc*. 2008:130;9006-9012.
29. Patra JK, Das G, Fraceto LF, et al. Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnology* 2018;16(1):71.
30. Fang M, Peng CW, Pang DW, Li Y. Quantum dots for cancer research: current status, remaining issues, and future perspectives. *Cancer Biol Med*. 2012;9(3):151-163.
31. Murar M, Albertazzi L, Pujals S. Advanced optical imaging-guided nanotheranostics towards personalized cancer drug delivery. *Nanomaterials* 2022;12(3):399.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.15

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 109–116

REVIEW PAPER

Olena Petrivna Sokolik [1], Galina Olexandrivna Prozorova [2]

Current research opportunities for potential phytotherapeutic agents for the treatment of pathologies of the female reproductive system

[1] Department of Pharmacology and Pharmacognosy, Odessa National Medical University, Odessa, Ukraine
[2] Department of Medical and Biological Disciplines, Pylyp Orlyk International Classical University, Mykolayiv, Ukraine

ABSTRACT

Introduction and aim. Herbal medicine is prescribed for various disorders of the menstrual cycle (uterine bleeding, dysmenorrhea), for the treatment of premenstrual and climacteric syndromes, inflammatory diseases of the genital organs, mastopathy and mastalgia and other pathological conditions. The aim of the study is to **a**nalysis and generalization of data from professional literature and own experience in the treatment of patients with pathologies of the female reproductive system by phytotherapeutic methods, taking into account the influence of medicinal plants on various links in the pathogenesis of the disease, as well as making recommendations for improvement and prospects for the use of phytotherapy in the treatment of this pathology.

Material and methods. To make an analysis of literary sources of domestic and foreign authors about usage of medicinal plants for the treatment of pathologies of the female reproductive system.

Analysis of the literature. In the treatment of primary (spasmodic) dysmenorrhea, herbal remedies with an antispasmodic, analgesic, hormone-mimetic effects are prescribed. It can be *Chamomile* (*Matricaria chamomilla* L.), *Achillea millefolium*, commonly known as yarrow, *Viburnum*, Shepherd's purse (*Capsella bursa-pastoris*), *Greater celandine*, *Atropa belladonna*, *Hyoscyamus niger*, commonly known as henbane, black henbane, or stinking nightshade and Abraham's tree. Many plants have bactericidal activity, and this property is used in the treatment of inflammatory diseases of the mucous membranes and skin. Such properties are possessed by flowers of *Chamomile* (*Matricaria chamomilla* L.), *Calendula officinalis*, infusion of Medicinal sage (*Salvia officinalis* L.). For the treatment of functional hyperprolactinemia phytopreparations are also successfully used. It is known that the medicinal plant *Vitex agnus castus* has dopaminergic properties, selectively blocking prolactin synthesis, and reduces follicle stimulating hormone levels. *Strychnos ignatia*, *Caulophyllum thalictroides*, *European cyclamen*, *Lilium tigrinum*, *Iris versicolor* provide a complex effect on the female body, effectively reduces the level of prolactin and the severity of mastalgia, which is confirmed not only by clinical data, but also by mammography data in fibrocystic breast disease. For the treatment of climacteric syndrome, a large number of medicinal plants are used, in particular, the most popular is the Cimicifuga racemose.

Conclusion. The effectiveness of phytotherapeutic drugs has been verified by many clinical trials. Modern phytotherapy is becoming more widespread in clinical practice, as an alternative to drug treatment.

Keywords. climacteric syndromes, female reproductive system, hyperprolactinemia, phytotherapy, premenstrual syndromes

Corresponding author: Olena Petrivna Sokolik, e-mail: sokolikep@gmail.com

Received: 2.12.2021 / Revised: 30.12.2021 / Accepted: 9.01.2022 / Published: 30.03.2022

Sokolik OP, Prozorova GO. *Current research opportunities for potential phytotherapeutic agents for the treatment of pathologies of the female reproductive system*. Eur J Clin Exp Med. 2022;20(1):109–116. doi: 10.15584/ejcem.2022.1.15

Introduction

Dyshormonal disorders are one of the very common functional pathologies of the reproductive system, which occurs in women of different ages. Successful treatment of dyshormonal disorders can restore a woman's reproductive potential and improve her quality of life. The manifestations of dyshormonal disorders are diverse: in young women there are more often cycle disorders, infertility, miscarriage, at an older age – uterine fibroids, endometriosis, chronic pelvic pain.[1] The high frequency of these problems in modern society is associated primarily with the deterioration of environmental conditions, the acceleration of the pace of life, chronic stress, unfavorable diet, work and rest; the role of the characteristics of a woman's reproductive behavior is also important. All this leads to a deterioration in the work of adaptation mechanisms, which leads to the development of dyshormonal disorders of the reproductive system. The manifestation of dyshormonal disorders most often occurs in active reproductive age and is based on subclinical disorders that usually begin during puberty.[2]

In a complex neuroendocrine system, the main role is played by five links, interacting with each other according to the principle of direct and reverse negative and positive interconnection, which is determined by the nature of the signals coming from the periphery. Physiology and pathology of the menstrual cycle in the clinical aspect most fully reflect the state of the woman's reproductive system. Physiological effects of structures of the highest level of regulation are carried out through nervous and humoral connections. The leading place in these relationships is given to brain neurotransmitters (catecholamines, serotonin, acetylcholine, g-aminobutyric acid, glutamic acid, enkephalins), which are located in extrapyramidal formations. Cerebral neurotransmitters regulate the hypothalamic-pituitary-ovarian level of reproductive function. So, they determine the circadian and circhoral rhythms, which are leading in the functioning of the entire reproductive system.[3]

The second level of regulation of the reproductive system is the hypothalamus. A special role belongs to luliberin, foliberin and prolactostatin, and the main role in the regulation of prolactin secretion is assigned to dopaminergic structures. Dopamine inhibits the release of prolactin from the pituitary lactophores, and its antagonists (Methyldopa, Reserpine, Chlorpromazine) increase it secretion. The pituitary gland produces gonadotropic hormones – follicle stimulating hormone (FSH) and luteinizing hormone (LH), which affect the function of peripheral endocrine glands. FSH stimulates the growth and maturation of follicles, secretion of estrogen. The formation and function of the corpus luteum are controlled by the secretion of LH and prolactin.[4]

Depending on the concentration and ratio of sex steroid hormones, the production of the corresponding tropic hormones of the pituitary gland is inhibited or activated. Peripheral endocrine organs (thyroid gland, adrenal glands) are also responsible for the regulation of ovarian function, where complex processes of sex steroid biosynthesis and follicular development take place. The female genitals and mammary glands, as well as the skin, bones and adipose tissue, are target organs with the corresponding regulatory mechanisms.[5]

The hypothalamic-pituitary-ovarian system has versatile adaptive mechanisms that change in the process of maturation, maturity and aging of the body. One of the important periods of a woman's life is reproductive, which is considered as period, intended for conceiving, carrying and feeding a child. In this age range, abortions and their complications, sexual infections, various gynecological diseases, breast diseases, infertility, complications during pregnancy and after childbirth are especially dangerous for a woman's health. The extinction of a woman's reproductive function signals the beginning of the climacteric period, in which premenopause, menopause and postmenopause are conventionally distinguished. It is characterized by a high frequency and severity of symptoms associated with a deficiency of sex hormones, which significantly reduce a woman's quality of life. Often this period is complicated by the development of climacteric syndrome of varying severity with the formation of vegetative-vascular, emotional-mental, metabolic-endocrine disorders and their combination.[6] The main symptoms of climacteric syndrome are vasomotor and psychovegetative climacteric disorders associated with estrogen deficiency, genitourinary disorders due to atrophy of the lower urinary tract, changes in bone density (osteoporosis). As we know, one of the leading methods of treating menopausal syndrome is hormone replacement therapy. However, not all women are able and willing to start treatment with hormonal drugs.

Throughout the history of medicine, the attention of doctors and scientists is riveted to herbal medicine. Avicenna as one of the founders of medicine, a renowned doctor and scientist, emphasized that there are three main tools of a doctor in medicine: the word, herbs and a knife. It is known that biologically active substances of a plant have much in common in their structure with substances formed in the cells of animals and humans, and therefore preparations based on them are well tolerated and have few contraindications with high efficiency. More and more phytopreparations are being created on the basis of plants. The terms "herbal medicine" and "herbal remedies" were first introduced by the French physician Henri Leclerc, who is rightfully considered the founding father of herbal medicine. He is the author of many books on the use

of medicinal herbs in clinical settings. Modern phytopreparations must meet the following requirements: have a standardized composition; be of impeccable quality, safe; do not contain foreign matter. Therefore, the plants used in the production of phytopreparations are cultivated and processed under strict control. The content and quality of substances in plant raw materials depend on the quality of the soil, climate, fertilizers, moisture content, the time of harvesting the plants, and the quality of processing. It is important that side reactions when taking phytopreparations are 5 times less common than when using others drugs, and the number of contraindications is significant smaller.[7] In this regard, phytopreparations can be used for a longer time, and when using them, the therapeutic effect occurs more slowly, but is long-lasting.

Phytohormonal preparations have a wide spectrum of action and balanced complex effect on metabolic processes in the body, and are devoid of pronounced side effects. Their influence is realized, first of all, at the cellular level, through the central nervous and endocrine systems. An important aspect of the pharmacodynamics of phytohormones is that they stimulate the function of the endocrine glands as a result of improving the energy supply of endocrine cells and normalizing the synthesis of ribonucleic acid and proteins, without disrupting the physiological hormonal mechanisms of regulation. Thanks to this, the glands continue to function actively at the end of the course of therapy.

Aim

Analysis and generalization of data from professional literature and own experience in the treatment of patients with pathologies of the female reproductive system by phytotherapeutic methods, taking into account the influence of medicinal plants on various links in the pathogenesis of the disease, as well as making recommendations for improvement and prospects for the use of phytotherapy in the treatment of this pathology.

Material and methods

To make an analysis of literary sources of domestic and foreign authors about usage of medicinal plants for the treatment of pathologies of the female reproductive system.

Most analyzed studies were clinical human (70%) and animal studies (30%), and also based on the subject of the papers, most papers on the effects of different plants focused on menstrual irregularities, premenstrual (PMS) and climacteric syndromes, inflammatory diseases of the genital organs, mastopathy and mastodynia (mastalgia), hyperprolactinemia, infertility. Based on time of publication of papers, most of the selected studies were published in the period 2017-2021 and the upward slope of studies has intensified from 2019 to 2021.

Analysis of the literature

The versatility of the action of herbal medicines and the safety of their use make herbal medicine indispensable in gynecology, obstetrics and perinatology, where the fundamental issues are harmlessness to the fetus with a very long duration of treatment, as well as obtaining several effects from a minimum amount of funds. In the overwhelming majority of cases, the body of a pregnant woman and the fetus do not need intensive therapy, but only the prevention of violations of the adaptive-homeostatic reactions of the fetoplacental system. Moreover, such prevention is necessary against the background of treatment of complications of pregnancy and the development of placental insufficiency, with the risk of perinatal infection.[8]

For the treatment of gynecological diseases, plants have also been used for a long time, and now their use in this area has received scientific justification. Herbal medicine is prescribed for various disorders of the menstrual cycle (uterine bleeding, dysmenorrhea), for the treatment of premenstrual and climacteric syndromes, inflammatory diseases of the genital organs, mastopathy and mastalgia and other pathological conditions. In case of menstrual irregularities, ergot alkaloids are used, they are direct-acting regulators of the menstrual cycle. In cases of uterine bleeding, in order to reduce blood loss, ergot preparations (methylergometrine, ergotamine) are used, as hemostatic agents – a decoction or infusion of Stinging Nettle (Urtica dioica).[9] In the treatment of primary (spasmodic) dysmenorrhea, herbal remedies with antispasmodic, analgesic, hormone-mimetic effect are prescribed. It can be *Chamomile* (*Matricaria chamomilla* L.), *Achillea millefolium*, commonly known as yarrow, *Viburnum*, Shepherd's purse (*Capsella bursa-pastoris*), *Greater celandine*, *Atropa belladonna*, *Hyoscyamus niger*, commonly known as henbane, black henbane, or stinking nightshade and Abraham's tree. Many plants have bactericidal activity, and this property is used in the treatment of inflammatory diseases of the mucous membranes and skin. Such properties are possessed by flowers of *Chamomile* (*Matricaria chamomilla* L.), *Calendula officinalis*, infusion of Medicinal sage (*Salvia officinalis* L.).[10]

Most often in gynecology, phytopreparations are used in the treatment of menstrual irregularities, premenstrual and climacteric syndromes, inflammatory diseases of the genital organs, mastopathy and mastodynia (mastalgia), hyperprolactinemia and urinary tract diseases. The range of indications for the use of phytopreparations is constantly expanding.

