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ORIGINAL PAPER

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Effect of high fat diet on structure of liver and gallbladder of adult male mice – an experimental study

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ABSTRACT

Introduction. High fat diet (HFD) intake induces obesity and adversely affects different body organs including liver and gallbladder.

Aim. It was to clarify the effects of HFD on the liver and gallbladder structure using light microscopic (LM) examination.

Material and methods. 16 healthy adult male mice were equally divided into 2 groups. Control group mice were fed normal diet. HFD group was fed using HFD. At the end of the 8-week experiment, mice were anesthetized. Liver and gallbladder were removed and prepared to histological processing. Sections were stained with hematoxylin and eosin (H&E) and immunostaining for cyclooxygenase-2 (COX-2) cellular localization. Oil Red O (ORO)-stained frozen liver sections were prepared.

Results. H&E-stained sections of HFD group revealed rounded swollen hepatic cells with pale cytoplasm suggesting cellular ballooning. Dilated congested sinusoids and portal vein, cellular degeneration and collection of inflammatory cells were observed between hepatic cells and in portal region. Gallbladder sections showed epithelial stratification and cellular vacuolation. Strong immunoexpression of COX-2 was observed in Kupffer and hepatic cells of the liver and gallbladder mucosal epithelial cells.

Conclusion. HFD is suggested to alter the normal histological features of liver and gallbladder represented by fatty liver and gallbladder epithelial hyperplasia and inflammatory reaction.

Keywords. COX-2, fatty liver, gallbladder epithelium, immunoexpression, Oil Red O staining

Introduction

Liver is the largest metabolizing organ in the body which regulates homeostasis of different body systems. Its main functions are synthesis, storage and metabolism of fats, carbohydrates and proteins, detoxification of hormones, drugs and toxins and excretion of bilirubin.[1] The gallbladder is a grey-blue pear-shaped organ found in a fossa at the right side of inferior surface of liver. Its main function is storage, concentration and then excretion of bile secreted by the liver; it has storage capacity of about 50 ml.[2] Despite the difference in the structure and functions of two organs, the liver and gallbladder are closely related in development where they firstly arise as a bud from the summit of duodenum. The bud then subdivides into cranial pars hepatica forming the liver and caudal pars cystica that dilates at its terminal end to form the gallbladder.[3]

High fat diet (HFD) intake is a large problem which

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faces modern societies where lifestyle has been changed and fast food and take-away foods become common. It induces obesity and adversely affects different body organs including liver and gallbladder.[4] Excess dietary fats could lead to fatty acids' deposition in hepatic cells with the possible development of nonalcoholic fatty liver disease (NAFLD).[5] It was recorded that about 25% of the world population are affected by NAFLD.[6]

Lipid deposition in tissues can be detected by use of ORO staining of frozen tissues' sections. ORO is a fat-soluble dye staining neutral lipids and cholesteryl esters but not membranes. In staining lipids, it depends on its hydrophobic character through moving away from the solvents to associate with lipids within tissues.[7]

Cyclooxygenase (COX) is an enzyme involved in production of prostaglandins (PGs) and other inflammatory mediators. It includes two subtypes; COX-1 and COX-2. COX-1 is responsible for physiological functions and cytoprotection of body organs. However, COX-2 is responsible for production PGs involved in inflammation. COX-2 is excessively expressed in pathological conditions of liver and gallbladder including hepatic inflammation, hepatitis, cirrhosis and cholecystitis while its expression is weak in physiological condition.[8] Therefore, COX-2 could be considered as an inflammatory marker.

Gall stones are a common public health problem; about 10-15% of the world adult populations are affected. Such disease is closely associated with the nutritional lifestyle. Its prevalence is increasing proportionate with the worldwide increase in obesity prevalence. The NAFLD is considered a risk factor for occurrence of gallbladder stone disease.[9,10] The main component of gallstones is cholesterol. Therefore, hypercholesterolemia is a predisposing factor for the development of gallstones and cholecystitis.[11,12]

In addition, it was reported that functional changes in the form of decreased gallbladder response to neurotransmitters and diminished contractility have been reported in association with high fat diet.[13] Also, Li et al. recorded association between fatty liver change and gallbladder diseases.[14] They stated that fatty liver can be considered together with age a predictor for the risk of developing gallbladder disease.

Inflammation in association with fatty diet has been reported by Yu et al. in the liver and also by Van Erpecum et al. in the gallbladder.[15,16] They recorded granulocytes and lymphocytic infiltrations in lamina propria. However, Lavoie et al. observed mucosal inflammation in the gallbladder of mice fed fatty diet; and they stated that inflammation was preceded by muscular dysfunction.[17]

Aim

Despite the well-documented adverse effects of HFD, previous studies examining its effect on the histological structure of the liver and gallbladder are scarce. Therefore, in this study, we aimed to elucidate the potential adverse effects of HFD administration on the structure of these organs using the LM examination. This was performed by routine histological examination using H&E as well as detection of lipid accumulation by ORO within tissues and immunohistochemical analysis of inflammation using COX-2.

Material and methods

Animals and study design

Sixteen healthy adult male mice were used in this study. Their weights ranged from 18 to 22 gm. The mice were bought from animal house unit of the faculty of Veterinary Medicine Suez Canal University. They were kept under good aseptic & healthy conditions and standardized environment (e.g., temperature 23±2°C). The study was approved by The Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC); reference number (Zu-IACUC/3/F/167/2019). Animals were divided into two groups; each contains eight mice; as the followings:

- G I (Control group): animals were fed normal chow diet for 8 weeks.
- G II (HFD group): animals were fed HFD; 15% total animal fat, 2% cholesterol (02780, LOBA Chemie, India) and 0.5% cholic acid (C-02682 Oxford Lab Chem) for eight weeks[18].

At the end of experiment, mice were anesthetized using thiopental. Their abdomens were opened to remove the liver and gallbladder that were immediately fixed in 10% neutral-buffered formalin solution for histological preparation. Parts of liver tissues were kept frozen (at -80°C) for the procedure of the ORO staining.

LM techniques

Liver and gallbladder were histologically processed and embedded in paraffin wax.[19] Then, 5 um thick sections were obtained and stained with H&E.[20]

Frozen liver tissue was embedded in Tissue-Tek as described by Mehlem et al.[7] Tissue sectioning was done and 12 µm thick sections were obtained to be stained with ORO and examined with LM.

Immunohistochemical staining for COX-2 (Inflammatory marker)

Liver and gallbladder tissues embedded in paraffin were cut into 5 µm thick sections, and then sections were processed for immunohistochemical staining by avidin biotin peroxidase method for COX-2 (DAKO, Germany) immunodetection.[21]

Morphometrical study

The area percentage (%) of ORO liver staining and COX-2 immunoexpression in liver and gallbladder were measured using Image J software.

Statistical study

The data were presented in the form of mean ± standard deviation (M±SD) statistical comparison of the mean values was done by independent sample student t test using Graph Prism 5.01 Software. The *P* value less than 0.05 was considered significant.

Results

H&E-stained sections

H&E-stained liver sections of the control groups showed normal hepatic lobule structure; polygonal acidophilic hepatic cells with single or two vesicular nuclei, arranged in cords around the central vein with hepatic sinusoids between the cords. (Fig. 1a). Portal vein that was a thin-walled vessel with wide lumen and bile ductile with its cuboidal epithelial lining were seen in the portal region (Fig. 1b). In the HFD group marked structural changes were observed; rounded swollen hepatic cells with pale cytoplasm were seen suggesting cellular ballooning. Dilated congested sinusoids, cellular degeneration and collection of inflammatory cells between the hepatic cells & in the portal region were observed. Also, marked dilatation and congestion of the Portal vein was seen (Fig. 1c, d).

Gallbladder sections stained with H&E showed in the control group mucosa consisted of one layer of simple columnar epithelium with cells having oval nuclei and lamina propria, underlying the mucosa, formed of loose connective tissue. The mucosal folds with central core of lamina propria were seen (Fig. 2a). In the HFD group epithelial stratification and cellular vacuolation were observed (Figs. 2b).

ORO-stained frozen liver sections

ORO-stained sections of the liver of control group revealed low lipid deposition specified by a weak cytoplasmic red staining of the hepatic cells (Fig. 2c). In the HFD group, there was excess lipid deposition specified by strong cytoplasmic red staining of the hepatic cells (Fig. 2d).

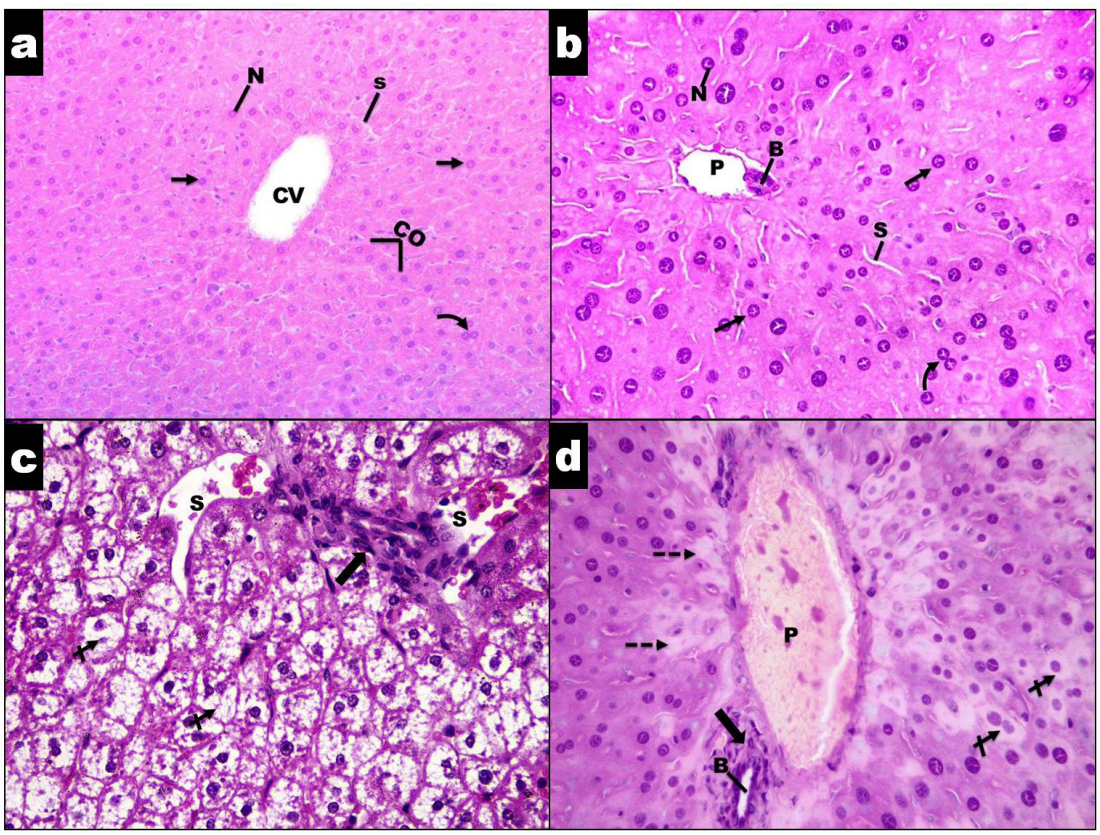

Fig. 1. Photographs of H&E-stained LM liver sections (x400) of adult male mouse: a – Control group showing normal hepatic lobule structure; polygonal acidophilic hepatic cells (arrow) with single (N) or two (curved arrow) vesicular nuclei, arranged in cords (co) around the central vein (CV) with the hepatic sinusoids (S) between the cords; b – Control group showing portal vein (P) that is thin-walled vessel with wide lumen and bile ductile (B) with its cuboidal epithelial lining in the portal region. Note: polygonal acidophilic hepatic cells (arrow) with single (N) or two (curved arrow) vesicular nuclei, hepatic sinusoids (S); c – HFD group showing marked structural changes; rounded swollen hepatic cells with pale cytoplasm suggesting cellular ballooning (crossed arrow); dilated congested sinusoids (S) and collection of inflammatory cells between hepatic cells (thick arrow) are seen; d – HFD group showing cellular degeneration (dotted arrow), aggregation of inflammatory cells (thick arrow) in the portal region and marked dilatation & congestion of the portal vein (P). Note: rounded swollen hepatic cells with pale cytoplasm (crossed arrow), bile ductile (B)

COX-2 immunohistochemically stained sections

COX-2 immunohistochemically stained liver sections of the control group of the liver showed a weak immunoexpression of COX-2 in hepatic cells marked by faint brown cytoplasmic staining in Kupffer cells. No immunoexpression of COX-2 was shown in hepatic cells (Fig. 3a). In HFD group a strong immunoexpression of COX-2 was observed as specified by markedly strong brown cytoplasmic staining in both Kupffer and hepatic cells was seen (Fig. 3b).

Examination of COX-2 immunohistochemical gallbladder-stained sections of the control groups showed weak immunoexpression of COX-2 noticed by a faint brown cytoplasmic staining in the mucosal epithelial cells (Fig. 3c). In HFD group strong immunoexpression of COX-2 specified by a strong brown cytoplasmic staining in the mucosal epithelial cells was observed (Fig. 3d).

Statistical analysis

Statistical analysis by independent student sample t test of the area % mean values of ORO staining in the liver tissue revealed a highly significant (P>0.001) increase in the HFD group as compared to control. Also, a very highly significant (P>0.001) increase in the area % mean values of COX-2 immunoexpression in the liver and gallbladder was recorded in the HFD group in comparison to the control group (Table 1; Fig. 4).