Hyperprolactinemia is one of the common causes of reproductive system dysfunctions, its clinical manifestations are well known: menstrual irregularities, anovulation, corpus luteum dysfunction, amenorrhea,

Table 1. Characterization of compounds identified in medicinal plant

Medicinal plant	Biological active substances	Pharmacological properties
Vitex agnus castus	Protocatechuic (3,4-dihydroxybenzoic) acid, penduletin/eupatorin, luteolin-7-glucoside, isochlorogenic acid a, kaftaric acid, chlorogenic acid, corosol acid, luteolin 1 and 2, vitexin, p-hydroxybenzoic acid, linolenic acid, luteolin glucoside, agnuzide, misodendron, casticin, 6'-o-p-hydroxybenzoylmusaenoside acid, chicory acid, apigenin, artemetin, hydroxy-tetramethoxyflavone, butyric acid	Antioxidant, chemopreventive, immunomodulatory and cytotoxicity, tumoricidal, antimutagenic, antimicrobial, antifungal, insect repellant, larvicidal, fracture healing, osteopenic, antinociceptive, opioidergic, antiepileptic, preventing non-alcoholic fat liver disease and oxidative stress, anti-inflammatory activities
Caulophyllum thalictroides	Magnoflorine, taspine, and boldine are contributed to aporphine alkaloids, quinolizidine alkaloids, triterpenoid saponins, palmitic acid, α-spinasterol, α-spinasterol-β-D-glucopyranoside, stigmasterol, lupeol, cholesterol	Antibacterial activity, anti-inflammatory and analgesic effects, antioxidant effects, antiacetylcholinesterase activity, antitumor activity, inhibitory cytochrome P450, Topoisomerase Inhibitor, Wound Healing,
European cyclamen	Triterpene saponins – isocyclamin and desglucocyclamin I, glycosides, phenolic components, anthocyanin and flavonoids	Antiproliferative activity, cytotoxic, spermicidal, antimicrobial, laxative, abortive, sedative, purgative, emmenagogue and antihelmintic, analgesic, anti-inflammatory, antimicrobial, antioxidative activities
Strychnos ignatia	Vomicine, loganin, mavacurine, novacine, icajine, α-colubrine, β-colubrine, isostrychnine, pserdostrychnine, seudobrucine, 16-hydroxy-β-colubrine, 18-hydroxy-sungucine, 18-hydroxyisosungucine, isobrucine N-oxide, isostrychnine N-oxide, 2-hydroxy-3-methoxystrychnine, cycloartenyl palmitate, fatty acid, proteins, polysaccharides	Inhibition of cyclooxygenase and lipoxygenase, cytotoxicity, anti-cancer activity, xanthine oxidase inhibitory activity, neurotransmitter receptors, antiplasmodial activity, antialcoholic effect and reduction of alcohol induced sleep time, antinociceptive effects, inhibitory effect of CYP450
Lilium tigrinum	Steroidal saponins, polysaccharides (galactose, mannose, arabinose, and galacturonic acid), alkaloids, flavonoids, organic acids	Anti-tumor, hypoglycemic, antibacterial, anti-oxidation, anti-depression, anti-inflammatory
Iris versicolor	(Iso)flavonoids, phenols, fatty acids, terpenoids, steroids, xanthones, quinones	Antibacterial, antioxidant, anti-inflammatory, anti-cancer, immunomodulatory
Tribulus terrestris L.	Flavonoids (kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, tribuloside, caffeoyl derivatives, quercetin glycosides, rutin), saponins (tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin, and sarsasapogenin), alkaloids, tribulusamides A and B, lignan amides, furostanol glycosides (protodioscin, protogracillin)	Diuretic, aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, absorption enhancing, hypolipidemic, cardiotonic, central nervous system, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, anticancer, antibacterial, anthelmintic, larvicidal, anticariogenic activities
Cimicifuga racemosa	Triterpene glycosides (acetin,cimicifugoside, 27-deoxyacetin), organic acids (isoferulic acid, cimicifugicacids) (A, B, E and F), fukinolic acid, caffeic acid, salicylic acid, cimicifugin, tannins, phytosterin	Labour-inducing effects, hormonal effects (estrogen-like), anti-hyperlipidemia and anti-osteoporosis effects, emmenagogue properties, anovulatory effects, anti-proliferative effects

infertility, galactorrhea, PMS.[11] Hyperprolactinemia is associated with diseases of the mammary glands (cyclic mastodynia, fibrocystic breast disease, galactorrhea) and menstrual irregularities (secondary amenorrhea – 60-85%, oligomenorrhea – 27-50%, hyperpolymenorrhea due to insufficient function of the corpus luteum, anovulation – 70%), as well as a pituitary tumor. Dopamine agonists are the most commonly used drug in the treatment of hyperprolactinemia. Their 1st generation includes Bromocriptine (Abergin), Lizurid, Pergolide; II – Quinagolide (Norprolac); III – Carbegoline (Dostinex).[12]

For the treatment of functional hyperprolactinemia phytopreparations are also successfully used. It is known that the medicinal plant *Vitex agnus castus* has dopaminergic properties, selectively blocking prolactin synthesis, and reduces FSH levels. Vitex agnus-castus, also called vitex, chaste tree (or chastetree), chasteberry, Abraham's balm, lilac chastetree, or monk's pepper, is a

native of the Mediterranean region. It is one of the few temperate-zone species of Vitex, which is on the whole a genus of tropical and sub-tropical flowering plants (table 1). Theophrastus mentioned the shrub several times, as agnos in Enquiry into Plants.[13] It has been long believed to be an anaphrodisiac, leading to its name as chaste tree, but its effectiveness for such action remains unproven. The preparations of *Vitex agnus castus* are effective in women of different ages with menstrual irregularities associated with hyperprolactinemia and luteal phase insufficiency, is used for mastalgia and PMS, in view of the possibility of a single daily intake, it is convenient for treatment and has a low number of side effects.

Phytopreparations are also used to treat premenstrual and climacteric syndromes.

PMS develops a few days before the onset of menstruation and manifests itself as both mental and somatic symptoms, which are expressed in fear, irritability, mood swings, drowsiness, headache, a feeling of chest swelling, bloating and swelling of the legs. The main hormones that regulate the menstrual cycle throughout a woman's life, as well as the growth and development of the mammary glands, are the hormones of the pituitary gland, ovaries (estrogens and progesterone), thyroid gland, adrenal glands and other biologically active compounds.[14] A large role belongs to prolactin – a hormone produced by the pituitary gland. Prolactin, together with estrogens and progesterone, controls the entire process of mammogenesis, providing both the formation of intraorgan structures and postpartum lactation. The role of prolactin is especially important during pregnancy, when the mammary gland is preparing for lactogenesis. Prolactin provides the synthesis of proteins, carbohydrates and milk lipids. After childbirth, the lactogenic effect of the hormone increases sharply. At the end of lactation, prolactin production drops to baseline. A pathological increase in the level of this hormone outside pregnancy and lactation can cause the development of mastopathy.[15] Often there is not a constant, but the so-called latent increase in the level of prolactin, leading to the development of a symptom complex called PMS (soreness and engorgement of the mammary glands, especially in the second phase of the menstrual cycle or just before menstruation, as well as vegetative disorders, migraine-like headaches, edema extremities, abdominal pain, flatulence). With the onset of menstrual bleeding, all of these symptoms usually disappear.[16]

In the pathogenesis of the development of PMS the leading role also is played by the violation of the secretion of serotonin. Serotonin and ovarian hormones have a close reciprocal relationship. Estrogens have a potent modulating effect on monoamine metabolism and central nervous system (CNS) function. The clinical manifestations of premenstrual tension syndrome are associated with a lack of serotonin in the CNS, relative hyperestrogenism and a violation of the ratio of estrogen and progesterone in the luteal phase of the menstrual cycle.[17]

Mastalgia occurs in 45-50% of patients.[18] The most common cause of mastalgia is a disturbance in the estradiol/progesterone relationship and an increase in prolactin secretion. Pain in the mammary glands is observed in 15-30% of women receiving hormone replacement therapy, as well as combined oral contraceptives (COCs), and are observed in the first months of their intake, which is associated with a slight increase in prolactin secretion and its level in blood serum in healthy women.[19]

Strychnos ignatia, *Caulophyllum thalictroides*, *European cyclamen*, *Lilium tigrinum*, *Iris versicolor* provide a complex effect on the female body, effectively reduces the level of prolactin and the severity of mastalgia, which is confirmed not only by clinical data, but also by mammography data in fibrocystic breast disease (table 1). *Strychnos ignatia* is a tree in the family *Loganiaceae*, native to the Philippines, particularly in Catbalogan and parts of China.[20] The plant was first described by the Moravian (Czech) Jesuit working in the Philippines, brother Georg Kamel who named its seeds "the beans of St. Ignatius", in honour of the founder of his religious order. *Caulophyllum thalictroides*, the blue cohosh, a species of *Caulophyllum* (family *Berberidaceae*) is a flowering plant in the *Berberidacea*e (barberry) family. It is a medium-tall perennial with blue berry-like fruits and bluish-green foliage. The name cohosh is probably from an Algonquian word meaning "rough". *Cyclamen purpurascens*, the Alpine, European or purple cyclamen, is a species of flowering plant in the genus *Cyclamen* of the family *Primulaceae*, native to central Europe, northern Italy, and Slovenia. It is an evergreen tuberous perennial with (usually) variegated leaves, and deep pink flowers in summer. *Lilium lancifolium* (syn. *L. tigrinum*) is an Asian species of lily, native to China, Japan, Korea, and the Russian Far East. It is widely planted as an ornamental because of its showy orange-and-black flowers, and sporadically occurs as a garden escapee in North America, particularly the eastern United States including New England, and has made incursions into some southern states such as Georgia.[21] Iris versicolor is also commonly known as the blue flag, harlequin blueflag, larger blue flag, northern blue flag, and poison flag, plus other variations of these names, and in Britain and Ireland as purple iris. It is a species of Iris native to North America, in the Eastern United States and Eastern Canada. It is common in sedge meadows, marshes, and along streambanks and shores. The specific epithet versicolor means "variously coloured".[22]

Infertility, which occurs in 15-20% of all married couples, should be considered one of the most significant

problems in medicine. It is estimated that approximately 10 million new infertile couples emerge each year. Moreover, in 55-65% of cases, the cause of infertility lies in the woman. Endocrine forms of female infertility are determined primarily by violations of ovulation and account for 35-40% of all forms of infertility. At the same time, 25% of the examined women with infertility of inflammatory genesis have normal hormonal function, and the overwhelming majority (75%) have its disorders. Menstrual cycles are of the type of failure of both phases (35%) or the second phase (45%). Changes in circadian rhythms and folliculogenesis are noted. Herbal preparations containing steroid glycosides (saponins) differ from synthetic hormonal preparations in a balanced complex effect on metabolic processes, due to which there is no violation of the physiological mechanisms of hormone regulation in the body. It is very important that the effect of phytohormones is manifested only against the background of the existing dysfunction of the endocrine glands, without affecting the normally functioning glands. One of the medicines containing steroidal saponins is a herbal preparation derived from the plant *Tribulus terrestris* L. (*Zygophyllaceae*) (Table 1). In terms of the content of furostalone saponins, Tribestan, consisting of n-butanol (n-BuOH) extract of the aerial parts of the plant, exceeds other analogues by 5-30 times. The content in the preparation of protodioscin (the main compound by which Tribestan is standardized) is ≥45%. *T. terrestris* is a native of the Mediterranean region, widespread in warm regions of Europe, Asia, America, Africa and Australia. *T. terrestris*, known in ancient Greece, is still used in traditional medicine in India, China, Bulgaria and other countries in the treatment of a wide range of diseases, including the urinary tract and sexual dysfunctions. In open clinical trials, *T. terrestris* has shown a positive effect in the treatment of female infertility. Recently, *T. terrestris* herb extract has become one of the most popular herbal products. The drugs based on the saponin fraction of *T. terrestris* are used in the treatment of infertility and libido disorders in men and women.[23]

Menopausal syndrome is understood as a set of complaints that women over the age of 45 apply to. The most common disorders are poly- and oligomenorrhea, dysfunctional bleeding, hot flashes, sweating, sleep disturbances, depression. One of the main treatments for menopausal disorders caused by estrogen deficiency is hormone replacement therapy (HRT). However, there are subjective and objective factors that reduce the acceptability of HRT.[24] These include contraindications for its implementation, a subjective negative reaction of a woman to taking hormonal drugs, the possibility of side effects, as well as intolerance to certain drugs. Therefore, in recent years, much attention has been paid to alternative methods of treating menopausal disorders. Improving health with hot flashes and other complaints of a climacteric nature, protection from osteoporosis without side effects – this is what women in menopause expect from an ideal drug. There has been research in the global pharmaceutical industry for many years to "improve estrogen", but nature has dealt with it faster. After the so-called selective estrogen receptor modulators (SERMs) have become a sensation in recent years, similar properties have been found in the extract of Cimicifuga racemose (table 1).[25] For the treatment of climacteric syndrome, a large number of medicinal plants are used, in particular, the most popular is the *Cimicifuga racemosa* (black cohosh). *C. racemosa* has long been used to treat the effects of snakebites, as a remedy for rheumatism, and later in climacteric syndrome, PMS, and prolonged formation of menstrual function. It has been proven that extracts of *C. racemosa* have a central serotonergic effect (contain components that bind serotonin receptors). There is evidence of the possibility of stimulation of certain substances by *C. racemosa* (estrogen-binding proteins), indirectly suppressing or stimulating estrogen receptors. There is a hypothesis about possible binding with other (not a and not b), not yet identified subtypes of estrogen receptors. The adverse effects caused by *C. racemosa* ranged from mild reactions, such as nausea, vomiting, headaches, dizziness, mastalgia, and weight gain, to acute liver damage, breast cancer metastasis. *C. racemosa* displayed side effects on the liver, cardiovascular, central and peripheral nervous system, gastrointestinal tract.[26] The herbal origin of a drug does not automatically mean its safety – it still needs to be proven. Factors that can cause toxicity include misidentification of plant species, different times and locations of collection, incorrect selection of plant parts, improper storage, contamination during preparation, errors in nomenclature and labeling, and falsification. For example, *C. racemosa* has an area of distribution in North America and there are many preparations based on it. In recent years, many drugs have appeared on the market that "use" cheaper species of *Cimicifuga*, which do not have the same effects as *C. racemosa*, despite the differences in chemical composition between them.