Table 1. Statistical analysis by independent sample t test of the area % of ORO staining in the liver and COX-2 immunoexpression in liver and gallbladder between the studied groups

Parameter	Control, mean ± SD (range)	HFD treated, mean ± SD (range)	t- test	P value
number of mice	8	8		
area % of ORO staining in the liver	14.13 ± 0.41 (12.58-15.74)	28.87 ± 0.44 (26.89–30.46)	24.47	0.0001
area % of COX-2 immunoexpression in liver	23.23 ± 1.61 (15.7-29.4)	47.01 ± 3.58 (33.49-60.09)	6.05	0.0001
area % of COX-2 immunoexpression in gallbladder	1.99 ± 0.2 (1.09-2.81)	14.63 ± 0.76 (11.33-17.96)	16.15	0.0001

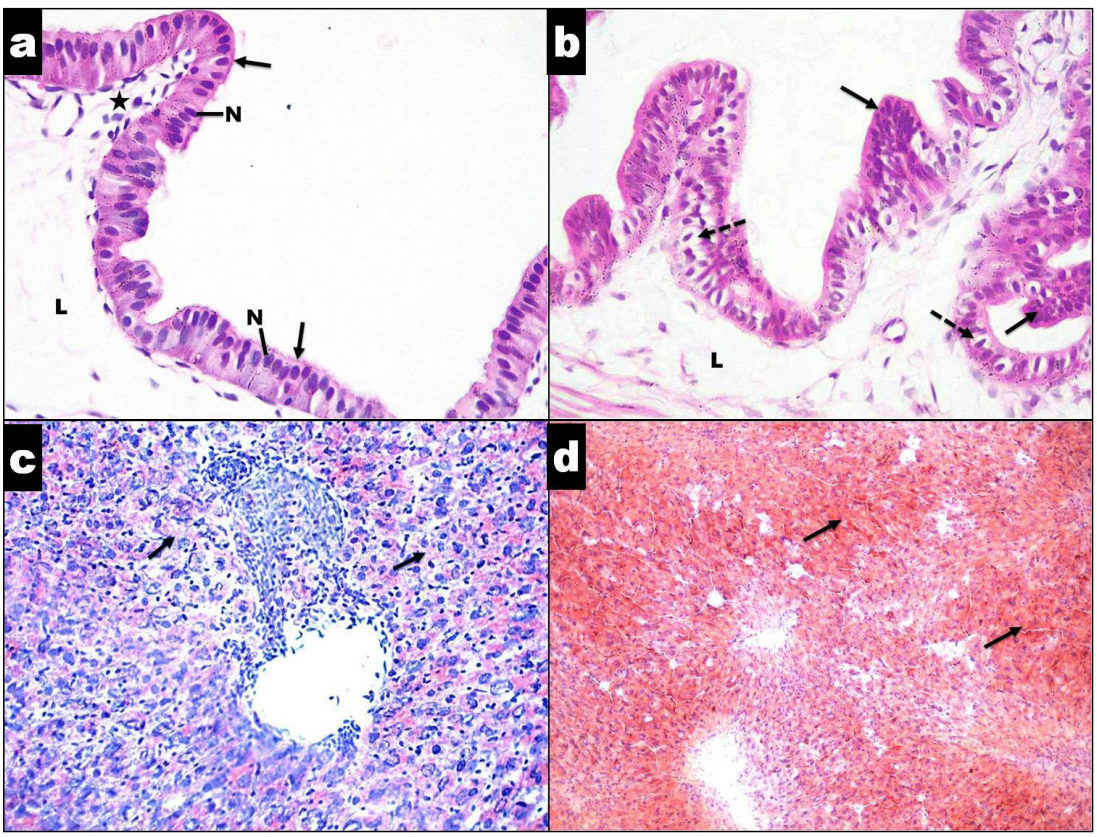

Fig. 2. Photographs of LM sections: a – H&E-stained gallbladder section (x400) of control group showing (showing) mucosa consisted of one layer of simple columnar epithelium with cells (arrow) having oval nuclei (N). Lamina propria (L), underlying the mucosa, formed of loose connective tissue. Mucosal folds (★) with central core of lamina propria are seen; b – H&E-stained gallbladder section (x400) of HFD group showing epithelial stratification (arrow) and cellular vacuolation (dotted arrow). Note: lamina propria (L); c – ORO-stained liver section (x200) of adult male mouse of control group showing low lipid deposition specified by weak cytoplasmic red staining of the hepatic cells (arrow); d – ORO-stained liver section (x200) of adult male mouse of HFD group showing excess lipid deposition specified by strong cytoplasmic red staining of the hepatic cells (arrow)

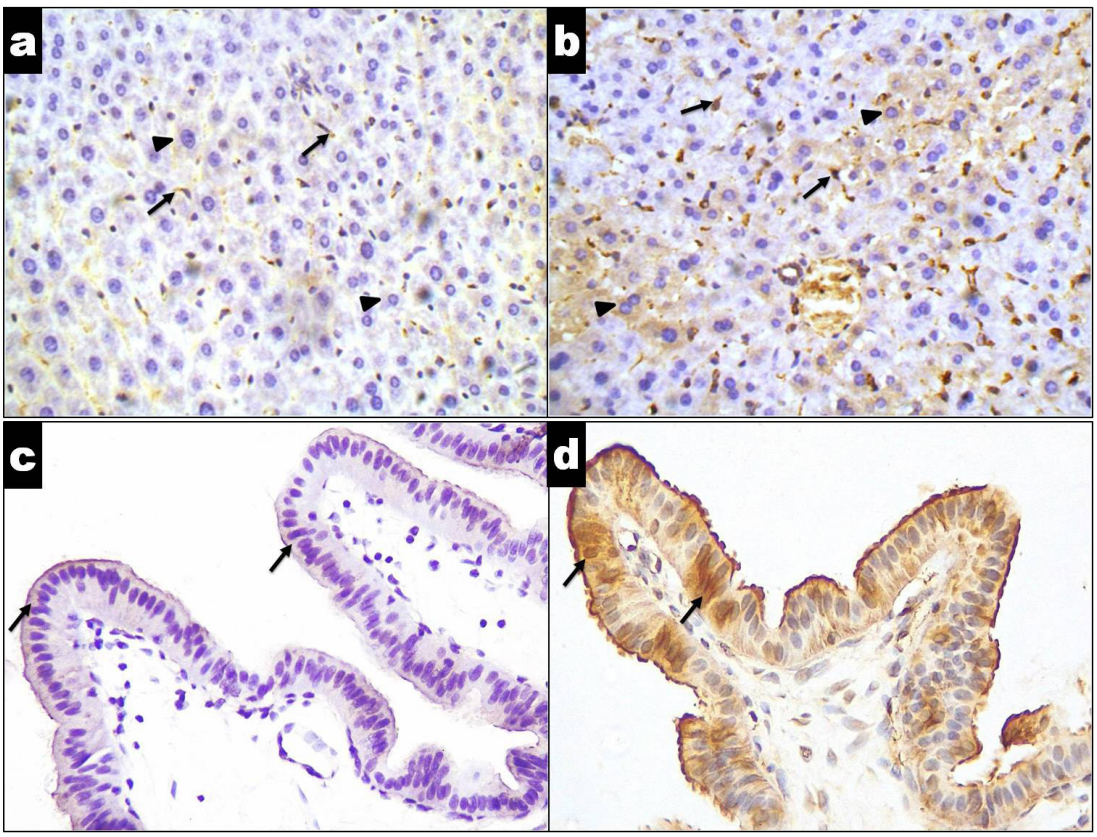

Fig. 3. Photographs of COX-2 immunohistochemically-stained LM sections: a – COX2 immunohistochemically-stained liver section (x400) of the control group showing a weak immunoexpression of COX-2 in hepatic cells specified by faint brown cytoplasmic staining in Kupffer cells (arrow). no immunoexpression of COX-2 is shown in hepatic cells (arrow head); b – COX2 immunohistochemically-stained liver section (x400) of the HFD group showing strong immunoexpression of COX-2 was observed specified by strong brown cytoplasmic staining in both Kupffer (arrow) and hepatic cells (arrow head); c – COX2 immunohistochemically-stained gallbladder section (x400) of the control group showing weak immunoexpression of COX-2 specified by faint brown cytoplasmic staining in the mucosal epithelial cells (arrow); d – COX2 immunohistochemically-stained gallbladder section (x400) of HFD group showing strong immunoexpression of COX-2 specified by markedly strong brown cytoplasmic staining in the mucosal epithelial cells (arrow)

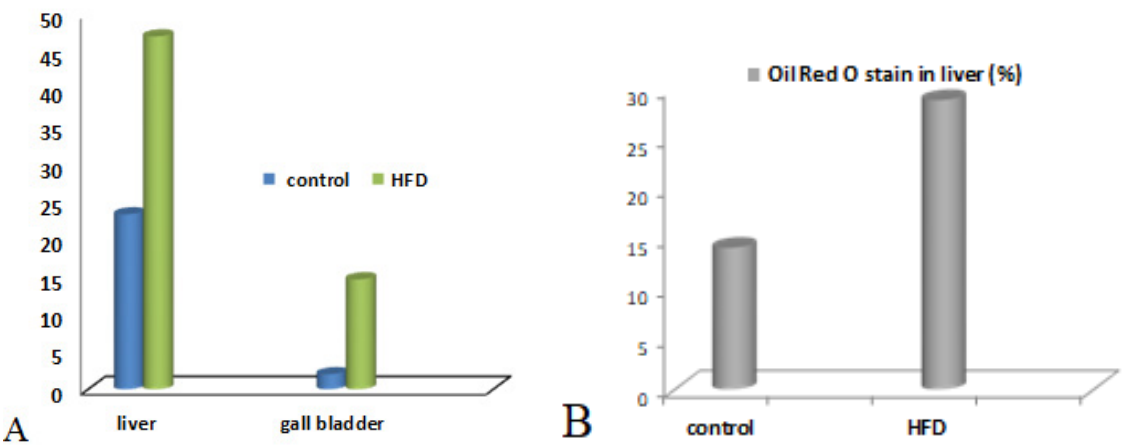

Fig. 4. Representative columns of the two groups: A – area percentages of COX-2 immunoexpression of liver and gallbladder; B – area percentages of ORO in liver

Discussion

HFD is a common bad food habit among many different societies. In this study, we aimed to investigate its effects on liver and gallbladder. We used male animals and not females for this purpose to avoid possible hormonal changes in female estrus cycles that might affect the results achieved.[22,23]

In this study, hematoxylin and eosin-stained liver sections of control group revealed normal hepatic lobule structure; polygonal acidophilic hepatic cells with vesicular nucleus, arranged in cords around the central vein with the hepatic sinusoids between the cords. Thin-walled portal vein and bile ductile with cuboidal epithelial lining were seen in the portal region. These results are consistent with the normal liver histology described by Ross and Pawlina.[24]

In the present study rounded swollen hepatic cells with pale cytoplasm were observed suggesting cellular ballooning. This picture is indicative of steatosis. Steatosis or fatty change of the liver is the accumulation of abnormal amounts of lipids in 5% or more of hepatic cells.[25]

In the present work, we assessed lipid deposition by examination of ORO-stained hepatic sections Low lipid deposition as specified by weak concise cytoplasmic red staining of the hepatic cells was seen in the control groups, while in the HFD group there was excess lipid deposition as specified by intense wide cytoplasmic red staining of the hepatic cells. In addition, statistically the area % mean values of ORO staining showed a very highly significant (P<0.001) increase in HFD group in comparison to the control group. These results are in accordance with findings reported by Meli et al. and Liu et al.[26,27]

In our work, dilated congested portal vein and hepatic sinusoids were observed in HFD group. This is in accordance with Altunkaynak et al. who attributed this finding to be a result of necrosis and inflammatory changes.[28] Arvanitidis et al. explained that hypoxia and ischemia induced by HFD intake could lead to vascular dilatation.[29] However, Elahi et al. stated that the cause of the vascular dilatation was hypertension occurring due to obesity induced by HFD.[30]

The current work revealed collection of inflammatory cells within portal area and between hepatic cells and strong immunoexpression of COX-2 was observed in HFD group which was weak in the control group. Statistically the area % mean values of COX-2 immunoreaction showed a very highly significant increase in HFD group in comparison to the control group. These results are in accordance with study of Yu et al.[15]

Our results suggest hepatic inflammation in HFD group that can be explained by Cyuela et al. who has stated that intracellular accumulation of fatty acids is associated with development of inflammatory response in addition to mitochondrial dysfunction that causes tissue ischemia.[31] Ischemic injury of the liver is associated with the inflammatory response that involves stimulation of Kupffer cells with subsequent production of proinflammatory cytokine like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and collections of neutrophils and T lymphocytes.[32] TNF-α and IL-6 are considered main mediators responsible for hepatic inflammation, necrosis and fibrosis.[33] In addition, excess inflammatory PGs production by COX-2 enzyme stimulates hepatocytes for excess formation of triglycerides that accumulate in liver tissue.[8] Furthermore, Zhang et al. found that HFD intake during pregnancy might adversely affect the liver of male offspring through acceleration of fatty acid synthesis; and the damage could extend to second generation.[34] On the other hand, Romeijn et al. demonstrated the effective role of low-calorie diet (LCD) in reducing liver size and weight.[35] The authors added that LCD contains 800 to 1200 kcal/day for at least 5 days. The LCD is less effective than very-low-calorie diet (VLCD) of 450–800 kcal per day. However, LCD is preferred over VLCD to avoid unnecessary dietary restrictions and subsequent negative aspects such as LBM loss and other side effects.

In this work, H&E gallbladder-stained sections of the control group showed mucosa consisted of one layer of simple columnar epithelium with cells having oval nuclei and lamina propria, underlying the mucosa, formed of loose connective tissue. Mucosal folds with central core of lamina propria were seen. These findings are in accordance with that reported by Lindberg and Lamps.[36]

In the present study, stratified epithelium and cellular vacuolations were observed in the gallbladder of HFD group. It was stated that excess cholesterol in bile irritates gallbladder epithelium.[37] This can explain epithelial metaplasia observed. Also, Zaki and Al-Refeidi reported cellular vacuolation and distorted gallbladder epithelium in association with gall stone disease.[38] Van Erpecum et al. stated that histological changes and affection of gallbladder cause a decrease in its ability to concentrate bile and this malfunction may reach up to gall stones formations.[16] HFD might cause gallbladder hypomotility and changes in composition of bile.[39] These could be additional factors for risk of gallbladder stones. Moreover, stress and anxiety during the COVID-19 pandemic that mostly associated with high HFD consumption could increase the incidence of acute calculous cholecystitis.[40]

Our study showed a strong COX-2 immunoexpression in gallbladder mucosal epithelial cells of HFD group which was weak in control group. Statistically the optical density mean values of COX-2 immunoreactions in gallbladder showed a very highly significant (P<0.001) increase in HFD group compared to control group. Our

result of inflammatory reaction is in general accordance with Van Erpecum et al. who reported granulocytes and lymphocytic infiltration in lamina propria.[16] According to Patel et al., HFD intake with elevated cholesterol level can induce gall stone formation and increase level of cholecystitis occurrence.[41] It was stated that inflammation and over production of PGE2 induced by COX-2 production has a cytoprotective role against cholesterol effects, but still, it may not be working as high levels of cholesterol decrease receptor binding capacity with resultant COX-2 overproduction.[42]

Conclusions

HFD could cause detrimental changes in the structure of liver and gallbladder in the form of fatty liver, gallbladder epithelial hyperplasia and inflammatory reaction. Therefore, it might be essential to lower fats in the usual diet. Furthermore, future studies are recommended to explore which drug or protocol can be administered in order to improve the detected changes.

Declarations

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Author contributions

Conceptualization, A.A.H, N.M.Q. and E.M.E.; Methodology, N.M.Q., N.M.A, and E.M.E.; Software, N.M.A.; Validation, A.A.H, N.M.Q. and E.M.E.; Formal Analysis, N.M.A, and E.M.E.; Investigation, All authors; Resources, N.M.A.; Data Curation, N.M.A. and E.M.E.; Writing – Original Draft Preparation, N.M.A. and E.M.E.; Writing – Review & Editing, A.A.H.; Visualization, N.M.Q. and E.M.E.; Supervision, A.A.H., N.M.Q. and E.M.E.; Project Administration, A.A.H., N.M.Q., N.M.A. and E.M.E.

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

The study was approved by The Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC); reference number (Zu-IACUC/3/F/167/2019).

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ORIGINAL PAPER

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The use of medial clavicular epiphysis ossification stages for bone age determination

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ABSTRACT

Introduction. Bone age determination is a radiological method investigating the compatibility of ossification processes of bones with chronological ages.

Aim. We aimed to investigate the use of CT staging of the medial clavicular epiphyseal ossification in bone age determination in Turkish adolescents and young adults.

Material and methods. Chest CT exams of 2018 patients between 11 and 35 years of age were retrospectively evaluated for epiphyseal ossification stages of the bilateral medial clavicles (4036 clavicles) on both axial&coronal images and compared with the sex and chronologic age of the individuals in Turkey.

Results. For stage 2,3 and 4 the ages of women were greater than men and it was statistically significant. For an individual classified as stage 4,it can be said with certainty that he or she has already reached the age of 18.There was no statistically significant difference between left&right sides and between the axial&coronal images.In addition, it was found that the medial clavicular head epiphyses showed a lot of variation.

Conclusion. CT evaluation of the medial clavicular epiphysis ossification stages is helpful in determination of the individuals over the age of 18. Regardless of the sex, the stage 4 can be used as a criterion to make the prediction that an individual is older than 18 years.

Keywords. bone age, clavicle, medial clavicular epiphysis, skeletal age

Introduction

Age estimation has an important role in both diagnosis and treatment and also decision making processes in forensic applications in which the accuracy of age determination is an important subject in criminal issues.[1] The age determination methods include mainly bone ossification, dental mineralization and sexual maturation characteristics. Bone age (skeletal age) determination is a radiological examination method investigating the compatibility of ossification processes of bones with chronological ages. The most commonly used method is hand bone ossification. However, completed hand and wrist bone ossification in cases over 18 years of age makes bone age estimation difficult. Sexual maturation and mineralization of teeth in these cases have also been completed. Because the clavicular medial head has the most lately fused epiphysis in the entire body, by looking at the union of the medial clavicular epiphysis (sternal end), bone age determination can be made up to a certain age, even after the age of 18.[2] The utility of radiological imaging methods primarily radiograhy and computed tomography (CT) and also magnetic resonance imaging (MRI) have been examined in various studies. In a study conducted in Korean adolescents and young adults chest ra-

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diographs were used to estimate the age with marginally moderate interobserver agreement and high diagnostic accuracy.[3] However, CT has advantages of more accurate evaluation of ossification centers with lack of artifacts due to soft tissue superposition. It is a method recommended by Forensic Age Diagnostics of the German Association of Forensic Medicine (AGFAD).[4]

Aim

In this study, we investigated the use of CT staging of the medial clavicular epiphyseal ossification in bone age determination in adolescents and young adults by retrospectively examining the CT images of 2018 patients in Turkey, using a modified staging system.

Material and methods

This retrospective study was approved by the local ethics committee of the hospital with the IRB number of 621. Chest CT and chest high resolution CT (HRCT) examinations of a total of 2018 patients between 11 and 35 years of age were retrospectively evaluated for epiphyseal ossification stages of the bilateral medial clavicles (4036 clavicles) and compared with the sex and chronologic age of the individuals. Patients with diseases affecting bone development, with clavicle fracture and mass lesions involving the medial head of the clavicle, patients who could not be evaluated clearly due to motion or contrast material artifacts were excluded from the study. The final study population consisted of 2018 patients; 43.4% (876) female and 56.6% (1142) male cases between 11 and 35 years of age. Examinations were carried out with 16 slice Philips and 64 slice Toshiba multidetector CT scanner with parameters of 220 kV, 120 mAs and a collimated slice width of a 1 mm. The slice thicknes varied between the range of 0.3-7 mm. In both gender, the ossification stage of both left and right medial clavicular epiphysis were classified with a modified staging system based on Schmeling and his colleagues staging at both axial and coronal planes, seperately (fig. 1-7).[5] In addition, presence of variations were also noted.

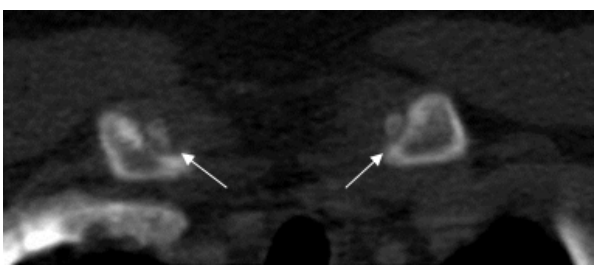


Fig. 1. CT image of medial clavicles of a14 year old female shows stage 2a ossification of bilateral epiphyseal cartilage (white arrows)

Stage 1: epiphyseal ossification is not yet visible (nonossified).

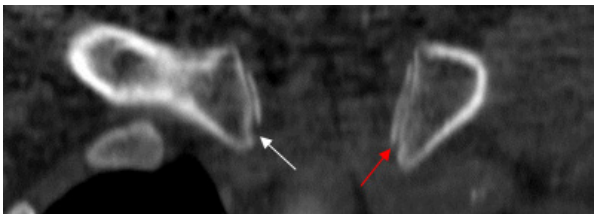


Fig. 2. CT image of medial clavicles of a 17 year old female shows stage 2a (white arrow) ossification of the right and stage 2c of the left epiphyseal cartilage (red arrow)

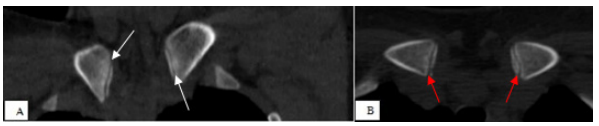


Fig. 3. (A, B) CT image of medial clavicles in a 17 year old female shows stage 2c ossification of bilateral epiphyseal cartilage on the coronal image (A, white arrow) and on the axial image (B, red arrows)

Stage 2a: epiphyseal ossification center has begun to appear but, its diameter is smaller than the 1/3 of the diameter of the metaphysis (Fig. 1, 2).

Stage 2b: the diameter of the epiphyseal ossification center is more than half the diameter of the metaphysis but less than its 2/3.

Stage 2c: the diameter of the epiphyseal ossification center is more than 2/3 of the diameter of the metaphysis but less than its 2/3 or has fully grasped the metaphysis, but fusion has not yet begun (Fig. 2, 3).

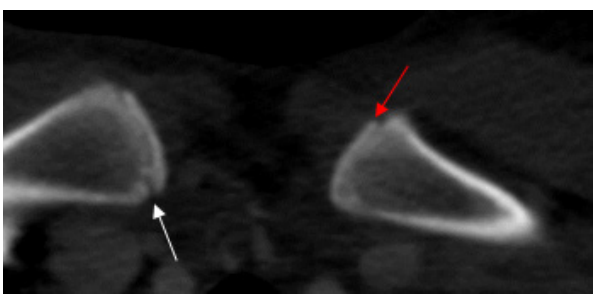


Fig. 4. CT image 18 year old female shows stage 3a ossification (white arrow) of the right and stage 3b of the left epiphyseal cartilage (red arrow)

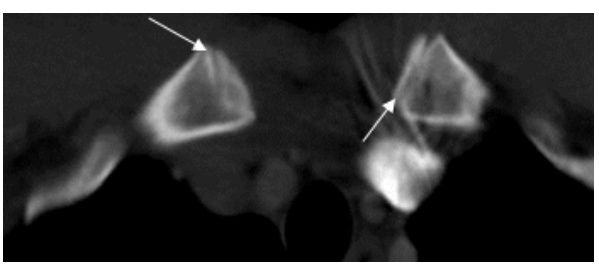


Fig. 5. CT image of medial clavicles of a19 year old male shows stage 3b ossification of bilateral epiphyseal cartilage (white arrows)

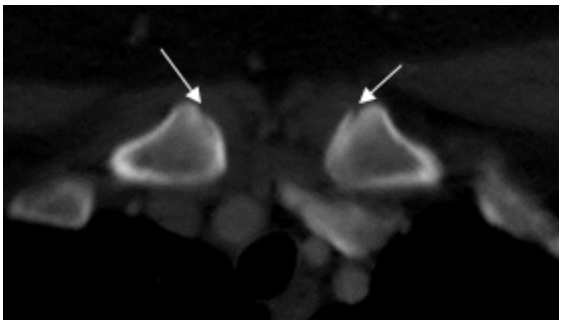


Fig. 6. CT image of medial clavicles of a 20 year old female shows stage 3c ossification of bilateral epiphyseal cartilage (white arrows)

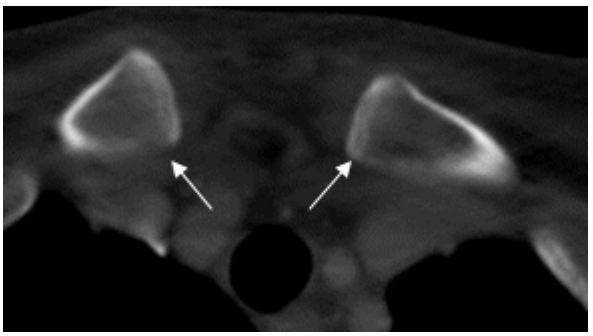


Fig. 7. CT image of medial clavicles of a 22 year old male shows stage 4a ossification of bilateral epiphyseal cartilage (white arrows)

Stage 3a: Epiphyseal fusion has begun, the fused part is less than the 1/3 of the diameter of the epiphysis (Fig. 4).

Stage 3b: Epiphyseal fusion is more than half the diameter of the epiphysis but less than its 2/3 (Fig. 4, 5).

Stage 3c: Epiphyseal fusion is more than 2/3 of the diameter of the epiphysis but it is not completed (Fig. 6).

Stage 4: Epiphyseal fusion is complete but epiphyseal scar (epiphyseal line) can be detected.

Stage 5: Epiphyseal scar can not be detected.

In our study, the stage 4 has been subdivided into 2 groups;

Stage 4a: Epiphyseal fusion is complete but an obvious epiphyseal scar (epiphyseal line) can be detected (Fig.7).

Stage 4b: Epiphyseal fusion is complete and some of the epiphyseal scar (epiphyseal line) can be detected.

Statistical analysis

In the descriptive statistics of the data, the mean, standart deviation, frequency and ratio values were used. The distribution of the variables was controlled with the Kolmogorov Simirnov test. Kruskal-wallis, Mann-Whitney u test for the analysis of quantitative data and Kappa test for the aggrement analysis was applied. SPSS 21.0 program was used in the analyzes.

Results

The ages of all stages differed significantly from each other. The age increased significantly ($p<0.05$) with each increasing stage (Table 1).

Table 1. The ages of the stages

	Age			p
	The lowest	The higest	Mean ± SD	
Stage 1	11	20	13.22 ± 1.92	<0.00001
Stage 2	11	24	16.78 ± 2.32	
Stage 3	14	27	20.59 ± 2.42	
Stage 4	18	35	25.48 ± 4.57	
Stage 5	21	35	29.30 ± 3.49	
Kruskal-Wallis/Mann-Whitney U test				

SD – standard deviation

Table 2 shows the mean ages and standard deviation values among the stage 2, 3 and 4 subgroups themselves. In stage 2a, the age of the patients was significantly ($p<0.05$) lower than stage 2b and 2c. The age of the patients in stage 2b and 2c did not differ significantly as in stage 3b and 3a did not differ significantly ($p>0.05$). In stage 3c, the age of the patients was significantly ($p<0.05$) higher than stage 3a and stage 3b. In stage 4b the age of the patients was significantly ($p<0.05$) higher than stage 4a (Table 2).