There are relatively few medicinal plants available for herbal medicine in this area. Their action can be considered as the action of hormone mimetics. The therapeutic effect of these medicinal plants is due to the still unknown "plant hormones" – substances similar to hormones, but not replacing hormones. Thus, herbal medicines can be effectively used in various obstetric and gynecological pathologies. It should be noted that the advantages of herbal remedies are good tolerance and a small number of contraindications; efficiency comparable to evidence-based medicine therapy.[27]

Conclusions

The relevance of studying the reproductive health of patients with dyshormonal non-inflammatory pathology of the female reproductive system is due to the steady increase in the share of this pathology in recent years in the structure of gynecological morbidity, the rejuvenation of the patient population, and the lack of a unified algorithm for rehabilitation measures. The choice of adequate tactics for managing such patients from the existing variety of treatment is the key to the success. The effectiveness of phytotherapeutic drugs has been verified by many clinical trials. Since phytopreparations practically do not cause side effects, they can be prescribed for a long time both as an independent treatment and in combination with other drugs. Modern phytotherapy is becoming more widespread in clinical practice, as an alternative to drug treatment. Phytotherapy is not only a scientifically based method of treatment and prevention of diseases, but also a method that has all the rights to creative development.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, O.P.S. and G.O.P.; Methodology, G.O.P.; Software, O.P.S.; Validation, O.P.S.; Formal Analysis, O.P.S.; Investigation, O.P.S. and G.O.P.; Resources, G.O.P.; Data Curation, O.P.S.; Writing – Original Draft Preparation, O.P.S.; Writing – Review & Editing, O.P.S.; Visualization, O.P.S.; Supervision, O.P.S.; Project Administration, G.O.P.; Funding Acquisition, G.O.P.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Vidal JD. The Impact of Age on the Female Reproductive System. *Toxicol Pathol*. 2017;45(1):206-215.
2. Oyola MG, Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. *Stress*. 2017;20(5):476-494.
3. Joseph DN, Whirledge S. Stress and the HPA Axis: Balancing Homeostasis and Fertility. *Int J Mol Sci*. 2017;18(10):2224.
4. Ferreira SR, Motta AB. Uterine Function: From Normal to Polycystic Ovarian Syndrome Alterations. *Curr Med Chem*. 2018;25(15):1792-1804.
5. Goldsammler M, Merhi Z, Buyuk E. Role of hormonal and inflammatory alterations in obesity-related reproductive dysfunction at the level of the hypothalamic-pituitary--ovarian axis. *Reprod Biol Endocrinol*. 2018;16(1):45.
6. Mattina GF, Van Lieshout RJ, Steiner M. Inflammation, depression and cardiovascular disease in women: the role of the immune system across critical reproductive events. *Ther Adv Cardiovasc Dis*. 2019;13:1753944719851950.
7. Naseri L, Akbari bazm M, Khazaei M. A Review on Therapeutic Effects of Tribulus terrestris. *J. Med. Plants*. 2019;18 (72):1-22.
8. Sharifzadeh F, Kashanian M, Koohpayehzadeh J, Rezaian F, Sheikhansari N, Eshraghi N. A comparison between the effects of ginger, pyridoxine (vitamin B6) and placebo for the treatment of the first trimester nausea and vomiting of pregnancy (NVP). *J Matern Fetal Neonatal Med*. 2018;31(19):2509-2514.
9. Moini Jazani A, Nasimi Doost Azgomi H, Nasimi Doost Azgomi A, Nasimi Doost Azgomi R. A comprehensive review of clinical studies with herbal medicine on polycystic ovary syndrome (PCOS). *Daru*. 2019;27(2):863-877.
10. Della Corte L, Noventa M, Ciebiera M, Magliarditi M, Sleiman Z, Karaman E, Catena U, Salvaggio C, Falzone G, Garzon S. Phytotherapy in endometriosis: an up-to-date review. *J Complement Integr Med*. 2020;17(3). doi: 10.1515/jcim-2019-0084
11. Appleton SM. Premenstrual Syndrome: Evidence-based Evaluation and Treatment. *Clin Obstet Gynecol*. 2018;61(1):52-61.
12. Verkaik S, Kamperman AM, van Westrhenen R, Schulte PFJ. The treatment of premenstrual syndrome with preparations of Vitex agnus castus: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2017;217(2):150-166.
13. Ring M. Women's Health: Polycystic Ovarian Syndrome, Menopause, and Osteoporosis. *Prim Care*. 2017;44(2):377-398.
14. Farahmand M, Khalili D, Ramezani Tehrani F, Amin G, Negarandeh R. Effectiveness of Echium amoenum on premenstrual syndrome: a randomized, double-blind, controlled trial. *BMC Complement Med Ther*. 2020;20(1):295.
15. Chiaramonte D, Ring M, Locke AB. Integrative Women's Health. *Med Clin North Am*. 2017;101(5):955-975.
16. Stute P, Bodmer C, Ehlert U, et al. Interdisciplinary consensus on management of premenstrual disorders in Switzerland. *Gynecol Endocrinol*. 2017;33(5):342-348.
17. Iba H, Watanabe T, Motomura S, et al. A Japanese herbal medicine attenuates anxiety-like behavior through GABAA receptor and brain-derived neurotrophic factor expression in a rat model of premenstrual syndrome. *J Pharmacol Sci*. 2021;145(1):140-149.
18. Fisher CY, Adams J, Frawley JE, Hickman LD, Sibbritt DW. Is there a role for Western herbal medicine in treating cyclic perimenstrual pain and discomfort? *Aust N Z J Obstet Gynaecol*. 2019;59(1):154-156.
19. Morehead A, McInnis LA. Herbal Supplements for Common Women's Health Issues. *Nurs Clin North Am*. 2021;56(1):69-78.

20. He W, Wang P, Chen J, Xie W. Recent progress in the total synthesis of Strychnos alkaloids. *Org Biomol Chem*. 2020;18(6):1046-1056.
21. Sim WS, Choi SI, Jung TD, Cho BY, Choi SH, Park SM, Lee OH. Antioxidant and anti-inflammatory effects of Lilium lancifolium bulbs extract. *J Food Biochem*. 2020;44(5):e13176.
22. Hoang L, Beneš F, Fenclová M, et al. Phytochemical Composition and In Vitro Biological Activity of Iris spp. (Iridaceae): A New Source of Bioactive Constituents for the Inhibition of Oral Bacterial Biofilms. *Antibiotics (Basel)*. 2020;9(7):403.
23. Akbaribazm M, Goodarzi N, Rahimi M. Female infertility and herbal medicine: An overview of the new findings. *Food Sci Nutr*. 2021;9(10):5869-5882.
24. Lee E, Jang M, Lim TG, et al. Selective activation of the estrogen receptor-β by the polysaccharide from Cynanchum wilfordii alleviates menopausal syndrome in ovariectomized mice. *Int J Biol Macromol*. 2020;165(Pt A):1029-1037.
25. Wang C, Huang Q, Liang CL, et al. Effect of cimicifuga racemosa on menopausal syndrome caused by LHRH-a in breast cancer. *J Ethnopharmacol*. 2019;238:111840.
26. Guo Y, Yin T, Wang X, et al. Traditional uses, phytochemistry, pharmacology and toxicology of the genus Cimicifuga: A review. *J Ethnopharmacol*. 2017;209:264-282.
27. Mohsen A, Fatemeh K, Leila N, Mona P, Mohammad Z, Mozafar K. Pharmacological and therapeutic properties of the Red Clover (Trifolium pratense L.): an overview of the new finding. *J Tradit Chin Med*. 2021;41(4):642-649.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.16

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 117–121

REVIEW PAPER

Magdalena Szpunar

Paired box 8 in organogenesis and oncogenesis – a review

English Division Science Club, Medical College of The University of Rzeszów, University of Rzeszów, Poland
supervisor: David Aebisher

ABSTRACT

Introduction and aim. Paired box 8 (PAX-8) is a specific transcription factor known as a protein product gene that plays an essential role in organogenesis and oncogenesis. The aim of this paper was to discuss structure and function of PAX-8. The aim of this study is to determine the utility of PAX-8 in cytology effusions with metastatic tumor.

Material and methods. This article is a review done in regards to discuss the role knowledge on PAX-8 especially in oncogenesis and organogenesis.

Analysis of the literature. Current information about PAX-8 is presented.

Conclusion. The PAX family of genes plays an important role in the formation of tissues and organs during embryonic development and in maintaining the proper functioning of certain cells after birth

Keywords. oncogenesis, organogenesis, paired box 8, PAX-8, transcription factor

Introduction

Paired box 8 (PAX-8) is a specific transcription factor known as a protein product gene that plays an essential role in organogenesis and oncogenesis. Location of PAX-8 in chromosome 2 is presented in Fig. 1. This gene belongs to the family of paired box transcription factors (PAX). The PAX genes provide instructions for making proteins that attach to specific regions of DNA, and these proteins help to control the activity of particular genes – their expression. Based on this action, PAX proteins are called transcription factors. The PAX family of genes plays an important role in the formation of tissues and organs during embryonic development and in maintaining the normal function of some cells after birth.[1-3] PAX-8 is a transcription factor involved in the regulation of organogenesis of the thyroid, kidneys and paramesonephric ducts (Müllerian ducts).[4-6] PAX-8 releases hormones important for regulating growth, brain development and metabolism. After birth, the PAX-8 protein regulates several genes that are responsible for the production of thyroid hormones.[7-9]

Aim

The aim of this study is to determine the utility of PAX-8 in cytology effusions with metastatic tumor.

Material and methods

This article is a review done in regards to discuss the role knowledge on PAX-8 especially in oncogenesis and organogenesis.

Analysis of the literature

The roles of PAX-8 in oncogenesis are diverse and include epigenetic remodeling, stimulation of proliferation, inhibition of apoptosis, and regulation of angiogenesis. PAX-8 may interact with various protein partners during tumor progression and may exhibit significant function-altering alternative splicing.[10-15] Al-

Corresponding author: Magdalena Szpunar, e-mail: szpunarmag@gmail.com

Received: 15.01.2022 / Revised: 08.02.2022 / Accepted: 13.02.2022 / Published: 30.03.2022

Szpunar M. *Paired box 8 in organogenesis and oncogenesis – a review.* Eur J Clin Exp Med. 2022;20(1):117–121. doi: 10.15584/ejcem.2022.1.16

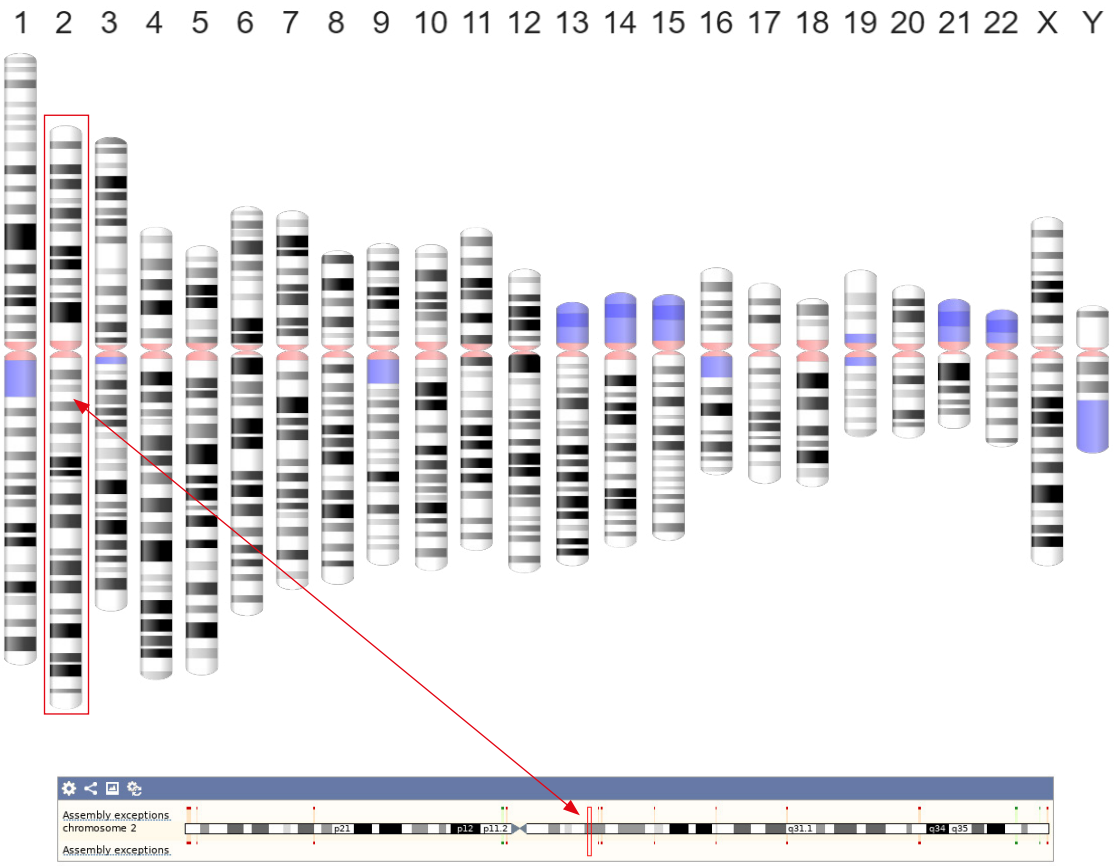

Fig. 1. PAX-8 location

ternative splicing is a fundamental regulatory process of gene expression. Defects in alternative splicing can lead to a variety of diseases, and modification of disease-causing splicing events holds great therapeutic promise.[16-19] Outcome of splicing is often influenced by extracellular stimuli and signaling cascades that converge in RNA-binding splicing regulators. in epithelial tumors of the thyroid and parathyroid glands, kidneys, thymus and female genitals. PAX-8 expression has also been reported in some neuroendocrine neoplasms, including well-differentiated pancreatic neuroendocrine tumors, duodenal and rectal carcinoids, and also in B-cell lymphomas. It has been shown that PAX-8 positivity in these tumors was due to a cross-reactivity of the antibody with the N-terminus region of PAX-6 and PAX-5 respectively. The PAX-8 gene often influences the formation of thyroid tumors (cancers). The abnormal growth then affects individual cells called follicular thyroid cells. Some of these growths, called follicular adenomas, are non-cancerous (benign). Other cancers known as follicular carcinomas are malignant.[21-26]

The combined PAX-8/PPARG gene occurs when the PAX-8 gene on chromosome 2 is linked to the PPARG gene on chromosome 3. The way PAX-8/PPARG influences thyroid follicular cell growth or why some lesions become cancerous while others are benign has not been fully elucidated. On the other hand, it is likely that the joined gene interferes with the normal control of cell division or triggers new cell actions that promote tumor formation. Rearrangements occurring within the PAX-8/PPARG genes most often concern follicular thyroid carcinomas. The PPARG gene encodes a nuclear receptor protein that regulates the expression of genes responsible for glucose and fatty acid metabolism.[26-28] The PAX-8 protein is one of the transcription factors regulating the synthesis of thyroid hormones (functional differentiation markers e.g. Tg, TPO, Slc5a5 and Tshr). As a result of the fusion involving the majority of the coding sequences of both genes, they result in the constant expression of the fusion protein, while maintaining the function of both proteins, which leads to deregulation of the metabolic processes of the cell. PAX-8/PPARG rearrangements are also correlated with lower age of onset and exposure to ionizing radiation.[27] Thyroid dysgenesis is the leading cause of congenital hypothyroidism, a condition characterized by abnormally low levels of thyroid hormones from birth, affecting nearly 1 in 2,000-3,000 newborns worldwide, as the most common endocrine disorder in newborns. PAX-8, expressed at all stages of thyroid follicular cell development, plays a

key role in thyroid morphogenesis through a complex regulatory network.[8]

At least 15 PAX-8 mutations contribute to congenital hypothyroidism, others may only slightly decrease thyroid hormone levels or have no detectable effect. Most mutations change one of the amino acids used to make the PAX-8 protein. Other mutations disrupt protein production, causing an abnormally small version of the PAX-8 protein. Almost all mutations in the PAX-8 gene prevent the PAX-8 protein from binding effectively to DNA. One mutation alters the interactions between the PAX-8 protein and other transcription factors. As a result, the PAX-8 protein cannot fulfill its role in regulating the activity of some genes.[11]

The thyroid gland is extremely small in people with the PAX-8 gene mutation. This finding suggests that mutations in the PAX-8 gene interfere with the normal growth or survival of thyroid cells during embryonic development. As a result, the thyroid gland is reduced in size and may not be able to produce the normal amount of thyroid hormones. Since cases caused by mutations in the PAX-8 gene are caused by a problem with thyroid development, they are classified as thyroid dysgenesis.[17] PAX-8 expression is increased in neoplastic renal tissues, Wilms tumors, ovarian cancer, and Müllerian carcinomas. For this reason, PAX-8 immunodetection is widely used in the diagnosis of primary and metastatic kidney tumors. Reactivation of PAX-8 (or PAX-2) expression has been reported in pediatric Wilms' tumors, nearly all subtypes of renal cell carcinoma, renal adenomas, ovarian, bladder, prostate and endometrial cancer cells. PAX-8 expression is also induced during the development of cervical cancer.[9] Ovarian cancer is one of the most dangerous and widespread gynecological cancers. It is the seventh leading cause of all cancer deaths in the world. High-grade serous cancer (HGSC) accounts for 70% of all ovarian cancer deaths. PAX-8 becomes an important histological marker in most epithelial ovarian cancers as it is present in approximately 90% of cases, especially in HGSC. PAX-8 is necessary for the proper development of the Müller's duct, which includes the fallopian tube, uterus, cervix, and the top of the vagina. In adults, it is expressed in the fallopian tube and uterine epithelium. Considering recent studies that look at events preceding HGSC tumor formation from the fallopian tube, PAX-8 appears to play an important role in the development of ovarian cancer.[10]

PAX genes code for growth regulators that are expressed in a variety of tissues and control critical events in morphogenesis. In kidneys, PAX-8 and PAX-2 are expressed in embryonic development and in certain kidney diseases related to abnormal proliferation (multiplication) of epithelial cells.[11] Genetic and cell biological studies suggest that reducing PAX protein activity in kidney cancer or polycystic kidney disease may slow the progression of these conditions. PAX proteins may be critical for tissue specificity and for epigenetic modifiers that control gene expression and chromatin structure. It may be possible to target PAX proteins to inhibit their function using small molecules. In the absence of effective treatments for kidney cancer and cystic disease, the PAX family of proteins represents new pharmaceutical targets that deserve research and further development.[20-21]

Various previous studies have provided that PAX-8 expression occurs at high levels in specific types of tumor, including thyroid and renal carcinomas and pancreatic neuroendocrine tumors. PAX-8 has been reported to be useful for the detection and differential diagnosis of ovarian carcinoma.[9-10,29]

PAX-8 has been recognized as a potential immunohistochemical marker of pancreatic neuroendocrine tumors. Haynes et al were establishing whether PAX-8 immunohistochemistry can be used as an ancillary marker of pancreatic origin for neuroendocrine tumors. Among welldifferentiated neuroendocrine tumors, only tumors from the pancreas were PAX-8 positive for 56% cases. It can help distinguish pancreatic primary tumors from tumors of other anatomic sites. Among poorly differentiated neuroendocrine carcinomas, PAX-8 expression was identified in all cases of pancreatic and thymic carcinomas.[30]

Metastatic breast carcinoma is known to morphologically mimic primary ovarian carcinoma, resulting in difficulty in distinguishing between these forms of cancer. Study with microarray analysis revealed that PAX-8 and EPAC are expressed at higher levels in ovarian compared with breast cancer.[29,32] Before that WT1 was considered to be a suitable marker to distinguish metastatic breast cancer from ovarian carcinoma. However, WT1 was later observed in focal breast cancer, causing false positive results. By contrast, PAX-8 was stained in none of the breast and almost all ovarian cancer samples, indicating that PAX-8 is a more superior marker for the differential diagnosis of ovarian and breast cancer. [29,31]

Conclusion

Expression of PAX-8 in cancer can serve as a biomarker for diagnostic and prognostic purposes. Due to its limited expression, PAX-8 is a useful immunohistochemical marker with a wide range of diagnostic applications in surgical pathology. Understanding the different mechanisms of action of PAX-8 in development and oncogenesis may identify novel malignancies that currently lack effective therapies.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, M.Sz., DA.; Writing – Original Draft Preparation, M.Sz., DA; Writing – Review & Editing, M.Sz., DA.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data supporting the results of this study shall, upon appropriate request, be available from the corresponding author.

References

1. Schwartz PE, Zheng W. Neoadjuvant chemotherapy for advanced ovarian cancer: the role of cytology in pretreatment diagnosis. *Gynecol Oncol*. 2003;90:644-650.
2. Laury AR, Perets R, Piao H, et al. A comprehensive analysis of PAX-8 expression in human epithelial tumors. *Am J Surg Pathol*. 2011;35:816-826.
3. Laury AR, Hornick JL, Perets R, et al.: PAX-8 reliably distinguishes ovarian serous tumors from malignant mesothelioma. *Am J Surg Pathol.* 2010;34:627-635
4. Li J, Abushahin N, Pang S, et al. Tubal origin of 'ovarian' low-grade serous carcinoma. *Mod Pathol.* 2011;24(11):1488-1499.
5. Kurman RJ, Vang R, Junge J, Hannibal CG, Kjaer SK, Shih IeM. Papillary tubal hyperplasia: the putative precursor of ovarian atypical proliferative (borderline) serous tumors, noninvasive implants, and endosalpingiosis. *Am J Surg Pathol*. 2011;35(11):1605-1614.
6. Vang R, Shih IeM, Kurman RJ. Fallopian tube precursors of ovarian low- and high-grade serous neoplasms. *Histopathology*. 2013;62(1):44-58.
7. DeMay RM. Cytopathology of false negatives preceding cervical carcinoma. *Am J Obstet Gynecol*. 1996;175(4 Pt 2):1110-1113.
8. Knoepp SM, Kunju LP, Roh MH. Utility of PAX-8 and PAX2 immunohistochemistry in the identification of renal cell carcinoma in diagnostic cytology. *Diagn Cytopathol*. 2012;40(8):667-672.
9. Butnor KJ, Nicholson AG, Allred DC, et al. Expression of renal cell carcinoma-associated markers erythropoietin, CD10, and renal cell carcinoma marker in diffuse malignant mesothelioma and metastatic renal cell carcinoma. *Arch Pathol Lab Med.* 2006;130(6):823-827.
10. McKnight R, Cohen C, Siddiqui MT. Utility of paired box gene 8 (PAX-8) expression in fluid and fine-needle aspiration cytology: an immunohistochemical study of metastatic ovarian serous carcinoma. *Cancer Cytopathol.* 2010;118(5):298-302.
11. Kurman RJ, Shih IeM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol*. 2010;34(3):433-443.
12. Koshiyama M, Matsumura N, Konishi I. Recent concepts of ovarian carcinogenesis: type I and type II. *Biomed Res Int*. 2014;2014:934261.
13. Przybycin CG, Kurman RJ, Ronnett BM, Shih IeM, Vang R. Are all pelvic (nonuterine) serous carcinomas of tubal origin? *Am J Surg Pathol*. 2010;34(10):1407-1416.
14. Barnes BM, Nelson L, Tighe A, et al.: Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes. *Genome Med*. 2021;13(1):140.
15. Garrido MP, Fredes AN, Lobos-González L, Valenzuela-Valderrama M, Vera DB, Romero C. Current Treatments and New Possible Complementary Therapies for Epithelial Ovarian Cancer. *Biomedicines*. 2021;10(1):77.
16. Gansmo LB, Sofiyeva N, Bjørnslett M, et al. Impact of the APOBEC3A/B deletion polymorphism on risk of ovarian cancer. *Sci Rep*. 2021;11(1):23463.
17. Schwartz PE, Zheng W. Neoadjuvant chemotherapy for advanced ovarian cancer: the role of cytology in pretreatment diagnosis. *Gynecol Oncol.* 2003;90(3):644-650.
18. Freedman OC, Dodge J, Shaw P, et al. Diagnosis of epithelial ovarian carcinoma prior to neoadjuvant chemotherapy. *Gynecol Oncol*. 2010;119(1):22-25.
19. Wang Y, Wang Y, Zheng W. Cytologic changes of ovarian epithelial cancer induced by neoadjuvant chemotherapy. *Int J Clin Exp Pathol*. 2013;6(10):2121-2128.
20. Sneige N, Fanning CV. Peritoneal washing cytology in women: diagnostic pitfalls and clues for correct diagnosis. *Diagn Cytopathol*. 1992;8(6):632-640.
21. Blok F, Roes EM, van Leenders GJ, van Beekhuizen HJ. The lack of clinical value of peritoneal washing cytology in high risk patients undergoing risk-reducing salpingo-oophorectomy: a retrospective study and review. *BMC Cancer*. 2016;16:18.
22. Wang Y, Wang Y, Li J, et al. PAX-8: a sensitive and specific marker to identify cancer cells of ovarian origin for patients prior to neoadjuvant chemotherapy. *J Hematol Oncol.* 2013;6:60.
23. Lin O. Challenges in the interpretation of peritoneal cytologic specimens. *Arch Pathol Lab Med.* 2009; 133(5):739-742.
24. Vergote I, Tropé CG, Amant F, et al. European Organization for Research and Treatment of Cancer-Gynaecological Cancer Group; NCIC Clinical Trials Group. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. *N Engl J Med*. 2010;363(10):943-953.
25. Laury AR, Hornick JL, Perets R, et al. PAX-8 reliably distinguishes ovarian serous tumors from malignant mesothelioma. *Am J Surg Pathol.* 2010;34(5):627-635.
26. Ordóñez NG. Value of estrogen and progesterone receptor immunostaining in distinguishing between peritoneal mesotheliomas and serous carcinomas. *Hum Pathol.* 2005;36(11):1163-1167.
27. Tong GX, Devaraj K, Hamele-Bena D, et al. PAX-8: a marker for carcinoma of Müllerian origin in serous effusions. *Diagn Cytopathol*. 2011;39(8):567-574.
28. Waters L, Crumley S, Truong L, Mody D, Coffey D. PAX2 and PAX-8: useful markers for metastatic effusions. *Acta Cytol*. 2014;58(1):60-66.