Table 2. The mean ages and standard deviation values among the stage 2, 3 and 4 subgroups

	Age			p
	The lowest	The highest	Mean ± SD	
Stage 2a	11	20	15.56 ± 2.27	<0.00001
Stage 2b	12	22	17.72 ± 1.82	
Stage 2c	14	24	17.87 ± 1.81	
Stage 3a	14	22	19.03 ± 1.83	<0.00001
Stage 3b	17	25	19.86 ± 2.03	
Stage 3c	17	27	21.52 ± 2.32	
Stage 4a	19	35	24.39 ± 3.91	<0.00001
Stage 4b	18	35	26.92 ± 4.98	
Kruskal-Wallis/Mann-Whitney U test				

SD – standard deviation

Table 3 shows the mean and standard deviation values of the earliest and the latest ages of the medial clavicular epiphyseal ossification stages. In stage 1, 2a, 2b, 3a, and 3c the average age of the men was significantly ($p<0.05$) higher than the women. In other stages, the ages of the men and women did not differ significantly ($p>0.05$) (Table 3).

There was a significant agreement between the right and left sides with accordance of the 89.1% of the stages on the axial images ($p=0.000$ <0.001; Kappa: 0.855) and with accordance of 89.3% of the stages on coronal images ($p=0.000$ <0.001; Kappa: 0.858) (Table 4). There was

also a significant agreement between the axial and coronal images with accordance of 98.9 % of the stages of the right side (p=0.000 <0.001; Kappa: 0.985) and with accordance of 99.1 % of the stages of the left side agreement (p=0.000 <0.001; Kappa: 0.989) (Table 5).

Table 3. The mean and standard deviation values of the earliest and the latest ages of the medial clavicular epiphyseal ossification stages

Age	Female	Male	
	Mean ± SD	Mean ± SD	p
Stage1	12.79 ± 1.94	13.39 ±1.89	0.004
Stage 2a	14.94 ± 1.94	15.97 ± 2.38	0.001
Stage 2b	16.58 ± 1.86	18.39 ± 1.43	<0.001
Stage 2c	17.44 ± 1.82	18.35 ± 1.68	0.007
Stage 3a	18.62 ± 1.84	19.58 ± 1.70	0.041
Stage 3b	18.91 ± 2.26	20.44 ± 1.69	0.052
Stage 3c	20.59 ± 2.14	22.14 ± 2.24	<0.001
Stage 4a	24.25 ± 4.20	24.57 ± 3.52	0.195
Stage 4b	27.69 ± 5.29	26.46 ± 4.77	0.484
Stage 5	29.44 ± 3.50	29.18 ± 3.47	0.194

Mann-Whitney U test

SD – standard deviation

Table 4. The aggreement between left anf right sides on axial and coronal images

	Left axial/right axial	
	accordance	difference
n	1799	220
%	89.1%	10.9%

	Left coronal/right coronal	
	accordance	difference
n	1803	216
%	89.3%	10.7%

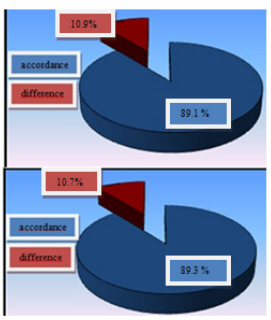
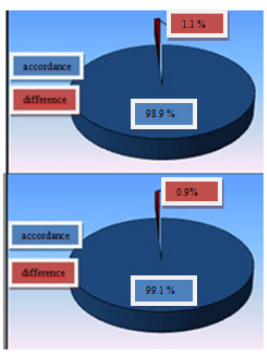


Table 5. The aggreement on axial/coronal images regarding the right and left sides

	Right axial/Right coronal	
	accordance	difference
n	1996	23
%	98.9%	1.1%

	Left axial/Left coronal	
	accordance	difference
n	2001	18
%	99.1%	0.9%



In table 6, the lowest and the highest ages, mean age, standard deviation and median values of women and men in each stages were shown.

In our study, no statistically significant difference was found between the right and left side on axial and coronal images of the same case in terms of the stage (Table 4, 5).

Table 6. The lowest and the highest ages, mean age, standard deviation and median values of women and men in each stages

		The lowest	The highest	Mean ± SD	Median (Q1/Q3)		
Stage 1	female	11	20	12.79 ± 1.94	12.0	11.0	14.0
	male	11	20	13.39 ± 1.89	13.0	12.0	14.0
Stage 2a	female	11	19	14.94 ± 1.94	15.0	14.0	16.0
	male	11	20	15.97 ± 2.38	17.0	14.0	18.0
Stage 2b	female	12	20	16.58 ± 1.86	16.0	15.3	18.0
	male	15	22	18.39 ± 1.43	19.0	17.5	19.0
Stage 2c	female	14	24	17.44 ± 1.82	18.0	16.0	18.0
	male	15	23	18.35 ± 1.68	18.0	17.0	20.0
Stage 3a	female	14	22	18.62 ± 1.84	19.0	17.0	20.0
	male	15	22	19.58 ± 1.70	20.0	18.0	21.0
Stage 3b	female	17	23	18.91 ± 2.26	18.0	17.0	21.0
	male	18	25	20.44 ± 1.69	21.0	19.0	21.0
Stage 3c	female	17	26	20.59 ± 2.14	20.0	19.0	22.0
	male	17	27	22.14 ± 2.24	22.0	20.8	24.0
Stage 4a	female	19	35	24.25 ± 4.20	23.0	21.3	25.8
	male	21	33	24.57 ± 3.52	23.0	22.0	25.8
Stage 4b	female	20	35	27.69 ± 5.29	28.0	22.0	33.0
	male	18	35	26.46 ± 4.77	25.0	23.0	31.0
Stage 5	female	21	35	29.44 ± 3.50	30.0	27.0	32.0
	male	21	35	29.18 ± 3.47	29.0	26.0	32.0

SD – standard deviation, Q1 – Quartile 1, Q3 – Quartile 1

In addition, there are some difficulties encountered during the evaluation in our study. The most common problem among these was the pronounced notching at the head of the clavicle (Fig. 8). In almost all of these cases the epiphyses were hypoplastic or evaluated as a smaller stage despite the advanced age. Another problem was the appearance of more than one small ossification centers (Fig. 9). In addition, the sclerotic bands parallel to the metaphysis, formed in relation with growth in pediatric cases create an appearance similar to the physeal scars observed in stage 4a (Fig. 10). In such cases, it will be necessary to know the estimated age of the person and to evaluate accordingly. Another important point to note is that, the occurance of accessory ossicle which is very rare, imitates the epiphyseal ossification center. In our study, it was found adjacent to the medial head of the right clavicle in two cases (Fig. 11). In addition, the diameter of the epiphysis is about half the diameter of the metaphysis in some cases, but the fusion is complete (Fig. 12). In our study, these cases were evaluated as stage 3c. If these cases came before the

fusion, they would be considered as stage 2a due to the small diameter of the epiphysis.

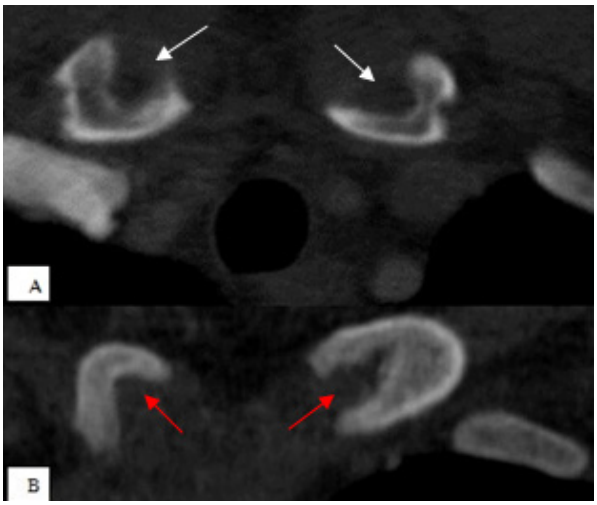


Fig. 8. (A, B) CT images of medial clavicles. A significant notching is seen on the heads of both clavicles on the axial image (A, white arrows) and the coronal image (B, red arrows). The epiphyseal ossification center is not visible

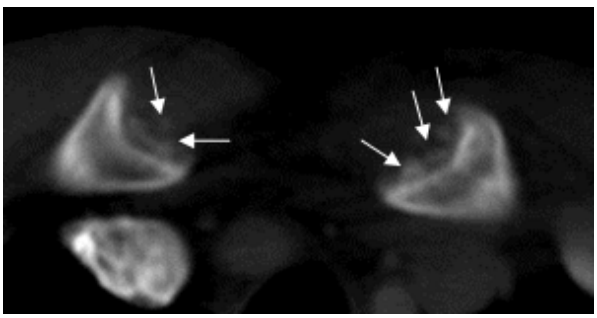


Fig. 9. CT image of medial clavicles of a18 year old male shows more than one ossification center on both sides (white arrows)

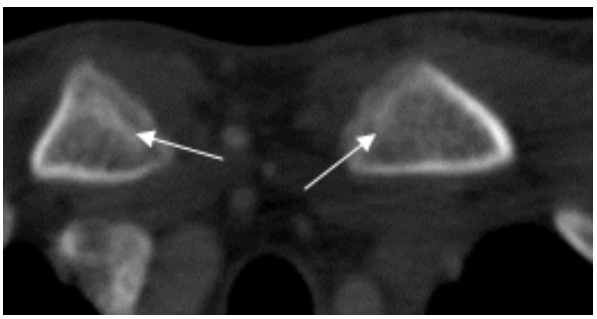


Fig. 10. CT image of medial clavicles of a13 year old male shows sclerotic band associated with growth (white arrows) creating an appearance similar to the physeal scars seen in stage 4a

Discussion

In legal cases, the chronological age is tried to be estimated with different methods. The most important marker showing the development from the birth to the maturity is the bone age.[6] In Turkey, the most commonly used method for determination of age is the left hand and wrist radiographs. Studies have been conducted in the literature to suggest that the ossification stage of medial clavicular head can be used for age determination in people over 18 years of age with closed hand skeletal epiphysis, especially in forensic issues.[3,7,8] Since long, in various studies, it was investigated whether there is any difference by using various methods such as, X-ray, CT and MRI. In the study conducted by Kreitner et al. in 1998 by using CT, ossification of the clavicle was divided into 4 stages (the 4 stage classification system).[9]

Stage 1: The epiphyseal ossification center is not seen.
Stage 2: Ossification center is started to be seen.
Stage 3: There is partial epiphyseal fusion.
Stage 4: The fusion is completed.

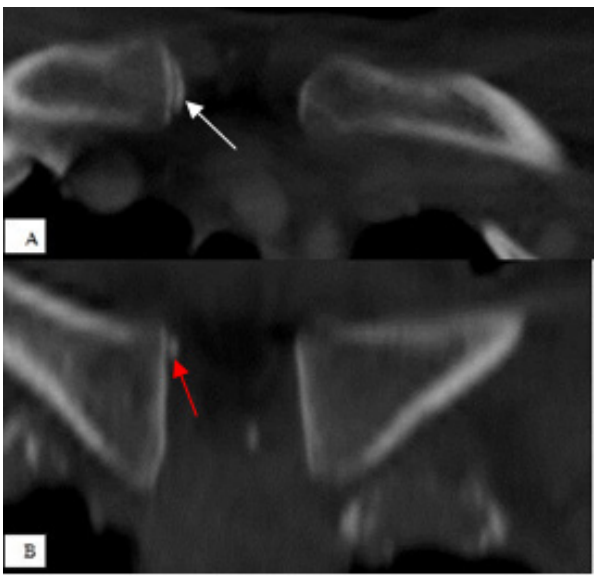


Fig. 11. (A, B) CT images of medial clavicles of a 31 year old female show the accessory ossicle imitating the epiphyseal ossification center on the right side on axial (A, white arrow) and coronal (B, red arrow) images

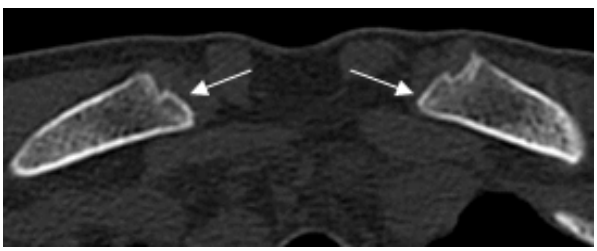


Fig. 12. CT image of medial clavicles of 20 year old male shows that the diameter of the epiphysis is about half the diameter of the metaphysis but the fusion is complete (white arrows)

In this study, it was stated that stage 1 was seen up to the age of 16, stage 2 was seen between the ages of

13 and 22, stage 3 was seen between the ages of 16 and 26 and the age of first appearance of the stage 4 was 26. However, since no gender discrimination was made, its use in forensic medicine was limited. This 4 stage classification system was also used in the study conducted by Zhang et al. analyzing the CT scans of 752 individuals in 2015.[10] They found that the epiphysis was observed to commence fusion in females at 16.28 years and 16.74 years in males and be fully ossified by 25.97 years in females and 25.81 years in males, suggesting that ossification of medial clavicular epiphyseal cartilage can be used in age estimation for West China Han population with the age threshold of 18 years. However, in many of the studies performed on the ossification stages of the medial clavicular head, Schmeling's 5-stage system is used (the 5 stage classification system).[5]

Stage 1: Non-ossified epiphysis.

Stage 2: ossification is started without growth plate ossification.

Stage 3: Growth plate is partially fused.

Stage 4: complete fusion of the epiphysis and metaphysis; physeal scar is visible.

Stage 5: complete fusion of the epiphysis and metaphysis; the physeal scar is no longer visible.

In the study conducted by Patil et al. in 2018, CT images of 462 individuals aged between 10 and 30 years were evaluated retrospectively in an Indian population using the 5 stage classification mentioned by Schmeling.[7] They concluded that the clavicular maturation stage 2 represent age >13 years, stage 3 represent age >16 years, stage 4 represent age >22 years and stage 5 represent age >25 years for an Indian. The study conducted on 142 subjects by El Morsi et al. in Egyptian population evaluating multislice CT on the medial end of clavicles of both sides recommended using stage 1 to be 15 years; Stage 3 to be >15 years; stage 4 of maturation to be >19 years and stage 5 to be >21 years.[8] This study revealed no significant differences between males and females (except for stage 1) or right and left sides as regard age of ossification of medial end clavicles.

In the study conducted by Kellinghaus et al. using thin slice CT in 2009, the difference between the genders was found to be significant for stage 2.[11] It was determined that female cases reached stage 2, 18 months earlier than male cases. It was also stated that the race has no effect on ossification but low socioeconomic status delays the ossification. Since our study is a retrospective study, no information was obtained about the socioeconomic levels of the cases. In 2010 Kellinghaus et al. divided each of the ossification stages 2 and 3 into an early, intermediate and late phase (a, b, c; respectively) by evaluating thin-slice CT scans of 185 patients aged between 13 and 26 years and formed the '9 stage classification system'.[12] In this study, no significant difference was found between genders. It was determined that the earliest age of appearance of stage 3c was 19.5 in women and 19.7 in men and the average age of this stage was 22.5 in women and 22.9 in men. Based on these findings, it was emphasized that even if the epiphyseal fusion of the case is incomplete, if the ossification stage is 3c, it is possible to say that the individual has already reached the legally important age threshold of 18 years. We used the Schmeling's 5 stages in our own modified form by subdividing the stage 4 into two subgroups. We found that the age of the first appearance of stage 4a is 19 for females and 21 for males. Based on this findings, for the individual who is classified as stage 4, it can be said with certainty that he or she has already reached the age of 18. The age of the last appearance of the stage 3b was found to be 23 for females and 25 for males. Based on this findings, it can be said that the age of the person with stage 3b is less than 22 years old. Since the other age ranges in the epiphyseal development and closure stages of the medial clavicular head were very wide, we can give a certain bone age range for a given individual but it was imposible to determine the exact bone age precisely by looking at the medial clavicular head epiphyseal development. In addition, we also found that after 20 years old or older, that is after the closure of iliac and iscial epiphysis (when we actually need to use medial clavicular ossification method) the standard deviation is higher in comparison to the earlier stages. The first and the latest seen age ranges of each seperate stage in our study are shown in table 6. In our study, the ages of the males in stage 1, 2a, 2b, 2c, 3a and 3c was significantly higher than females. No statistically significant difference between left and right sides was found. In the study conducted by Schulz et al. by using CT in 2005, the difference between genders was found to be significant for stage 2. In addition, in this study the age of the first appearance of stage 5 was found to be 21 in women and 22 in men.[13] In terms of the effect of CT slice thickness on the stage, a study conducted by Mühler et al. was published in 2005. In this study, it was determined that increasing the slice thickness (between 1 mm and 3 mm, between 3 and 5 mm, between 5 mm and 7 mm) caused evaluation differences and it was stated that the most appropriate slice thicknes should be 1 mm.[14]

Limitations

The main limitation of our study is its retrospective design, which caused differences in CT slice thickness among the study patients and made us accept the officially recorded age as the real chronological age of the study participants. In addidion we could not evaluate the effect of socioeconomic or nutrition status on the epiphyseal ossification, although low socioeconomic or nutrition status affecting skeletal maturation is a quite rare possibility in Tukey. Nevertheless, we think that our study is valuable in terms of having a very large patients series that has a power to reflect the general population.

In addition, our study is also different in terms of revealing the anatomical variations that may interfere the estimation of the medial clavicular ossification stages.

Conclusion

CT evaluation of the medial clavicular epiphysis ossification stages is helpful in determination of the individuals over the age of 18 and can be used in forensic medicine. Regardless of the sex, the stage 4 can be used as a criterion to make the prediction that an individual is older than 18 years. However, it is not possible to determine the bone age clearly, as the development and closure stages of the medial clavicle epiphysis are seen in a wide age range and the variations are common. Therefore a certain bone age range can be given, but, in this regard it should be taken into account that the standard deviation is higher after the closure of the iliac and ischionic epiphyses (20 years and over) compared to the previous periods.

Declarations

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Author contributions

Conceptualization, N.N.W.; Methodology, N.N.W.; Validation, N.N.W. and B.E.; Formal Analysis, B.E., N.N.W.; Investigation, B.E. and N.N.W.; Data Curation, N.N.W.; Writing – Original Draft Preparation, B.E.; Writing – Review & Editing, B.E. and N.N.W.; Supervision, B.E. and N.N.W.

Conflicts of interest

The authors declare no conflict 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

This retrospective study was approved by the local ethics committee of the Istanbul Medical Faculty with the IRB number of 621.

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ORIGINAL PAPER

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Serum levels of vitamin D and tumour necrosis factor-alpha in adults with metabolic syndrome

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ABSTRACT

Introduction. Reports continue to show that a significant association exists between serum vitamin D level and metabolic syndrome (MS)-associated inflammation. However, information on the serum levels of vitamin D and alterations in inflammation in different vitamin D status is presently lacking.

Aim. To determine the serum levels of vitamin D and TNF-α, and assess their possible relationship with gender in individuals with MS.

Material and methods. Sixty adults with MS and 40 controls were enrolled into this case-control study. Serum vitamin D and TNF-α levels were measured and participants stratified into different vitamin D status.

Results. None of the participants had vitamin D deficiency and the mean vitamin D level was similar in MS compared with the controls. However, TNF-α level was significantly higher in MS compared with the controls. Serum vitamin D level had significant inverse correlation with serum TNF-α level in MS. Also vitamin D level was significantly lower while TNF-α level was significantly higher in female-MS compared with the male-MS.

Conclusion. Adults with MS have elevated TNF-α level which appears to be associated with the serum level of vitamin D. Also, females with MS have low vitamin D level and this may exacerbate the MS-associated inflammation in them.

Keywords. inflammation, metabolic syndrome, vitamin D

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Introduction

Metabolic syndrome (MS) is a constellation of disorders which increases the propensity for developing various cardiometabolic diseases including cardiovascular diseases (CVD), insulin resistance (IR), and diabetes mellitus (DM).[1] It is a major public health problem affecting approximately 30% of adults and 25% of the worldwide population.[2] Genetic predisposition, lack of physical activity, increased consumption of high calorie-low fibre fast food, overweight and obesity underlie the aetiology of MS.[1,3]

Despite the availability of reports on MS preventive and therapeutic strategies, the prevalence of MS continues to rise worldwide even, in the developing world where poverty is widespread.[4-10] This has been attributed to technological advances favouring sedentary lifestyle and non-adherence to therapeutic lifestyle changes (TLCs).

There is an avalanche of reports indicating that inflammation plays a vital role in the pathophysiology of MS.[11-14] The reports of Alberti et al., Zimmet et al. and Grundy et al. showed that elevated circulated inflammatory markers such as tumour necrosis factor-alpha (TNF-α) and reduced levels of anti-inflammatory molecules are components of multiplex risk factors associated with MS.[15-17] The altered inflammatory responses in MS favours pro-inflammation and it is initiated by positive energy balance, manifesting as central adiposity, which causes adipocyte hypertrophy and a dysregulation of adipokine secretory patterns resulting in an imbalance between pro- and anti-inflammatory adipokines production, and infiltration of the adipose tissue by macrophages.[11,13]

Prominent among the inflammatory peptides produced by the adipocytes and the adipose tissue infiltrating macrophages is TNF-α which has been shown to impair insulin signalling in insulin sensitive tissues.[13,18] This results in insulin resistance (IR), one of the hallmarks of MS, which stands out as the main end point underlying the clustering of CVD risk factors in MS.[19,20] TNF-α promotes IR via serine phosphorylation of insulin receptor substrate 1 (IRS-1), as against the usual tyrosine phosphorylation. This phosphorylation in serine prevents IRS activation by the insulin receptor, blunts downstream signalling and facilitates the degradation of IRS protein.[21-26]

Understanding the interplay between molecules with anti-inflammatory properties and disordered inflammatory responses in MS is of the essence in developing preventive and therapeutic approaches to MS. One of the substances with profound anti-inflammatory properties is vitamin D.[27] It is a pleiotropic hormone with critical roles in health maintenance.[28] It contributes to the regulation of the proliferation, differentiation and function of various cells of the immune system hence; plays important roles in the regulation of both the innate and adaptive immune system.[29] Martens et al. reported that several cells of the immune system including neutrophil, monocytes, macrophages, B-cells and T-cells express vitamin D receptor (VDR) which directly or indirectly modulates their activities towards achieving immunity.[30] Reports have shown that an inverse relationship exists between vitamin D and MS and that an optimal serum vitamin D level lowers the risk.[31-33]

Although the mechanisms by which vitamin D reduces inflammation remains poorly understood, the reported lower serum level of vitamin D in individuals with MS indicates the possible role of vitamin D deficiency in the pathogenesis of MS-associated pro-inflammation.[27,34,35] Presently, there is the dearth of information on the vitamin D status of Nigerians with MS. Similarly, information on the possible interplay between the serum levels of vitamin D and TNF-α is presently lacking.

Aim

To determine the serum levels of vitamin D and TNF-α, and assess their possible relationship with gender in individuals with MS.

Materials and methods

Study design

The study was a case-control study.

Study population

A total of 100 participants were enrolled into this study. They include 60 patients with metabolic syndrome (MS) diagnosed using the International Diabetes Federation (IDF) criteria 36 and 40 apparently healthy adults randomly selected from the cohorts of traders involved in a study titled "Risk Assessment of Type 2 Diabetes Mellitus and Dementia in Nigerians with Metabolic Syndrome".[37] All the study participants had no history of cardiovascular disease, diabetes mellitus and were not on any medication as earlier reported.[38]

Ethical consideration

Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. Also, informed consent was obtained from the study participants.

Sample collection

Venous blood (10 ml) was obtained from each study participant and serum samples obtained were stored at -20^{0}C until analyzed.

Laboratory analyses

The serum levels of vitamin D was determined using HPLC as described by Kand'ár and Záková while the serum TNF-α level was determined using ELISA (Boster Biological Technology, USA) following the Manufacturer's instruction.[39]

Vitamin D classification

The serum vitamin D level was classified as reported by Holick.[40] Vitamin D levels ≤20, 21 – 29, 30 – 150 and >150 ng/ml were considered as deficiency, insufficiency, sufficiency and intoxication respectively.

Statistical analysis

Continuous variables were subjected to Kolmogorov-Smirnov Test of Normality and differences in means or medians of the variables were compared using the independent Student's t-test or Mann Whitney U depending on the Gaussian distribution of the variable. Association between dichotomous variables was determined using the Fisher's Exact test. Correlation between vitamin D and TNF-α was determined using the Spearman correlation. Results are presented as mean ± standard deviation, number (percentages) and median (interquartile range) as appropriate. *P*-values less than 0.05 were considered as statistically significant.

Results

The vitamin D status of the study participants is shown in Table 1. None of the study participants had VDD. The proportion of MS patients with vitamin D sufficiency was similar to that of controls. Only 1 patient had vitamin D intoxication among the MS patients (Table 1).

The possible effect of serum vitamin D concentration on serum TNF-α level was assessed by classifying the MS patients into two categories based on the mean level of vitamin D; 37.70 ng/mL. This classification became necessary as none of the patients had VDD and only a few had vitamin D insufficiency and intoxication. The median level of TNF-α was significantly higher in MS patients with vitamin D level lower than the mean compared with the MS patients with vitamin D level equal or greater than the mean (Fig. 1).

The median level of TNF-α was significantly higher in MS compared with the controls. However, the mean level of vitamin D was similar in both groups (Table 2).

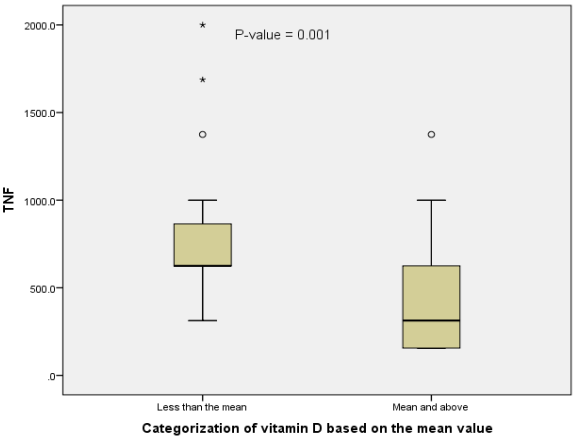

Fig. 1. Serum TNF-α levels in MS patients with below or above the mean vitamin D level (37.70 ng/mL)

A significant inverse correlation was observed between vitamin D and TNF-α levels (Fig. 2).

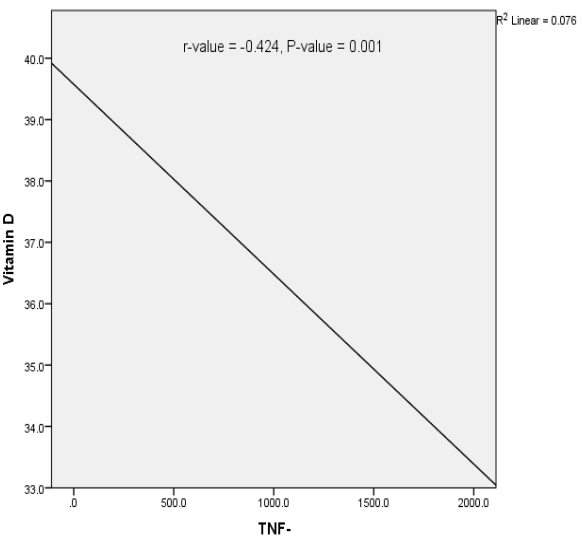

Fig. 2. Correlation between serum levels of vitamin D and TNF-α

Table 1. Vitamin D status of the study participants

	MS	Controls	n	χ^2	*P*-value
Vitamin D deficiency	0 (0.0%)	0 (0.0%)	0	2.835	0.242
Vitamin D insufficiency	2 (3.3%)	0 (0.0%)	2		
Vitamin D sufficiency	58 (96.7%)	39 (97.5%)	97		
Vitamin D intoxication	0 (0.0%)	1 (2.5%)	1		

Table 2. Serum levels of vitamin D and TNF-α in MS and controls

	MS (n = 60)	**Controls** (n = 40)	***P*-value**
Vitamin D (ng/mL)	37.70 ± 4.32	37.96 ± 3.02	0.742
TNF-α (pg/mL)	625.00 (313.00 – 728.20)	312.50 (156.00 – 312.50)	<0.001*

*Significant at P<0.05, MS = Metabolic syndrome, TNF-α = Tumour necrosis factor-alpha

Differences in the serum levels of vitamin D and TNF-α in MS based on gender is shown in Table 3. The mean serum level of vitamin D was significantly lower in females with MS compared with the males with MS. In contrast, the median serum level of TNF-α was significantly higher in females with MS compared with the males with MS (Table 3).