29. Xiang L, Kong B. PAX8 is a novel marker for differentiating between various types of tumor, particularly ovarian epithelial carcinomas. *Oncol Lett*. 2013;5(3):735-738.
30. Haynes CM, Sangoi AR, Pai RK. PAX8 is expressed in pancreatic well-differentiated neuroendocrine tumors and in extrapancreatic poorly differentiated neuroendocrine carcinomas in fine-needle aspiration biopsy specimens. *Cancer Cytopathol*. 2011;119(3):193-201.
31. Nikiforov YE. Molecular diagnostics of thyroid tumors. *Arch Pathol Lab Med*. 2011;135:569-577.
32. Schaner ME, Ross DT, Ciaravino G, et al. Gene expression patterns in ovarian carcinomas. *Mol Biol Cell*. 2003;14:4376-4386.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.17

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 122–125

CASUISTIC PAPER

Berrin Erok, Taha Oğuz Keklikoğlu, Naciye Kış, Hakan Önder

Non-ketotic hyperglycemia and diabetic striatopathy – a rare presentation with hemichorea-hemiballismus

Department of Radiology, University of Health Sciences Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey

ABSTRACT

Introduction and aim. Non-ketotic hyperglycemia (NKHG), also known as hyperosmolar hyperglycemic state (HHS) is a serious metabolic complication of diabetes mellitus (DM).The mortality rate can be up to 20% and this is much more higher than that of diabetic ketoacidosis (DKA). It is usually precipitated by an event such as pulmonary/urinary infection, myocardial infarction (MI) or stroke. In this state of metabolic derangements, central nervous system (CNS) manifestations including altered mental status with or without focal neurological deficits are prominent clinical presentations. On the other hand, HHS may also be complicated with various other CNS events. Herein, a quite rare presentation of HHS with hemichorea – hemiballismus in a 71 year old female patient with type 2 DM is presented.

Description of the case. A 71-year-old female patient type 2 DM presented to our emergency department with progressive involuntary movements on the right upper and lower extremities accompanied by semiconsciousness during the last 24 hours. On neurological examination, cranial nerves and cerebellar signs were found to be normal, as the deep tendon reflexes. However, involuntary non-rhythmic writhing movements at rest were present on her right sided extremities. The fingerstick evaluation showed marked hyperglycemia (HG). The laboratory findings were characterized with high blood glucose level without obvious acidosis compatible with HHS. In urine analysis, glucosuria without significant ketonuria was detected. On head CT, subtle hyperdensity was noted in the left neostriatal regions without any mass effect or perilesional edema, compatible with left sided diabetic striatopathy (DS).

Conclusion. Diabetic striatopathy is a quite rare presentation of HHS with hemichorea – hemiballismus. The characteristic computed tomograhy (CT) findings of associated striatopathy should be differentiated from vascular lesions that may also present with unilateral findings in the course of HHS and should not be overlooked in diabetic patients to recognise the ongoing HHS before the coma precedes.

Keywords. diabetic striatopathy, hemiballismus, hemichorea, hyperdense basal ganglia

Introduction

Non-ketotic hyperglycemia (NKHG), also known as hyperosmolar hyperglycemic state (HHS) is a serious metabolic complication of diabetes mellitus (DM). It is particularly seen in elderly diabetic women, mostly with T2DM but can also occur in type 1 DM. It was first described by Won Frerichs and Dreschfeld in 1880s.[1] It is a potentially fatal complication which can progress to coma, which is called as non-ketotic hyperosmolar coma (NKHC). The mortality rate can be up to 20% and this is much more higher than that of diabetic ketoacidosis (DKA).[2,3] It is usually precipitated by an event such as pulmonary/urinary infection, myocardial infarction (MI) or stroke. HHS is characterized with

Corresponding author: Berrin Erok, e-mail: drberrinerok@hotmail.com

Received: 30.09.2021 / Revised: 07.11.2021 / Accepted: 18.11.2021 / Published: 30.03.2022

Erok B, Keklikoğlu TO, Kış N, Önder H. *Non-ketotic hyperglycemia and diabetic striatopathy – a rare presentation with hemichorea--hemiballismus.* Eur J Clin Exp Med. 2022;20(1):122–125. doi: 10.15584/ejcem.2022.1.17

profound hyperglycemia (HG) without significant ketoacidosis & Kussmaul breathing or fruity urine. In HHS, high blood glucose level leads to severe dehydration and increased osmolarity resulting in decreased circulation with compromised end organ perfusion. As a response to decreased cerebral blood flow, the brain starts to produce osmotically active organic substances (idiogenic osmoles) in order to ensure intracellular volume by preventing fluid from moving into the extracellular space from the intracellular space.[4] In this state of metabolic derangements, central nervous system (CNS) manifestations including altered mental status with or without focal neurological deficits are prominent clinical presentations. On the other hand, HHS may also be complicated with various other CNS events, some of which are early presenting clinical manifestations before the coma.

Aim

Herein, we aimed to present a quite rare presentation of HHS with hemichorea - hemiballismus in a 71-year old female patient with type 2 DM.

Description of the case

A 71-year-old female patient with hypertention, hypercholesterolemia and long-standing poorly controlled type 2 DM presented to our emergency department with progressive involuntary movements on the right upper and lower extremities that started 4-5 days ago and were accompanied by semiconsciousness during the last 24 hours. There were no history of movement disorders, previous history of stroke or trauma. She was ill appearing with findings of dehydration including hypotension (105/65 mmHg), tachycardia (113/minute) and weak pulses. The skin and the oral mucosa were all dry with poor skin turgor. Her body temperature was raised (38.3°C). On neurological examination, cranial nerves and cerebellar signs were found to be normal, as the deep tendon reflexes. However, involuntary non-rhythmic writhing movements at rest were present on her right sided extremities. The fingerstick evaluation showed marked HG. The laboratory findings were characterized with high blood glucose level (524 mg/dL) without obvious acidosis (pH: 7.347 and HCO_3: 12 mmol/L) compatible with HHS. Serum sodium (Na^+) level was 131 mg/dl with increased anion gap of 20 mmol/L. The low level of Na was compatible with pseudohyponatremia and the corrected level was calculated as 137.8 mg/dL. The serum osmolarity was calculated as 345 mOsm/L. The other electrolytes including potassium (4.6 mg/dL), phosphorous and magnesium were all in the normal ranges, except the increased level of Cl (113 mg/dL). The creatinine, urea and uric acid levels were elevated reflecting prerenal azotemia. In urine analysis, glucosuria without significant ketonuria was detected. The white blood cell count (WBC) was increased ($25x10^3$/μL) with neutrophil predominance (90%) in addition to increased level of procalcitonin (6.45 μg/L), which reflects a pyogenic infection. Neither pneumonia on chest computed tomography (CT) nor urinary tract infection in urine analysis were detected, but methicillin resistant staphylococcus aureus (MRSA) was established in the blood culture analysis (Table 1).

Table 1. The laboratory values

	Patient's results	Normal reference values
Blood glucose	524 mg/dL	74-100 mg/dL
pH	7.347	7.37-7.45
HCO_3	12 mmol/L	21-26 mmol/L
Na^+	131 mmol/L	136-145 mmol/L
K^+	4.6 mmol/L	3.5-5.1 mmol/L
Cl^-	113 mmol/L	95-105 mmol/L
WBC	$25x10^3$/uL	$3.8\text{-}10x10^3$/uL
neutrophils	90%	45-78 %
procalcitonin	6.45 μg/L	0-0.5 μg/L

On head CT, there were no intracranial hemorrhage or cerebral edema. However, when looked at carefully, subtle hyperdensity was noted in the left neostriatal regions without any mass effect or perilesional edema, compatible with left sided diabetic striatopathy (DS) which explains the right sided hemichoreic movements (Fig. 1). She was managed with wide spectrum intravenous antibiotherapy in addition to isotonic fluid and insulin to correct the HG with close monitoring of the glucose level. Her clinical findings were resolved upon glucose control completely at first month on clinical follow up.

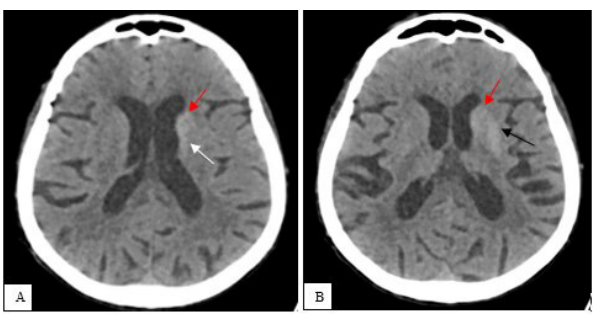

Fig. 1. Head CT showing subtle abnormal hyperdensity involving the left striatal regions; The head of caudate nucleus (a,b; red arrow), the body of caudate nucleus (a,white arrow) and putamen (b, black arrow)

Discussion

HHS is defined as plasma glucose level greater than 600 mg/dL, plasma effective osmolarity greater than 320 mOsm/L at the absence of significant ketoacidosis.[5] In the pathophysiology, the main underlying factor is decreased utilization of glucose in peripheral tissues

caused by the deficient effect of insulin. The resultant HG becomes more pronounced with gluconeogenesis and glycogenolysis by the release of counter-regulatory hormones as a response to tissue starvation, as in the pathogenesis of DKA. Also, increased serum osmolarity causes free water to be withdrawn from extracellular space and excreted in the urine, along with glucose and electrolytes, resulting in dehydration.[6] In HHS, as insulin is still produced, ketogenesis is greatly inhibited, and thus ketonemia & acidosis. In severe HG, the osmotic gradient leads to passage of intracellular water into the extracellular space resulting in Na^+ level to be measured incorrectly low, which should be corrected to assess the severity of dehydration and to adress the management. On the other hand, the anion gap must be calculated by using the measured Na^+ level instead of the corrected Na^+ and if it is found to be increased as in our patient, the cause is lactic acid production from compromised end-organ perfusion, which is also the cause of mild acidosis in HHS, if present.[7] Acute neurological manifestations in HHS are not uncommon. First of all, patients are at increased risk of thromboembolic events due to hyperosmolarity. Moreover, the earliar stages of HHS, may be complicated with various CNS events. Seizure is seen in up to 25% of the cases and is called as nonketotoic hyperglycemic seizure.[8] Much more rare than this, hemichorea-hemiballismus may also be the early presentation in HHS. It is also called as nonketotic hyperglycemic hemichorea, chorea-hyperglycemia-basal ganglia syndrome or DS. It is more commonly reported in elderly type 2 diabetic female patients as compared to males, and more frequently in Asian patients, which may suggest a genetical predisposition or may be a publication bias.[9,10] The neostriatum is composed of caudate nucleus and putamen, which involve in voluntary motor control and timing of the movement among many other functions. Hemichorea-hemiballismus is unilateral involuntary and non-rhytmic movements on one side of the body caused by contrlateral disorders of the neostriatum. DS is a very rare cause of hemichorea-hemiballismus associated with contrlateral neostriatal imaging abnormalities.[9,11] A metanalysis reported bilateral chorea with bilateral neuroimaging abnormalities in a small subset of the cases. Hemichorea with ipsilateral neuroimaging findings was very rare which was seen in only 2% of the patients while 98% of the cases had contrlateral findings.[9] Although the exact pathogenesis is not clear, the augmented sensitivity of dopaminergic receptors in neostriatum due to decline in the estrogen receptors is considered regarding the higher prevelance in elderly women.[12] Cerebrovascular insufficiency causing transient ischemia and petechial microhemorrhages were also proposed as the underlying causes. Additionally, secondary metabolic changes like depletion of gamma-aminobutyric acid and acetylcholine associated with metabolic acidosis secondary to shifting of cerebral metabolism to anaerobic pathway is also considered.[13-15] Neuroimaging findings are very typical and include contrlateral hyperdensity in the neostriatum on CT and corresponding T1w hyperintensity on magnetic resonance imaging (MRI) in the same areas.[13,15-17] Although the precise pathophysiological causes of these neuroimaging abnormalities are also not clear, petechial microhaemorrhages due to regional blood-brain barrier disruption induced by hyperviscosity resulting in erythrocyte diapedesis was suggested.[18] In addition, mineral deposits, myelin destruction and infarction with astrocytosis were also proposed.[13,17,19] Petechial microhemorrhages can explain the characteristic CT finding but some reports including susceptibility images show that hemorrhages are not present consistently. Calcification is another explanation for CT hyperdensity but reversibility of the imaging findings makes this also unlikely. Therefore, reactive astrocytosis and an abundance of gemistocytes was suggested as the most possible underlying mechanism to explain the high T1 signal on the MRI and protein desiccation during Wallerian degeneration to explain CT findings.[17] Neuroimaging studies are crucial in the evaluation of acute onset hemichorea-hemiballismus in order to differentiate vascular lesions which may also present as a unilateral basal ganglia findings. The hyperdensity conforming to the shape of the caudate nucleus and the putamen with no perilesional edema and no mass effect on CT is very characteristic to distinguish DS from hemorrhage involving basal ganglia. DS is also differentiated from a striatocapsular infarct caused by the occlusion of middle cerebral artery (MCA) by the sparing of the anterior limb of the internal capsul.[9] Besides, on CT the abnormality is not hyperdense in case of ischemic infarction and instead, faint hypodensity may present. The reversibility of neuroimaging findings upon correction of HG in contrast to the vascular lesions is the other characteristic feature of DS and usually seen on follow up imaging studies. The time for the resolution of the imaging findings was reported as three months for CT and over eight months on MRI in a study.[13] Unfortunately, our patient had no follow up imaging studies till the three monts after her presentation to show the reversibility. In addition, electroencephalography had not been performed during the clinical follow up of the patient to evaluate the changes. In the management of DS there is no need for specific treatment. The prognosis is excellent in most of the cases and clinical findings resolve with the correction of HG – hyperosmolarity. In a case series, the complete resolution of the chorea was shown in 74% of the patients over a period of one hour to ten months.[9] However, some patients may require dopamine antagonists for the relief of the longstanding symptoms. The other important aspect of HHS regarding neurological complications is the

prevention of cerebral edema during management. The serum osmolarity should be decreased so rapidly that it does not exceed the rate at which the brain can remove the idiogenic osmoles. Therefore blood glucose level should be closely monitored and should be prevented from sudden drop.

Conclusion

In conclusion, hemichorea-hemiballismus syndrome can be encountered as an early presenting clinical manifestation in HHS and imaging plays an important role in dfiferentiation of DS from vascular lesions which may also occur in the course of HHS as a triggering factor or as a complication related with hyperosmolarity and may also present with unilateral imaging findings. It should not be overlooked in diabetic patients to recognise the existing HHS and to prevent coma.

Acknowledgements

The corresponding author, Berrin Erok thanks Prof Dr Aytekin Oğuz for his great teachings on pathophysiology of diabetes.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, B.E. and H.Ö.; Methodology, B.E. and T.O.K.; Formal Analysis, B.E., N.K. and H.Ö.; Investigation, B.E. and T.O.K.; Writing – Original Draft Preparation, B.E. and T.O.K.; Writing – Review & Editing, B.E. and T.O.K.; Supervision, B.E. and H.Ö.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable.

References

1. Pasquel FJ, Umpierrez GE. Hyperosmolar hyperglycemic state: a historic review of the clinical presentation, diagnosis, and treatment. *Diabetes Care*. 2014;37(11):3124-3131.
2. Milionis HJ, Elisaf MS. Therapeutic management of hyperglycaemic hyperosmolar syndrome. *Expert Opin Pharmacother*. 2005;6(11):1841-1849.
3. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care*. 2009;32(7):1335-1343.
4. Rosenbloom AL. Hyperglycemic crises and their complications in children. *J Pediatr Endocrinol Metab.* 2007;20(1):5-18.
5. Zeitler P, Haqq A, Rosenbloom A, Glaser N., Drugs and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Hyperglycemic hyperosmolar syndrome in children: pathophysiological considerations and suggested guidelines for treatment. *J Pediatr.* 2011;158(1):9-14:14.e1-2.
6. Delaney MF, Zisman A, Kettyle WM. Diabetic ketoacidosis and hyperglycemic hyperosmolar nonketotic syndrome. *Endocrinol Metab Clin North Am*. 2000;29(4):683-705.
7. Gabow PA. Disorders associated with an altered anion gap. *Kidney Int.* 1985;27:472-483.
8. Singh BM, Strobos RJ. Epilepsia partialis continua associated with nonketotic hyperglycemia: clinical and biochemical profile of 21 patients. *Annals of neurology.* 8(2):155-160.
9. Oh SH, Lee KY, Im JH, et al. Chorea associated with non--ketotic hyperglycemia and hyperintensity basal ganglia lesion on T1-weighted brain MRI study: a meta-analysis of 53 cases including four present cases. *J Neurol Sci.* 2002;200:57-62.
10. Yacoub HA. Abnormal magnetic tesonance imaging and hemichorea asssociated with non-ketotic hyperglycemia. *J Neurol Res.* 2013;3:146-149.
11. Cheema H, Federman D, Kam A. Hemichorea–hemiballismus in non-ketotic hyperglycaemia. *J Clin Neurosci.* 2011;18:293-294.
12. Narayanan S. Hyperglycemia-induced hemiballismus hemichorea: a case report and brief review of the literature. *J Emerg Med.* 2012;43(3):442-444.
13. Chua CB, Sun CK, Hsu CW, et al. "Diabetic striatopathy": clinical presentations, controversy, pathogenesis, treatments, and outcomes. *Sci Rep.* 2020;10:1594.
14. Bhagwat NM, Joshi AS, Rao G, et al. Uncontrolled hyperglycaemia: a reversible cause of hemichorea-hemiballismus. *BMJ Case Rep.* 2013;2013:bcr2013010229.
15. Herath, H., Pahalagamage, S. & Senanayake, S. Case report of hyperglycemic nonketotic chorea with rapid radiological resolution. *BMC Med. Imaging.* 2017;17:54.
16. Wintermark M, Fischbein NJ, Mukherjee P, et al. Unilateral putaminal CT, MR, and diffusion abnormalities secondary to nonketotic hyperglycemia in the setting of acute neurologic symptoms mimicking stroke. *AJNR Am J Neuroradiol*. 2004;25(6):975-976.
17. Homaida M, Kanodia AK, Young N, et al. Diabetic striatopathy: a rare condition and diagnostic dilemma. *BMJ Case Reports CP.* 2021;14:e240141.
18. Mestre TA, Ferreira JJ, Pimentel J. Putaminal petechial haemorrhage as the cause of non-ketotic hyperglycaemic chorea: a neuropathological case correlated with MRI findings. *J Neurol Neurosurg Psychiatry.* 2007;78:549-550.
19. Ray S, Howlader S, Chakraborty S, et al. Hemichorea-hemiballism as the first presentation of type 2 diabetes. *Clin Diabetes.* 2015;33:87-89.

© Wydawnictwo UR 2022
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2022.1.18

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 126–128

CASUISTIC PAPER

Naciye Kış, Berrin Erok, Harun Kılıç, Hakan Önder

A benign entity – cerebral multinodular and vacuolating neuronal tumor

Department of Radiology, University of Medical Sciences, Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey

ABSTRACT

Introduction and aim. Multinodular and vacuolating neuronal tumor (MVNT) of the cerebrum is a rare benign, mixed glial/neuronal lesion which has been included in the recent (2016) World Health Organization (WHO) Classification of the central nervous system tumors.Most of the reported cases are remarkable with adult onset seizure in the literature.They can also be found incidentally in nonepilepsy patients with or without headache We aimed to present this unique entity with its typical magnetic resonance imaging (MRI) features.

Description of the case. A 21-year old man presented with complaint of headache that increased in frequency within the last few months.No relevant seizure or any other signs of note.He was diagnosed with MVNT by imaging andstarted to be followed-up.The repeat MRI 6 months later showed no interval changes.

Conclusion. Clinicians should be aware of that it is a do not touch lesion in asymptomatic patients with no need for biopsy or surgery and follow up imaging is sufficient when presented with the typical MRI manifestations. Surgical resection may be required for seizure control and was reported in few cases with no tumoral regrowth in the literature.

Keywords. do not touch brain tumors, magnetic resonance imaging, multinodular and vacuolating neuronal tumor

Introduction

Multinodular and vacuolating neuronal tumor (MVNT) of the cerebrum is a rare benign, mixed glial/neuronal lesion which has been included in the recent (2016) World Health Organization (WHO) Classification of the central nervous system tumors (WHO grade I).[1] However, pathological characteristics are thought to be more closer to a developmental malformation.[2] In addition, lesions with identical imaging features have been described in the cerebellum but have not been histologically confirmed and have, therefore, prudently been named as multinodular and vacuolating posterior fossa lesions of unknown significance (MV-PLUS).[3] Most of the reported cases are remarkable with adult onset seizure in the literature.They can also be found incidentally in nonepilepsy patients with or without headache.[4,5]

Aim

We aimed to present this unique entity with its typical MRI features detected in a 21-year old man.

Description of the case

A 21-year old male patient presented with complaint of headache with increased frequency within the last few months. No relevant seizure or any other signs of note. Brain MRI revealed innumerable, small, well-defined, coalescent nodules extending from the juxtacortical white matter to the juxtaventricular area of the left frontal lobe sparing the overlying cortex. They were T2w/Flair hyper-

Corresponding author: Berrin Erok, e-mail: drberrinerok@hotmail.com

Received: 18.11.2021 / Revised: 17.12.2021 / Accepted: 30.12.2021 / Published: 30.03.2022

Kış N, Erok B, Kılıç H, Önder H. *A benign entity – cerebral multinodular and vacuolating neuronal tumor.* Eur J Clin Exp Med. 2022;20(1):126–128. doi: 10.15584/ejcem.2022.1.18

intense without restricted diffusion and blooming on susceptibility weighted imaging (SWI) (Fig.1).

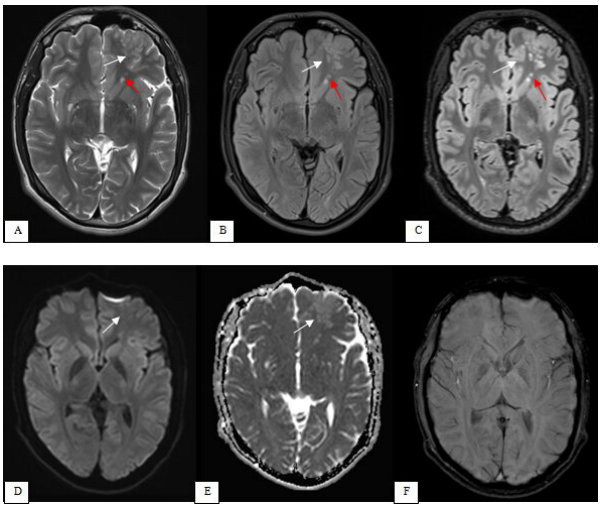

Fig. 1. Axial T2w (A), Flair (B), Flair 3D reconstruction (C) images showing a left frontal intraaxial subcortical lesion consisting of multiple, well marginated coalescent hyperintense nodules (A,B,C, white arrows). Note the lower signal intensity of the lesions in comparison to CSF on T2w images (A) and the absence of surrounding white matter hyperintensity on T2w/Flair images (A,B,C). Axial DWI (D) and ADC mapping (E) showing no restricted diffusion (D,E, arrows). Axial SWI (F) image shows no blooming. Note the superficial subcortical localization of the lesions following the gyral contour with extention to the deeper white matter areas (A,B,C, red arrow)

The lesions were isointense to gray matter on T1w images with no pathological enhancement following contrast administration and no increased perfusion on cerebral blood flow (CBV) map on perfusion imaging (Fig. 2).