Table 3. Serum levels of vitamin D and TNF-α in males and females with MS

	Males (n = 15)	**Females** (n = 45)	***P*-value**
Vitamin D (ng/mL)	40.87 ± 1.92	36.64 ± 4.39	0.000*
TNF-α (pg/mL)	156.00 (156.00 – 312.50)	625.00 (625.00 – 1000.00)	0.000*

*Significant at P<0.05, MS = Metabolic syndrome, TNF-α = Tumour necrosis factor-alpha

Discussion

Understanding the dynamics of MS-associated pro-inflammation in different vitamin D status could facilitate potential therapeutic modulation of cytokine systems in MS.

In this study, the proportion of MS patients with vitamin D sufficiency and the mean serum vitamin D level were observed to be similar to that of controls and no single case of VDD was observed. This observation contradicts the reports of Farrell et al. and Godala et al. which showed lower level of vitamin D in individuals with MS.[34,35] Incongruities in our report and that of previous reports could be due to selection of study participants. In our study, the study participants were all traders whose skins are usually exposed to solar ultraviolet B (UVB) radiation as majority of them trade in open market space for hours daily. It is well known that cutaneous synthesis of vitamin D via solar UV radiation is the major source of vitamin D with only a small proportion derived from dietary intake.[41] Alagöl et al. showed that vitamin D deficiency (VDD) is more prevalent among individuals with little sun exposure.[42] Similarly, a Manchester study which used whole-body irradiation for 6 weeks to simulate summer sunlight exposure showed that UV radiation equivalent to 30 min of sunlight 3 times per week achieved vitamin D level of >50 nmol/L in >90% of the study participants.[43]

TNF-α is an immunomodulatory inflammatory cytokine with pleiotropic effects. It is produced by numerous cells including macrophages/monocytes, natural killer cells (NKCs), lymphocytes and adipocytes during inflammation. It performs diverse range of intracellular signalling.[44-47] The observed elevated serum level of TNF-α in MS compared with the controls was in line with previous reports.[48-50] This observation could be due to the heightened phagocytosis of necrotic adipocytes in the adipose tissue. It could also be attributed to MS-associated IR which results in dyslipidaemia and metabolic endotoxaemia favouring pro-inflammation.

In a bid to understand the effect of serum vitamin D level on serum level of TNF-α, MS patients were classified into 2 groups using the mean vitamin D level of the group as most of the patients were vitamin D sufficient and comparison of serum TNF-α level based on vitamin D status was impossible. The observed elevation in serum TNF-α level in the below mean group supports and the significant inverse correlation observed between vitamin D and TNF-α levels in MS support the reports of Kayaniyil et al. and Milovanovik et al. who reported high level of TNF-α in their vitamin D deficient study participants.[51,52] Our observations, which confirm the established anti-inflammatory properties of vitamin D, indicate that vitamin D could serve therapeutic purpose in MS and this could be mediated via upregulation of MAPK phosphatase-1 resulting in the inhibition of lipopolysaccharide (LPS)-induced p38 activation and cytokine production by monocytes/macrophages.[27]

Reports have shown that gender differences exist in vitamin D status with female gender negatively associated with vitamin D sufficiency.[53,54] The observed higher vitamin D level in males with MS compared with the females with MS corroborates the reports of Borissova et al. and Yan et al. showing significantly lower level of vitamin D in females compared with males.[55,56] Our observation could be as a result of differences in the amount of subcutaneous fat between males and females with MS. Vitamin D is fat soluble and could be stored in large amounts in the subcutaneous adipose tissue thereby, causing a reduction in the circulating level.[57] Several reports have shown that women have more subcutaneous fat than men.[37,58,59] Similarly, our observation could be due to differences in lifestyle and outdoor activities. Furthermore, the common use of sunscreen, which absorbs UVB, among women, could be partly responsible for our observation. Faurschou et al. showed that the use of sunscreen may lead to VDD as an exponential increase in serum vitamin D level was observed with decreasing thickness of sunscreen layer in response to UVB exposure.[60]

The observed low vitamin D level in females with MS could be responsible for the observed elevated level of TNF-α in females with MS compared with the males with MS. This observation further confirms the inverse relationship between vitamin D and TNF-α.

Conclusion

It could be concluded from this study that adults with MS have elevated TNF-α level which appears to be associated with the serum level of vitamin D. Furthermore, females with MS have low vitamin D level and this may exacerbate the MS-associated inflammation in them. Therefore, females with MS may benefit from vitamin D supplementation.

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Declarations
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Conflicts of interest
The authors declare no conflict of interest.

Data availability
Data available in the repository of the Department of Chemical Pathology, College of Medicine, University of Ibadan.

Ethics approval
Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee.

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ORIGINAL PAPER

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The Charlson Comorbidity Index: predicting readmission and severity in emergency departments

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ABSTRACT

Introduction. The Charlson Comorbidity Index (CCI) is a comorbidity scale used widely throughout the world. Despite its widespread use, its relationship with patient readmission to the Emergency departments (ED) has not been evaluated previously.

Aim. To show whether there is a correlation between the CCI score and the number of repeated admissions to ED and that the CCI score can be used as a predicted factor for the serious patients.

Material and methods. This was a prospective observational cross-sectional study. Age, gender, vital signs of the patients who agreed to participate in the study was recorded. Numbers of ED readmissions of patients within six months after discharge and CCI scores have been recorded.

Results. The study was completed with 1420 patients. The admission rates of patients in the ED in the six months were significantly higher in the CCI 5+ group than in other groups ($p<0.05$) There was a positive correlation between the number of visits and CCI scores ($p<0.01$; $C>0$).

Conclusion. We believe that the CCI scoring system can be used by ED clinicians to predict the risk of readmission of patients after discharge from ED.

Keywords. Charlson Comorbidity Index, emergency medicine, readmission

Introduction

Emergency Departments (ED) intervene in and regulate the treatment of sudden illness or acute exacerbations of chronic illnesses.[1] In recent years, an increasing number of patients have been observed in emergency services around the world, and delays are being experienced in their treatment.[2-5] Patients who have been evaluated in the ED sometimes return with the same complaint shortly after being discharged, leading to the opinion that the initial evaluation and treatment they receive is inadequate.[6] These recurrent admissions increase the workload of the EDs, contribute to overcrowding, reduce the quality of treatment, and raise the healthcare costs. Similar to the global situation, the number of readmissions to the EDs in Turkey is increasing rapidly. This situation has created serious problems for the hospitals in our country. There are multiple reasons for readmission to the ED. Most of these are patient, disease, health, and clinician factors. However, few studies have revealed other reasons for repeated applications to the ED. Risk factors, demographic and clinical characteristics of patients must be assessed to identify groups at high risk of morbidity and mortality.[7,8]

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The Charlson Comorbidity Index (CCI) is a comorbidity scale used widely throughout the world.[9] It uses patients' preoperative and intraoperative morbidity factors to evaluate morbidity and mortality risk.[10] It includes 19 comorbidity factors: Acquired immuno-deficiency syndrome (AIDS), cancer, heart attack, heart failure, lymphoma, dementia, peptic ulcer, leukemia, metastasis, hemiplegia, benign liver diseases, connective tissue diseases, cerebrovascular disease, complicated diabetes, non-complicated diabetes, peripheral vascular disease, chronic respiratory diseases, moderate or advanced kidney failure, and moderate or severe liver disease. Morbidity and mortality estimates are given according to patients' CCI scores. Though the CCI has widespread use, it has not been evaluated in patients attending at EDs, and its relationship with patient readmission has not been shown previously.

Aim

In this study, we aimed to show whether there was a correlation between the CCI values and the number of ED readmissions of the patients during next six months period of our study. In addition, considering that CCI scoring is predictive for poor clinical outcome, we also aimed to examine the relationship between number of patients' readmissions and poor outcome indirectly.

Material and methods

Study design and setting

This was a prospective observational cross-sectional study carried out on patients admitted to the ED of Atatürk University Research Hospital in Turkey between 01.10.2018 and 07.04.2019. Our study was conducted in accordance with good clinical practice standards and was evaluated and approved by the Ethics Committee of the Faculty of Medicine before the study started (25.04.2018/Decision number: 4/Session number: 4). Informed consent was obtained from all patients prior to their registration.

Patient selection

Our study was conducted on patients admitted to the ED during a seven days period at the first phase of the study. Admitted patients were informed about the study, and those who agreed to participate were included to the study. In the second phase of the study, the number of readmissions to ED within the next six months of the patients included in the study during this 7-day period was examined.

Exclusion criteria

- Patients who did not agree to participate in the study
- Patients who were unable to provide informed consent (altered mental state, non-Turkish-speaking).
- Patients with non-hospital cardiopulmonary arrest
- Patients who had accessed ED for the same or a similar complaint within the last seven days.
- Patients younger than 18 years old.

Physicians involved in the study were given training on the research. Pre-prepared study forms were filled in via face-to-face interviews with participants. Emergency medicine specialists collected the data.

Measurements

Participants' age, gender, and vital signs (blood pressure, heart rate, body temperature and oxygen saturation) were recorded. In addition, the complaints that led to ED admission, the number of ED readmissions of the patients' during the next six months, and their CCI score were recorded. The patients were classified into four ordinal groups according their CCI scores; CCI 0, CCI 1–2, CCI 3–4, and CCI 5+ (5 points and above). Among these groups, having a high CCI risk score (CCI 3-4 and CCI 5+) was accepted as an indicator of poor clinical outcome. The relationship between this situation and the number of readmissions in the second phase of the study was examined.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). The percentages and frequencies for the categorical variables and the mean (± standard deviation [SD]) values for the continuous variables were determined. Nonparametric tests were used, as the data did not conform to a normal distribution. This included the Kruskall Wallis test for multiple groups with Bonferroni correction. A Chi-square test was used for categorical data analysis. A Spearman's correlation was used to test for a correlation between CCI risk groups. Mean ± SD values were used, and $p < 0.05$ was considered statistically significant.

Results

In total, 2111 patients attended our ED during the first phase of our. Of these, 354 did not agree to participate in the study. Those excluded from the study were listed; 15 patients with cardiopulmonary arrest, 116 patients with unconsciousness, 119 patients aged lower than 18 years old, and 87 patients with repeated ED visits (38 of these had repeat admissions during the first phase, while 49 had visited the ED before the study period). A total of 1420 patients who applied to the ED with different complaints were eligible for this study. Demographics, vital parameters and the most frequent complaints of all patients by CCI groups and the number of readmissions to the ED in the second phase of the study are shown in Table 1.

The differences between the patient groups created according to the CCI risk scores were examined. It was observed that those with a CCI score of 5 and above

were mostly male patients and male patients had higher CCI risk scores than female patients ($p<0.05$). There was also a difference between the groups in terms of the age of the patients. The mean age of the patients in the CCI 3–4 and CCI 5+ groups was significantly higher than the other groups ($p<0.05$). The number of patients who applied to the ED in the second phase of the study was significantly higher in the CCI 5+ group than in other groups ($p < 0.05$).

Finally, we evaluated the correlation between patients' CCI scores and their number of readmissions to the ED during the second phase. There was a positive correlation showing that as the number of readmissions in the second phase increased, so did CCI score ($p< 0.01$; $C>0$).

Discussion

One of the most important problems faced by physicians in the ED is to detect serious patients despite the intensity of their services. In this study, our aim was to examine the relationship between the clinical severity of patients and the rate of readmissions to the ED. We wanted to determine which patients were at high risk for discharge from ED. We used the CCI risk scoring system to explain this high-risk situation with an objective scale. We used a timeframe of six months to avoid the effects of cases involving renal colic and simple infection, which can cause repeated applications in a short period of time. The results of our study showed a statistically significant relationship between high CCI scores and readmissions within the six months period.

The 19 item CCI was first described by Charlson and colleagues in 1987 and has been modified many times.[11-13] It is used in 10-year mortality estimation in patients with multiple comorbidities; however, it has been studied in many areas. Several studies have been carried out to investigate the effects of comorbidity on postoperative complications. The effect of CCI score on complications after various surgical procedures has also been examined, and a large number of studies with extensive patient groups are available in the literature. For example, postoperative complication rates, hospitalization periods, and mortality rates were evaluated in patients who underwent surgery for pancreatic cancer.[14,15] In another study, there was a significant difference in the parameters mentioned in patients with a CCI score of 4 and above. Patients with CCI scores of 6 and above were three times more likely to die in 1 year than other patients.[16] Ather et al. found that mortality was significantly higher in nephrectomy patients who had CCI scores of 5 and above compared to other patients.[17]

According to previous studies, after discharge in stroke patients, CCI score was an indicator of prognosis. Each one point increase in CCI score was associated with a 15% increase in poor outcome at discharge, a 29% increase in one year mortality rate, and a 60% increase in 30 day mortality rate.[18-21] In our study, the rate of ED visits in the last 6 months was around two per patient (the maximum was 25), and the ED visit rate of patients with CCI scores of 5 and above was around sev-

Table 1. Demographics, vital signs, complaints and readmissions of the patients according to the charlson comorbidity ındex scores

	Charlson Comorbidity Index Groups				
	0	**1-2**	**3-4**	**5 plus**	p values*
Characteristics					
Male sex, n (%)	421 (56.9%)	139 (18.8%)	111 (14.9%)	70 (9.5%)	0.038
Age (years), mean	32.6 ±10.1	50.0±12.1	65.1±11.4	70.0±11.7*	<0.0001
Vital signs					
SBP (mmHg), median	121 (71-216)	129 (89-217)	130 (89-245)	130 (87-212)*	0.046
DBP (mmHg), median	79 (45-112)	80 (46-133)	80 (48-146)	75 (45-119)*	0.004
HR (beat/min), mean	87 ±12.8	85±12.6	86 ±14.5	89±17.2	0.125
O_2 Saturation (%), median	95 (60-100)	94 (72-100)	93 (60-99)	92 (60-98)*	<0.0001
Body temperature (°C), median	36.7 (36-39)	36.7 (36-40)	36.7 (36-38)	36.7 (36-39)	0.229
Complaints (most frequent)					
Trauma	142 (17.0%)*	32 (11.5%)	4 (2.1%)	6 (5.3%)	<0.0001
Chest pain	63 (7.6%)	8 (2.9%)	29 (14.9%)	18 (15.8%)*	0.001
Stomachache	92 (11.0%)	33 (11.9%)	18 (9.3%)	11 (9.6%)	0.803
Myalgia	84 (10.1%)	27 (9.7%)	16 (8.2%)	5 (4.4%)	0.244
Dyspnea	19 (2.3%)	7 (2.5%)	22 (11.3%)	19 (16.7%)*	<0.0001
Readmissions during 6 months	1.45 ± 2.27	2.30 ± 3.45	2.38 ± 3.33	7.36 ± 6.58*	<0.0001

Values expressed as number (%), mean ± standard deviation, median (range)

DBP: Diastolic blood pressure; HR: Heart rate; SBP: Systolic blood pressure

*p value: for the statistically significant differences between selected category and other categories

en. There was a significant difference between groups. In particular, a positive correlation was observed between ED visits in the next six months and CCI score. In our study, increasing CCI scores may indicate severe cases, which may require additional care in their treatment. The development of new methods to estimate the severity of patients' clinical conditions is of great importance for improving patient health and reducing health costs.

In our study, patients with CCI scores 5 points and above were considered as high risk patients. The mean age of patients with a CCI score of 5 and above was around 70. In this group, most of the patients were elderly, and increasing age is given increasing scores in the CCI. In other words, an increase in age directly increases CCI score. A significant proportion of patients given CCI scores over 5 received them due to age, and age was the most important factor in determining the severity of patients' clinical conditions.

Patients aged 24 to 45 years had the highest frequency of reapplication to the ED, according to the National Hospital Ambulatory Medical Care Survey of America.[22] Dinh et al. stated that patients between 20 and 39 years had the highest frequency of repeat attendances within a 72 hour period. [23] Verelst et al. showed that the mean age of patients who attended for repeat examinations was 47.[24] In these studies, patients who visited ED repeatedly were usually in their thirties and forties. Unlike the literature, the majority of patients who reapplied in the six-month period in our study consisted of the 60-70 age group. We think that the main reason for this difference between our study and the common literature is the assessment of short-term readmission rates in previous studies. In our study, we examined the rate of re-admission for a long period of 6 months. And we confirmed this with an objective index. Thus, we think that we have achieved more objective and exact results compared to short term readmission rates. Walraven et al also discussed the 30-day short term results and found that the admission rate of elderly patients with chronic diseases was high, similar to our study.[25]

In our study, the distribution of CCI score by age was examined, and the mean age of patients increased in parallel with CCI score. In addition, in our study, the rate of reappearance at the ED increased as patients' age increased. There was a positive correlation between CCI and increasing age, and this was reflected in the rate of readmission. Older patients with high CCI scores had a high rate of readmission at the ED, so treatment protocols should be provided more carefully for elderly patients with CCI scores of more than 5 than for other groups.

Conclusion

This study presented a method of determining the severity of ED patients' conditions. We believe that the CCI scoring system can be used by ED clinicians to predict the risk of readmission of patients after discharge from ED. And patients with CCI values greater than 5 may be considered serious cases.

Declarations

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interest.

Data availability

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

Ethics approval

The Ethics Committee of the Ataturk University Faculty of Medicine, Erzurum, Turkey (25.04.2018/Decision number: 4/Session number: 4)

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REVIEW PAPER

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Singlet oxygen discovery

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ABSTRACT

Introduction. Singlet oxygen is perfectly suited to interact with biological macromolecules and cellular composition.

Aim. The goal was to present an information about singlet oxygen discovery.

Material and methods. In this article a narrative review regarding singlet oxygen discovery.

Analysis of the literature. The desire to summarize information about generation and basic application of singlet oxygen is presented.

Conclusion. The history of singlet oxygen is well documented in literature.

Keywords. discovery, history, singlet oxygen

Introduction

Oxygen was independently discovered by Scheele in about 1771 and Priestley in 1774. Carl Wilhelm Scheele's first publication, "Chemische Abhandlung von der Luft und dem Feuer" was delivered to the printing house in 1775, but was not published until 1777. During this time, Joseph Priestley and Lavoisier published their experimental data and conclusions regarding oxygen. Thus, Carl, who made the discovery chronologically earliest, was credited with the discoverer of oxygen along with Joseph Priestley, the English exponent, and Antoine Lavoisier, the father of modern chemistry. Undoubtedly, however, each of the three chemists made a contribution to the discovery of this element; Scheele was the first to isolate the gas, Priestley first published "An Account of further Discoveries in Air" in the journal Philosophical Transactions and pointing to the connection between air and blood, and the French physicist and chemist Lavoisier, describing it as "purified air itself in without changing ", giving it the name oxygenium and explaining, for the first time, its meaning without using the then erroneous theory of phlogiston, and at the same time putting an end to it.

Aim

The aim of this work is to present the review of oxygen discovery.

Material and methods

This article is a review done in regards to discuss the role of singlet oxygen.

Analysis of literature

Amadeo Avogadro in 1811 described oxygen as a diatomic molecule. In 1848 Michael Faraday announced that the oxygen molecule is a paramagnet and is attracted by a magnet, differing from other gases such as helium gases by a specific electronic structure.[1-3] In his 1867 publication, Fritzsche first described a reaction involving singlet oxygen. This was a precipitation reaction from a solution of 2,3-benzanthracene exposed to sunlight and air, but the true nature of the reaction was not yet suspected. The Frenchman Louis Cailletet was one step ahead of the Swiss Raoul Pictet by producing a few drops of liquid oxygen in 1877. The following year, Sir Dewar conducted an oxygen liquefaction demonstra-

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tion in front of members of the Royal Institute during the Friday Discussions. Over the next five years, he improved his method of liquefying oxygen and had a large amount of this element in the liquid state, thanks to which its characteristics could be studied comprehensively. He found that liquid oxygen (ozone) is attracted to magnetic poles. In 1891, at one of the "Friday Discussions", he presented his discovery, using a magnet of a large size and a vacuum bottle, known to this day in laboratories as the Dewar vessel. The liquid oxygen flowing from the bottle hangs in the air between the poles of the magnet, hanging like a large drop until it turns into gas and disappears.[4] It was only in 1925 that this phenomenon was explained by Robert Mulliken, thanks to the newly formulated quantum theory.

Robert S. Mulliken, Nobel laureate in chemistry, in 1928 showed that the paramagnetic property of oxygen is due to the parallel spins of two outer electrons in an oxygen molecule. This paramagnetic, unpaired pair of electrons is triplet oxygen. They determine its chemical properties, making it difficult to select electrons, which is related to the low capacity of oxygen to bind with other compounds. The electron spin reversal produces one pair of electrons and one free electron in the orbit, allowing oxygen to react by removing the constraining spin function. The first evidence of the existence of a metastable and highly reactive oxygen species was given in 1931 by Kautsky. Gerhard Herzberg, also a Nobel laureate in chemistry, thanks to infrared spectroscopy in 1934 confirmed the existence and accurately described the possible states of molecular oxygen, including singlet oxygen as a higher energy state of oxygen. In 1943, Schenck discovered the one reaction with singlet oxygen. This reaction is one of the most studied processes in organic chemistry today.[5,6] The first purposeful use of singlet oxygen took place in 1954. Schenck and Ziegler then carried out the oxidation of α-terpinene in the presence of chlorophyll, as a result of which ascaridol was produced.[7,8]

The next discoveries about singlet oxygen came from photo-oxidation experiments by Christopher Spencer Foote and Wexler and Elias Corey and Taylor in 1964. Foote, a faculty member at UCLA, made a groundbreaking discovery of the role of singlet oxygen as an excited form of oxygen in air in the reactions of organic molecules caused by light and ultraviolet radiation. Foote's discovery of developing an independent chemical pathway for the formation of this form of oxygen was made in 1964, while he was still a lead at the University of California, Los Angeles. This has led to a large amount of research into the interaction of singlet oxygen with a wide variety of chemicals, DNA and nanomaterials.[9] His research has led to important discoveries about why molecular oxygen is both essential to life processes and a major factor in biological damage. Many aspects of singlet oxygen chemistry are derived from the work of Christopher S. Foote and his colleagues. Singlet oxygen is an interesting molecule with an extraordinary history behind its discovery. Foote and Wexler conducted experiments in the 1960s, where they obtained evidence of singlet oxygen generation through two independent pathways: 1) photochemical reaction (photooxidation with dye) and 2) chemical reaction (NaOCl with H_2O_2). An important factor in the discovery of singlet oxygen as an intermediate in the photooxidation reaction with the dye was Foote's reassessment of the 1930s chemical literature, where it has already been suggested. Experiments using silica gel beads provided evidence for the presence of a volatile diffusive oxidant such as singlet oxygen.[10] Soon after Foote's first research was published in 1964, the idea of singlet oxygen as an intermediate in photooxidation chemistry gained more and more recognition and validation in organic, gaseous and biological processes. Foote's 43-year research career has led him to become a world leader in organic chemistry. His earliest work focused on the effect of bond angle deformation on the property of organic molecules. He established a quantitative correlation between spectroscopic properties and reactivity.[11]

Foote's main interest was the generation and reaction of reactive oxygen species. Foote has released over 250 research papers, many of which focus on singlet oxygen. Scientific advances in the chemistry of singlet oxygen and its reaction with organic compounds have occurred rapidly over the past 25 years. The great importance of the reaction with singlet oxygen has been noticed in medicine, biochemistry, organic chemistry, food chemistry and environmental chemistry.[12]

Molecular oxygen comes in two forms: singlet and triplet. In its ground state, an oxygen molecule has two electrons with opposite spin, occupying the π-bonding orbitals perpendicular to each other. Molecular oxygen exhibits paramagnetic properties due to a spin quantum number of 1. As a result of the supply of a certain amount of energy, a triplet oxygen molecule is excited and two electrons with opposite spin pairing. The spin quantum number is then zero. The oxygen molecule excited in this way is singlet oxygen and has a higher energy. The amount of delivered and absorbed energy determines the form of singlet oxygen, two of which are distinguished: delta and sigma with different distribution of electrons in molecular orbits: 1ΔgO2 - in which there are two paired electrons in one orbital π * 2p and 1Σg + O2 - in which it occurs after one electron in each of the π * 2p orbitals, and the electrons have opposite spins. The difference between the ground state and the excited states is respectively: 22.5 kcal mol-1 and 31.5 kcal mol-1. Singlet oxygen is an excited form of molecular oxygen, but it is not a free radical.[13]

Singlet oxygen is produced by a photosensitization

reaction in which an endogenous photosensitizer, such as a porphyrin, is excited by light. When a quantum of ultraviolet radiation (or higher energy radiation) is absorbed, the excitation energy is transferred to oxygen and transformed into singlet oxygen, and the photosensitizer returns to its ground state. In order to obtain singlet oxygen, therefore, one needs an oxygen source (it may be air), light of the appropriate wavelength (usually sunlight) and photoactive particles. The efficiency of the process is determined by a parameter called quantum efficiency. Singlet oxygen can also be generated in chemical reactions without the use of light, e.g. decomposition of calcium peroxide, molybdenum, tungsten and lanthanum peroxides, thermal decomposition of ozonophosphine adducts (Myrray method), thermal decomposition of aromatic endoperoxides or decomposition of hydrogen peroxide. Singlet oxygen is also produced by an oxygen explosion in phagocytes during an inflammatory reaction, while hypochlorous acid is formed, which reacts with hydrogen peroxide and as a result of lipid peroxidation, i.e. the reaction of two peroxyl radicals.[14]

Singlet oxygen interacts with other molecules by transferring excitation energy - this is the so-called quenching singlet oxygen, it then goes into a triplet state or by entering into a chemical reaction. It can easily react with other singlet molecules. Chemical reactions involving singlet oxygen are often accompanied by visible light emission - chemiluminescence (e.g. blue during the oxidation of luminol). The high reactivity of singlet oxygen is used in a number of chemical reactions in organic chemistry, especially with unsaturated compounds. The first step of the reaction with unsaturated compounds is usually the formation of R2C = C (R) -O-O-H allyl hydroperoxide or the R-O-O-R peroxide bridge. These compounds often undergo secondary reactions - simultaneous rupture of the O-O and C-C bond with formation of two carbonyl groups. The reaction products of singlet oxygen with cholesterol and tryptophan are characteristic. The possibilities of singlet oxygen are limited by inactivation with water. Heavy water is less likely to quench the excited state of oxygen, therefore it is used in experiments to determine the role of singlet oxygen in the studied reactions. If the reaction presumably caused by singlet oxygen works better in heavy water than in ordinary water, we have a right to consider our suspicions to be valid.[15-17]

Singlet oxygen is more electrophilic and has better oxidizing properties than basic oxygen, therefore it is considered a universal oxidant. It reacts with: lipids leading to peroxidation; proteins leading to oxidation of side chains, inactivation, misfolding, or enhanced degradation in proteasomes; nucleic acids, resulting in base modification and strand breaks. The most susceptible to damage by singlet oxygen are histidine, methionine, tryptophan, tyrosine, cysteine and guanine residues.