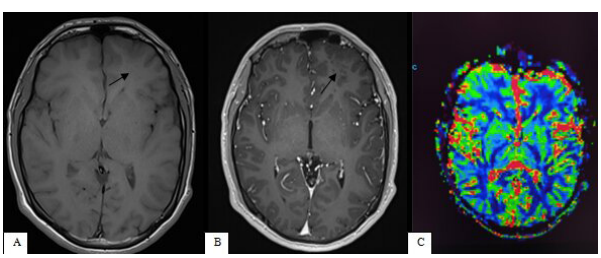

Fig. 2. Axial T1w (A) and postcontrast T1w (B) images showing the lesions are isointense to the gray matter (A, black arrow) with no pathological enhancement (B, black arrow). No elevated CBV was depicted on CBV perfusion image (C)

MR spectroscopy showed no obvious abnormal peaks (Fig. 3). With the typical radiological manifestations MNVT was considered and he was decided to be followed up without surgery. The repeat MRI 6 months later showed no interval changes.

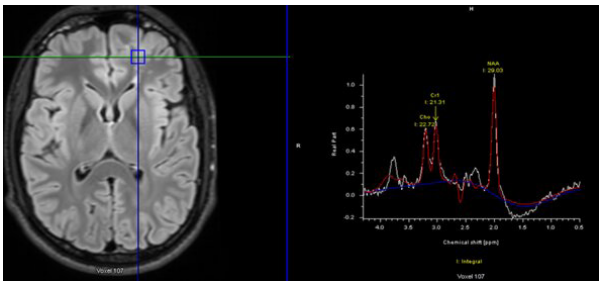

Fig. 3. The MRI Spectroscopy image shows no abnormal peaks in association with the lesion

Discussion

MVNT of cerebrum was first described with a case series of 10 patients by Huse et al. as non-neurocytic, purely neuronal tumors most commonly located in the temporal lobes (7 cases of 10) with presentation of adult onset seizure.[5] In the study of Nunes et al. that retrospectively evaluated 33 MVNT cases with compatible imaging features, 4 of which proven by biopsy and the remaining without interval change for at least 24 months follow up, the mean age of diagnosis was found to be 39 years with slight female predominance 1.4/1. In this study, 9 (27%) lesions were in the parietal lobe, 8 (24%) were in the frontal lobe, 6 (18%) were in the temporal lobe and 2 (4%) lesions were located in the occipital lobe with 8 (24%) involving more than one lobes.[6] On radiological imaging, this entity is characterized with a cluster of supratentorial, intraaxial, multiple rounded/ovoid nodules without mass effect located principally in the subcortical/juxtacortical white matter with usually normal overlying cortex.[4,6] However, there are also some reports showing that the overlying cortex may also be involved.[5,6] In our patient the lesions were also located predominantly in this characteristic location but also had extention to the deeper white matter areas as opposed to the more confined lesions reported in the literature, usually located within the deep cortical ribbon and the superficial white matter. The frequent presentation with late onset epilepsy can be explained with the juxtacortical location, but incidental detection is also common. Our patient was presented with non-focal headache as reported in one case having also left frontal MVNT in the case series of Nunes et al.[6] The signal characteristics include T2w hyperintensity but less than that of CSF with no suppression on Flair images. In our patient there was no surrounding white matter hyperintensity on T2W/Flair images as commonly reported in the literature but, confluent T2/FLAIR hyperintensity sparing the cortex may be present.[6] DWI shows increased signal intensity with increased corresponding ADC value in relation with T2-shine through effect

rather than true restricted diffusion. In addition, there is no blooming on SWI images. Following contrast administration they show no enhancement as in our case, but some faint focus of enhancement was demonstrated in a few cases.[5,6] In the qualitative evaluation of the CBV map, there was no increased perfusion compared to the contrlateral normal parenchyma. On MRS evaluation although the contrlateral normal parenchyma was not sampled there was no obvious pathological peaks in our patient. Furthermore, in some studies in the literature mildly increased choline/creatine ratio was also demonstrated.[4]

Although MVNT of cerebrum is considered as a unique cytoarchitectural pattern of gangliocytoma (WHO grade I), it is very different from gangliocytomas on imaging which had both cystic and enhancing solid components with cortical involvement indistinguihable from ganglioglioma.[1,7] The imaging differentials may include dilated perivascular spaces which are usually more elongated along vessel long axis and are differentiated with attenuation on Flair images. Dysembryoplastic neuroepethelial tumor (DNET) may also be considered with its peripheral location frequently in the frontotemporal lobes.However, DNET is generally cortical rather than subcortical and is associated with gyral expansion.In addition, there is usually some suppression on Flair images with frequent well defined bright FLAIR rim.[8] Focal cortical dysplasia may also have T2w hyperintensity deep to the cortex but is associated with overlying cortical thickening.[9]

In the management of MVNT in asymptomatic patients follow up radiological imaging is sufficient without need for biopsy. Surgical resection may be required for seizure control and was reported in few cases with no tumoral regrowth in the literature.[5,6,10]

Conclusion

MVNT is a benign entity that may be detected in patients presenting with seizure or may be found incidentally in patients with or without headache. Clinicians should be aware of that this is a don't touch lesion in asymptomatic patients with no need for biopsy or surgery when presented with the typical MRI manifestations.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, N.K. and B.E.; Methodology, N.K., B.E. and H.Ö.; Formal Analysis, B.E. and N.K.; Investigation, B.E., H.K. and N.K.; Writing – Original Draft Preparation, B.E. and N.K.; Writing – Review & Editing, B.E., N.K. and H.K.; Supervision, HÖ

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data sets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable.

References

1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131(6):803-820.
2. Thom M, Liu J, Bongaarts A, et al. Multinodular and vacuolating neuronal tumors in epilepsy: dysplasia or neoplasia? *Brain Pathology*. 2018;28(2):155.
3. Lecler A, Bailleux J, Carsin B, et al. Multinodular and Vacuolating Posterior Fossa Lesions of Unknown Significance. *AJNR Am J Neuroradiol*. 2019;40(10):1689-1694.
4. Makrakis D, Veneris S, Papadaki E. Multinodular and vacuolating neuronal tumor incidentally discovered in a young man: Conventional and advanced MRI features. *Radiology Case Reports*. 2018;13(5):960-964.
5. Huse J, Edgar M, Halliday J, Mikolaenko I, Lavi E, Rosenblum M. Multinodular and vacuolating neuronal tumors of the cerebrum: 10 cases of a distinctive seizure-associated lesion. *Brain Pathol.* 2013;23(5):515-524.
6. Nunes RH, Hsu CC, da Rocha AJ, et al. Multinodular and Vacuolating Neuronal Tumor of the Cerebrum: A New "Leave Me Alone" Lesion with a Characteristic Imaging Pattern. *AJNR A. J Neuroradiol.* 2017;38(10):1899.
7. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol.* 2021;23(8):1231-1251.
8. Parmar HA, Hawkins C, Ozelame R, Chuang S, Rutka J, Blaser S. Fluid-attenuated inversion recovery ring sign as a marker of dysembryoplastic neuroepithelial tumors. *J Comput Assist Tomogr.* 2007;31(3):348-353.
9. Kabat J, Król P. Focal cortical dysplasia - review. *Pol J Radiol*. 2012;77(2):35-43.
10. Fukushima S, Yoshida A, Narita Y, et al. Multinodular and vacuolating neuronal tumor of the cerebrum. *Brain Tumor Pathol*. 2015;32(2):131-136.

ISSN 2544-1361 (online)

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2022; 20 (1): 129–136

Instructions for Authors

About the Journal

The European Journal of Clinical and Experimental Medicine (Eur J Clin Exp Med) is an open access journal, and all articles are free to access, download, share, and re-use. The Eur J Clin Exp Med is a peer-reviewed, international scientific journal that publishes full-length articles on topics within medical science. The journal welcomes submissions of articles on current advances in life and health sciences, clinical and experimental medicine, and related disciplines.

Open access and creative commons

All articles are published with **free** open access under the CC-BY Creative Commons attribution license (the current version is CC-BY, version 4.0. If you submit your paper for publication by the Eur J Clin Exp Med, you agree to have the CC-BY license applied to your work. Under this Open Access license, you, as the author, agree that anyone may download and read the paper for free. In addition, the article may be reused and quoted provided that the original published version is cited. This facilitates freedom in re-use and also ensures that Eur J Clin Exp Med content can be mined without barriers for the research needs.

Article processing charges

The Eur J Clin Exp Med is an open access journal and does **not** levy an article processing charge. There are no submission, color, or page charges for all article types.

Copyright Statement

Authors of articles published in the Eur J Clin Exp Med retain copyright on their articles, except for any third-party images and other materials added by the Eur J Clin Exp Med which are subject to copyright of their respective owners. Authors are therefore free to disseminate and re-publish their articles, subject to any requirements of third-party copyright owners and subject to the original publication being fully cited. Visitors may also download and forward articles subject to the citation requirements. The ability to copy, download, forward or otherwise distribute any materials is always subject to any copyright notices displayed. Copyright notices must be displayed prominently and may not be obliterated, deleted or hidden, totally or partially.

Ethics in publishing

The Eur J Clin Exp Med takes responsibility of enforcing rigorous peer-review together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, inappropriate authorship credit, and the like, do arise. The Eur J Clin Exp Med takes such publishing ethics issues very seriously and our editors are trained to proceed in such cases with a zero tolerance policy. The Eur J Clin Exp Med is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE).

Editorial and submission policies

When you submit a manuscript to the Eur J Clin Exp Med we will take it to imply that the manuscript has not already been published or submitted elsewhere. If similar or related work has been published or submitted elsewhere, then you must provide a copy of this work with the submitted manuscript. You may not submit your manuscript elsewhere while it is under consideration in the Eur J Clin Exp Med. If the manuscript includes personal communications, please provide a written statement of permission from any person who is quoted. Permission by email is acceptable.

We reserve the right to reject a paper even after it has been accepted if it becomes apparent that there are serious problems with its scientific content, or our publishing policies have been violated.

Author responsibilities

Authorship provides credit for a researcher's contributions to a study and carries accountability. Authors are expected to fulfil the criteria below (adapted from McNutt et al., Proceedings of the National Academy of Sciences, 2018, 201715374; DOI: 10.1073/pnas.1715374115):

— **Each author** is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it
— **AND** to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);
— **AND** to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

The Eur J Clin Exp Med does not require all authors of a research paper to sign the cover letter upon submission, nor do they impose an order on the list of authors. Submission to the Eur J Clin Exp Med is taken by the publication to mean that all the listed authors have agreed to all of the contents. The corresponding (submitting) author is responsible for having ensured that this agreement has been reached, and for managing all communication between the publication and all co-authors, before and after publication.

Author contributions statements

Authors are required to include a statement of responsibility in the manuscript (at the end of the main text, before the 'References' section) that specifies the contribution of every author. For articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.".

Corresponding author – responsibilities

The corresponding (submitting) author is solely responsible for communicating with the Eur J Clin Exp Med and for managing communication between co-authors. Before submission, the corresponding author ensures that all authors are included in the author list, its order has been agreed by all authors, and that all authors are aware that the paper was submitted.

A confidential process

The Eur J Clin Exp Med treats the submitted manuscript and all communication with authors and referees as confidential. Authors must also treat communication with the Eur J Clin Exp Med as confidential: correspondence with the Eur J Clin Exp Med, referee reports and other confidential material must not be posted on any website or otherwise publicized without prior permission from the Eur J Clin Exp Med publishing team, regardless of whether or not the submission is eventually published. Our policies about posting preprints and post prints, and about previous communication of the work at conferences or as part of a personal blog or of an academic thesis, are described in the Confidentiality section.

Referee suggestions

During the submission process, please suggest three potential reviewers (names and institutional e-mail addresses) with the appropriate expertise to review the manuscript, but please keep in mind that we are not obliged to follow these recommendations. The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may suggest reviewers from among the authors that you frequently cite in your paper. You may also name a limited number of scientists who should not review your paper (up to 3 named individuals or laboratories); these exclusions will be honored. The decision of the Editorial Board Member on the choice of referees is final.

Ethics, use of experimental animals, and human participants

For articles in the Eur J Clin Exp Med reporting experiments on live vertebrates and/or higher invertebrates, the methods section must include a statement: (i) identifying the institutional and/or licensing committee approving the experiments, including any relevant details; (ii) confirming that all experiments were performed in accordance with relevant guidelines and regulations.

For research involving human participants, authors must identify the committee that approved the research, confirm that all research was performed in accordance with relevant guidelines/regulations, and include in their manuscript a statement confirming that informed consent was obtained from all participants and/or their legal guardians.

Authors may be required to submit, on request, a statement from the research ethics committee or institutional review board indicating approval of the research.

Competing interests policy

In the interests of transparency and to help readers to form their own judgements of potential bias, authors must declare any competing financial and/or non-financial interests in relation to the work described. For the purposes of this policy, competing interests are defined as financial and non-financial interests that could directly undermine, or be perceived to undermine, the objectivity, integrity and value of a publication, through a potential influence on the judgements and actions of authors with regard to objective data presentation, analysis and interpretation. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Competing interests statement format guidelines

The statement included in the article file must be explicit and unambiguous, describing any potential competing interest (or lack thereof) for EACH contributing author.

Examples of declarations are:

— Competing interests: The author(s) declare no competing interests.
— Competing interests: Dr X's work has been funded by A. He has received compensation as a member of the scientific advisory board of B and owns stock in the company. He also has consulted for C and received compensation. Dr Y and Dr Z declare no potential conflict of interest.
— Competing interests: "This work was supported by the [Funding Agency] under Grant [number]."

Peer-reviewers

The Eur J Clin Exp Med invites peer-reviewers to exclude themselves in cases where there is a significant conflict of interest, financial or otherwise. However, just as financial interests need not invalidate the conclusions of an article, nor do they automatically disqualify an individual from evaluating it. We ask peer-reviewers to inform the editors of any related interests, including financial interests as defined above that might be perceived as relevant. Editors will consider these statements when weighing peer-reviewers' recommendations.

Availability of materials and data

In order to maintain the integrity, transparency and reproducibility of research records, authors are encouraged to make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Data availability statement format guidelines

The statement should be provided as a separate section (titled 'Data Availability') at the end of the main text, before the 'References' section. Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Depending on the data described in the manuscript, data availability statements commonly take one of the following forms, or can be a composite of the statements below:

— The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].
— The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
— All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).
— The datasets generated during and/or analyzed during the current study are not publicly available due to [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
— No datasets were generated or analyzed during the current study.
— The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

Correction and retraction policy

The Eur J Clin Exp Med operates the following policy for making corrections to its peer-reviewed content.

Publishable amendments must be represented by a formal online notice because they affect the publication record and/or the scientific accuracy of published information. Where these amendments concern

peer-reviewed material, they fall into one of four categories: Publisher Correction (formerly Erratum), Author Correction (formerly Corrigendum), Retraction or Addendum.

Publisher Correction (formerly Erratum). Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper or the reputation of the authors or the journal.

Author Correction (formerly Corrigendum). Notification of an important error made by the author(s) that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.

Retraction. Notification of invalid results. All co-authors must sign a Retraction specifying the error and stating briefly how the conclusions are affected, and submit it for publication. In cases where co-authors disagree, the in-house editors may seek advice from independent referees and impose the type of amendment that seems most appropriate, noting the dissenting author(s) in the text of the published version.

Addendum. Notification of additional information. Addenda are published when the in-house editors decide that the addendum is crucial to the reader's understanding of a significant part of the published contribution.

Peer-review process

Initial checks

Once submitted, your manuscript will be assigned to a member of our Editorial Board, who will read the paper and decide whether it is appropriate for the journal. Manuscripts that are within scope and seem, on initial assessment, to be technically sound and scientifically valid, will be sent to external reviewers. Copies of any papers containing similar or related work under consideration or in press at other journals must be included with the submission.

Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission.

Peer review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. Reviewers will be able to access your manuscript securely using our online system, whilst maintaining referee anonymity. A double-blind review is applied, where authors' identities are unknown to reviewers and vice versa. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

Editorial Decision

After considering the reviewer reports the Editorial Board Member will make one of the following decisions:

— Accept outright,
— Request a minor revision, where authors revise their manuscript to address specific concerns,
— Request a major revision, where authors revise their manuscript to address significant concerns and perhaps undertake additional work,
— Reject outright.

The final decision is made by the Editor-in-Chief.

Revisions

In cases where the referees or Editorial Board Member has requested changes to the manuscript, you will be invited to prepare a revision. The decision letter will specify a deadline for submission of a revised manuscript. Once resubmitted, the manuscript may then be sent back to the original referees or to new referees, at the Editorial Board Member's discretion.

A revised manuscript should be submitted via the revision link provided in the decision letter, and not as a new manuscript. The revision should also be accompanied by a point-by-point response to referees explaining how the manuscript has been changed. Please ensure that all issues raised have been addressed in the first round of revision. Where the authors disagree with a reviewer, they must provide a clear response.

Final submission and acceptance

When all editorial issues are resolved, your paper will be formally accepted for publication. Once accepted, the manuscript will undergo professional copy-editing, English editing, final corrections, pagination, and, publication on the http://www.ejcem.ur.edu.pl/. The Eur J Clin Exp Med reserves the right to make the final decision about matters of style and the size of figures.

Appeals

Even in cases where the Eur J Clin Exp Med does not invite resubmission of a manuscript, some authors may ask the Editorial Board to reconsider a rejection decision. These are considered appeals, which, by policy, must take second place to the normal workload. In practice, this means that decisions on appeals often take several weeks. Only one appeal is permitted for each manuscript, and appeals can only take place after peer review. Final decisions on appeals will be made by the Editorial Board Member handling the paper.

Decisions are reversed on appeal only if the relevant Editorial Board Member is convinced that the original decision was a serious mistake. Consideration of an appeal is merited if a referee made substantial errors of fact or showed evidence of bias, but only if a reversal of that referee's opinion would have changed the original decision. Similarly, disputes on factual issues need not be resolved unless they were critical to the outcome.

If an appeal merits further consideration, the Editorial Board Member may send the authors' response and the revised paper out for further peer review.

ORCID

The Eur J Clin Exp Med supports the use of ORCID. The Eur J Clin Exp Med mandates ORCID iDs for all submitting authors; this is published on the final article to promote discoverability and credit. Please provide the ORCID iDs of the authors in the title page.

Submission guidelines

Submission Process

Manuscripts for the Eur J Clin Exp Med should be submitted online at https://mc04.manuscriptcentral.com/pmur. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Cover letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Accepted File Formats

Authors must use Microsoft Word to prepare their manuscript. Please insert your tables, graphics (schemes, figures, etc.) in the main text after the paragraph of its first citation.

In most cases, we do not impose strict limits on word count or page number. However, we strongly recommend that you write concisely and stick to the following guidelines:

— We encourage not exceeding 20 pages for original and review papers, and 8 pages for case reports of standard computer text (1800 signs on a page).
— The main text should be no more than 4,500 words (not including Abstract, Methods, References and figure legends).
— The title should be no more than 20 words.
— The abstract should be no more than 250 words.
— Recommended font: Times New Roman, 12 points.
— Manuscript text should be double-spaced. Do not format text in multiple columns.

Types of Publications

Manuscripts submitted to the Eur J Clin Exp Med should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information.

Reviews. These provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.

The Eur J Clin Exp Med accepts also the following types of submissions: case reports, letters to the editor, commentaries, book reviews, and reports from scientific meetings and conferences.

Reporting guidelines

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

Please refer to existing guidelines for reporting methodology; e.g.:

— AGREE guidelines for clinical practice guidelines
— ARRIVE guidelines for *in vivo* animal studies
— CARE guidelines for clinical case reports
— CONSORT guidelines for clinical trials
— PRISMA guidelines for systematic reviews and meta-analyses
— SPIRIT for clinical trials
— STARD guidelines for studies of diagnostic accuracy
— STROBE guidelines for observational studies

Manuscript Preparation

Your paper should consist of the following parts. Title page should be supplied as a **separate** file.

Research manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords.
— Research manuscript sections: Introduction, Aim, Materials and Methods, Results, Discussion, Conclusions.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions,

Conflicts of Interest, Data Availability, Ethics Approval, References.

Research manuscript sections:

— *Introduction*

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

— *Material and methods*

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

— *Results*

Results should be clear and concise. The section may be divided into subsections, each with a concise subheading. Tables and figures central to the study should be included in the main paper. Do not use the term "significant" unless p-values are provided. Show p-values to 2 or 3 decimal places. The Results section should be written in past tense.

— *Discussion*

This should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

— *Conclusions*

Summarize the work's findings, state their importance, and possibly recommend further research.

Review manuscripts should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords
— Literature review sections
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, References.

Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

Case reports should comprise:

— Title page: Title, Author list, Affiliations, Abstract, Keywords
— Case reports should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
— Back matter: Supplementary Materials, Acknowledgments, Funding Statement, Author Contributions, Conflicts of Interest, Data Availability, Ethics Approval, References.

Please note that tables with captions and figures with captions should be should be inserted immediately after the first paragraph in which they are cited.

Language Style

Manuscripts must be submitted in English (American or British usage is accepted, but not a mixture of these).

Title page

These sections should appear in all manuscript types:

Title: The title of your manuscript should be concise and informative. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

Author List and Affiliations: Authors' full first and last names must be provided. For each affiliation provide the details in the following order: department, institution, city, country. If available, the e-mail address of each author should also be provided. At least one author should be designated as *corresponding author*, and his or her email address and other details should be included at the end of the affiliation section.

Abstract: The abstract should be a total of about 250 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts: *Introduction and aim:* Place the question addressed in a broad context and highlight the purpose of the study; *Material and methods*: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. *Results:* Summarize the article's main findings; and *Conclusion*: Indicate the main conclusions or interpretations.

Keywords: Three to six pertinent keywords need to be added after the abstract in alphabetical order. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Back Matter

Supplementary Materials: Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate

the name and title of each element as follows Figure S1: title, Table S1: title, etc.

Acknowledgments: Thank all of the people who helped with the research but did not qualify for authorship. Acknowledge anyone who provided intellectual assistance, technical help, or special equipment or materials.

Funding Statement: All sources of funding of the study should be disclosed.

Author Contributions: Authors must supply an Author Contribution Statement as described in the *Author contributions statements* section.

Conflicts of Interest: Authors must supply a competing interests statement. For more details please see *Competing interests policy.*

Data Availability: Authors must include a Data Availability Statement in all submitted manuscripts; see *Availability of materials and data* section for more information.

Ethics approval: Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

References: References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, Reference Manager or Zotero to avoid typing mistakes and duplicated references.

References style

References should be prepared according to the American Medical Association style.

Examples: The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level.[1,2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3] Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

A citation should contain a maximum of 6 authors. When an article has more than six authors, only the first three names should be given by adding 'et al.' If the source does not have any authors, the citation should begin with the title.

Journal titles should be given in brief according to the Index Medicus standard.

The following are examples of individual citations made according to the required rules of editing and punctuation:

— **Article from a journal, number of authors from 1 to 6**

Author AA, Author BB, Author CC. Title of article. *Abbreviated Journal Title*. Year;Volume(Issue):Page-Page.

Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. *Comput Methods Programs Biomed*. 2016;134(2):72-78.

Wolf ZR. Nursing practice breakdowns: Good and bad nursing. *Medsurg Nursing*. 2012;21(1):16-36.

— **Article from a journal, number of authors more than 6**

Author AA, Author BB, Author CC, et al. Title of article. *Abbreviated Journal Title*. Year;Volume (Issue):Page-Page.

Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. *Cell Rep*. 2017;18:1215-1228.

Jordan J, Toplak H, Grassi G, et al. Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. *J Hypertens*. 2016;34:1678-1688.

— **Article from an online journal: DOI**

Author AA, Author BB. Title of article. *Abbreviated Journal Title*. Year;Volume(Issue):Page-Page. doi:xx.xxxx/xxxxxxxxxxxxx

Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13-year-olds. J Hum Nutr Diet. 2012;25:43-49. doi: 10.1111/j.1365-277X.2011.01184.x.

Cogulu O, Schoumans J, Toruner G, Demkow U, Karaca E, Durmaz AA. Laboratory Genetic Testing in Clinical Practice 2016. Biomed Res Int. 2017;2017:5798714. doi: 10.1155/2017/5798714.

— **Websites**

Webpage title. Name of Website. URL. Published or Updated date. Accessed date.

Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiticholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.

Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.

— **Book**

Author AA, Author BB. *Title of Work*. Location: Publisher; Year:Page-Page

Doane GH, Varcoe C. *Family Nursing as Relational Inquiry: Developing Health– Promoting Practice*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:25-28.

London ML, Ladewig PW, Ball JW, et al. *Maternal & Child Nursing Care*. Upper Saddle River, NJ: Pearson Education; c2011:101-103.

— **Chapter in a book**
Author AA. Title of Work. Editor AA, Editor BB, eds. Location: Publisher; Year:PagePage.
Goodman LS, Brunton LL, Chabner B, Knollmann BC. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. Brunton LL, ed. New York, NY: McGraw-Hill; 2011:99.
NOTE: The editorial board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

Preparing Figures, Schemes and Tables

File for Figures and Schemes must be provided during submission and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.

All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).

All Figures, Schemes and Tables should have a short explanatory title (not on the figure itself) and caption. Tables should present new information rather than duplicating what is in the text. Readers should be able to interpret the table without reference to the text.

All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Please supply editable tables in appropriate place in the main text. Do not submit your tables in separate files.

Abbreviations

The journal requires using only standard abbreviations. Abbreviations should be defined in parentheses the first time they appear in the abstract, main text and in figure or table captions and used consistently thereafter. Ensure consistency of abbreviations throughout the article. Keep abbreviations to a minimum.

SI Units

SI Units (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.