The very strong oxidizing properties of singlet oxygen can find application in environmental protection, e.g. treatment of industrial wastewater containing phenols derived from clothing from the paper industry. The singlet oxygen method to remove 2-chlorophenol from industrial wastewater uses oxygen from the air and sunlight to generate singlet oxygen, and the immobilization of photoactive Bengal red particles in silica gel enables their recovery and multiple use. We also find many uses of singlet oxygen in medicine, e.g. for the sterilization of blood donations by scientists working for the Swiss Red Cross. In medical applications, photoactive molecules must meet stringent requirements, such as high antimicrobial activity and non-toxicity to humans, e.g. methylene blue. The mechanism of action is based on the interaction of singlet oxygen with cells of foreign organisms. First, the photoactive molecule is excited, and then, as a result of energy transfer, molecular oxygen is formed, oxidizing bacterial cell walls, nucleic acid fragments and enzymes. Due to the high degree of adsorption of phenothiazines, such as methylene blue, they can be successfully used in local antibacterial therapy in the fight against microorganisms such as, for example, Helicobacter pylori, Escherichia coli, Staphylococcus aureus, Enterococcus faecium. Phenothiazine derivatives are called the "antibiotics of the future" because they may soon become a modern alternative to currently used antibacterial agents.[13-14]

Singlet oxygen is believed to be the primary reactive form of oxygen responsible for damage to leaf cell components and the light-induced loss of photosystem II activity.

Singlet oxygen, unlike its spin-limited sister form, quickly reacts with molecules of organic compounds, but if by chance oxygen existed only in singlet form, it would never accumulate in the atmosphere, and as a result, life would never come from the oceans to land . Singlet oxygen is not ROS produced in the cells of our body in physiologically significant amounts. However, there are situations in which the production and reactions of singlet oxygen become important. This is the case with porphyria, a disease caused by a defect in the metabolism of porphyrins, which causes them to accumulate in the skin. Some drugs are photosensitive and singlet oxygen acts as a mediator of their harmful side effects. St. John's Wort Hypericum contains hypericin, which produces singlet oxygen when exposed to light, so that large amounts of St. John's wort by cows or sheep in sunny pastures can lead to photosensitivity reactions.

Conclusion

Oxygen is hailed as the elixir of life, while arousing fear as a fuel to keep smoking and the poison that causes our death. Undoubtedly, however, it is very important in our lives - we cannot live without it for more than a few minutes.

Declarations

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Author contributions

Conceptualization, A.C.; Writing – Original Draft Preparation, A.C.; Writing – Review & Editing, A.C.

Conflicts of interest

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REVIEW PAPER

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New endoscopic treatment methods for PPI-resistant GERD

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ABSTRACT

Introduction. Gastroesophageal reflux disease (GERD) is a common disease with the highest prevalence in North America. Up to 40% of patients report persistent gastroesophageal reflux disease (GERD) symptoms despite proton pump inhibitor (PPI) therapy.
Aim. The aim of this article is to complete discuss the GERD characterized by heartburn and/or regurgitation symptoms.
Material and methods. We discuss here the evidence for medical therapy for PPI nonresponsive GERD.
Analysis of the literature. GERD may present with a variety of other symptoms, including water brash, chest pain or discomfort, dysphagia, belching, epigastric pain, nausea, and bloating. In addition, patients may experience extraesophageal symptoms like cough, hoarseness, throat clearing, throat pain or burning, wheezing, and sleep disturbances.
Conclusion. There has been an increase in GERD prevalence. GERD is one of the most common gastrointestinal disorders managed by gastroenterologists and primary care physicians.
Keywords. diagnostics, endoscopic treatment, gastrology

Introduction

Gastroesophageal reflux disease (GERD) is a chronic condition of the upper gastrointestinal tract. An international consensus group in Montreal has defined GERD as a condition that develops when reflux of stomach contents causes troublesome symptoms with or without complications.[1] The typical GERD syndrome is characterized by heartburn and regurgitation, and proton pump inhibitor (PPI) therapy represents the mainstay of medical treatment for typical GERD, with very high efficacy in heartburn relief and healing of erosive reflux disease (ERD).[2]

Aim

We performed a systematic literature search and present a narrative review. We investigated factors related to proton pump inhibitor (PPI)-refractory gastroesophageal reflux disease (GERD) symptoms.

Material and methods

According to the recommendations, a new endoscopic treatment method for PPI-resistant GERD have been investigated in data base such as Pubmed, Science Direct and Medline.

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Analysis of the literature

A recent systematic review showed that the prevalence of GERD is 18.1–27.8% in North America, 8.8–25.9% in Europe, 2.5–7.8% in East Asia, 8.7–33.1% in the Middle East, 11.6% in Australia, and 23.0% in South America.[3] Eusebi et al. made global meta-analysis of global prevalescence GERD stated the prevalence of gastro-oesophageal reflux symptoms varied strikingly among countries, prevalence was significantly higher in subjects ≥50 years, smokers, NSAID users and obese individuals, although these associations were modest.[4] Yamasaki et al noticed that over the last decade, there has been a significant increase in the proportion of younger patients with GERD, especially those within the age range of 30–39 years.[5]

There are several important factors like age ≥50 years, male sex, white rase associated with the risk of complications from GERD, but none are strongly associated with GERD symptoms. Environmental factors are strongly related to both GERD symptoms and complications, including obesity, tobacco use, and inversely with infection with *Helicobacter pylori*.[6]

GERD is mainly a clinical diagnosis based on typical symptoms. Patients with typical symptoms should first be given a trial of PPI treatment. Patients with alarm symptoms including dysphagia, anemia, weight loss, bleeding, and recurrent vomiting should proceed directly to upper endoscopy.[7]

To describing endoscopic assessment of oesophagitis there is used the Los Angeles scale (Tab. 1).

Table 1. The Los Angeles scale

Grade	Endoscopic view
A	One (or more) mucosal break no longer than 5 mm, that does not extend between the tops of two mucosal folds
B	One (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds
C	One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but which involves less than 75% of the circumference
D	One (or more) mucosal break which involves at least 75% of the oesophageal circumference

GERD can result in serious complications, including esophagitis and Barrett's esophagus which can vary widely in severity with severe cases resulting into gastrointestinal (GI) bleeding or potential to progress Barrett's esophagus to esophageal adenocarcinoma.[8]

The treatment gastroesophageal reflux disease included lifestyle modifications like weight loss, avoid smoking, chocolate, carbonated beverages, spicy food, fatty food, alcohol, and large meals.[9] Elevating the head of the bed and sleeping in the left decubitus position, has positive effect too.[10] Pharmacologic management of esophageal reflux is classified into five major categories: acid neutralizing medications, alginate-based barriers, sucralfate, adjunctive therapies and acid-suppressive medications. However, the efficacy of this intervention is often hampered by adherence, costs, and the risks of long-term PPI use.

In the case of treatment failure several surgical techniques are currently available for the treatment of GERD. We include among them Nissen funoplication, antireflux surgery, magnetic sphincter augmentation and Roux-en-Y gastric bypass. Studies show only minimal long-term symptomatic improvements with anti-reflux surgery over PPI therapy, paired with an increased incidence of dysphagia and dyspepsia.[7-11]

Researchers in last years have focused on the development of endoscopic therapies for the management of GERD, which are less invasive and safer than surgical treatment. The original endoluminal therapies have been broadly categorized to four different types; (1) fixation, (2) ablation, (3) injection, (4) mucosal excision and suturing.[11,12] These therapies include injectable agents, electrical stimulation of the lower esophageal sphincter, antireflux mucosectomy, radiofrequency ablation, and endoscopic suturing devices designed to create a fundoplication. The most popular endoscopic antireflux devices include the following: radiofrequency ablation (RFA), transoral incisionless fundoplication (TIF),endoscopic full-thickness plication and Medigus Ultrasonic Surgical Endostapler (MUSE).[13,14]

There are currently two approved endoscopic GERD therapies: Stretta - radiofrequency therapy for GERD uses low-energy radiofrequency ablation of the submucosal tissue and transient lower esophageal sphincter relaxation.

Stretta's four-channel RF generator and catheter system delivers pure sine-wave energy (465 kHz, 2 to 5 watts per channel, 80 volts maximum at 100 to 800 ohms). Each needle tip incorporates a thermocouple that automatically adjusts the power obtup to a desired target temperature in the muscle layer. Maintaining target temperatures below 100° minimizes any adjacent tissue damage due to vaporization and high impedance values. Temperature is similarly monitored with a thermocouple at each needle base abutting the mucosa and the power delivery ceases if such mucosal temperature exceeds 47°. A recent meta-analysis of 18 studies and 1441 patients concluded that: (1) Stretta is very effective in GERD symptom relief; (2) Is safe and well-tolerated; and (3) Stretta significantly reduces acid exposure to the esophagus, but does not consistently normalize pH. On this last point it is important to note that even PPIs do not normalize pH in up to 50% of symptomatically controlled GERD patients treated with PPIs.[12-15] Stretta is an outpatient endoscopic option with unique mech-

anisms of action, is effective, safe and durable. Stretta therapy has been shown to acid and volume reflux. Side effects are extremly rare and included fever, superficial mucosal injury, chest pain requiring opioid analgesic use, transient dysphagia, sedation-related hypotension and submental swelling related to topical analgesia allergy, serious complications are including esophageal perforation and aspiration pneumonia.[14,15] Transoral fundoplication uses polypropylene H fasteners to create a serosa-to-serosa fusion to create a fundoplication, it reconfigures the tissue to obtain a full-thickness gastro-esophageal valve from inside the stomach, by serosa-to-serosa plications which include the muscle layers; the new valve boosts the barrier function of the LES with potentially fewer procedure-related side effects than surgery. The device body is composed of the following: fasteners cartridge; retractor lock; vacuum connection; fastener pushers and helix retractor control; tissue mold knob. The device chassis and tissue mold provide fundic tissue rotation and compression during fastener firing. Fasteners over a stylet: the spear-like stylet penetrates approximated tissue planes, and fasteners ensure adequate tissue alignment and compression during the healing period. Helical retractor provides tissue retraction, anchoring of the GEJ during the creation of fundoplication. This retractor is stored inside the tissue mold during EsophyX-Z insertion into the stomach and during its withdrawal. The invaginator allows circumferential tissue retraction and reduction of small hiatal hernias, and ensures adequate localization of the fundoplication.[16,17]

New endoscopic methods for PPI-resistant GERD are in constant demand. For example Inouone et al. conducted a new method of treatment for gastroesophageal reflux - ARMA - minimally invasive anti-reflux mucosal ablation (Tab. 2). At first stomach was insufflated with CO_2 to visualize the cardia in retroflex view. Next they marked placed using the triangle-tip knife J connected to an electrocautery generator in spray coagulation mode (50W). Mucosal ablation was planned around the cardia on the gastric side in a butterfly shape with width of approximately 1.5 scope diameter, leaving two contralateral areas of normal cardia mucosa with approximately one scope diameter, to avoid stenosis. Saline with indigo carmine dye was injected into the submucosal layer along the markings using a 25-gauge needle. A submucosal cushion reduces thermal injury and the risk of perforation during ablation. Mucosal ablation was performed using the triangle-tip knife J in spray coagulation mode (50W). Adequate ablation depth was defined as reaching the submucosal layer, which could be confirmed by observation of the indigo carmine dye during ablation.[18]

In new study Mondragón used new ablative technique named antireflux ablation therapy - ARAT - for control of GERD in patients without hiatal hernia, the novel treatment using argon plasma coagulation, combined with a submucosal bleb creation (Tab. 2). Process started after marking two lines composed of 5 to 6 dots were placed at EGJ in retroflex view over the greater curvature, using soft coagulation (effect 2, 40W) with 1 to 1.5cm of distance between. Next submucosal bleb was created with the injection of saline solution combined with methylene blue all along the EGJ in retroflex position. High-power coagulation (forced coagulation effect 3, 100w) was applied all along the EGJ starting at z-line up to 3cms below this point in circumference direction, except for the marking lines previously performed, in order to make 270 to 320 degrees of ablation.[19]

Table 2. Comparision of new methods

Short-cut	ARMA	ARAT
Name	anti-reflux mucosal minimally invasive ablation	antireflux ablation therapy
Method	Mucosal ablation around the cardia on the gastric side, next injected aaline with indigo carmine dye into the submucosal layer along the markings and finally mucosal ablation.	Marking two lines dots placed at EGJ in retroflex view over the greater curvature, using soft coagulation. Next created submucosal bleb with the injection of saline solution combined with methylene blue all along the EGJ in retroflex position. In the end high-power coagulation applied all along the EGJ
Study patients	GERD in patients without sliding hiatal hernia >3cm	GERD in patients without hiatal hernia

Conclusion

Endoscopic therapies for the management of GERD have made significant advances in the past 20 years. However, more studies are needed to define optimal techniques and most appropriate patient selection criteria and to further evaluate device and technique safety.

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Author contributions

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Conflicts of interest

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REVIEW PAPER

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The role of new biomarkers for the diagnosis and treatment of colon cancer

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ABSTRACT

Introduction. Colorectal cancer may be benign or malignant. According to the World Health Organization and CDC, it is the second most common cancer worldwide, after lung cancer. The mortality of colorectal cancer has been dropping for more than 20 years due to the improvements in screening techniques and treatments.

Aim. The aim of this article is to discuss the role of new biomarkers for the diagnosis and treatment of colon cancer.

Material and methods. This article is a review done in regards to discuss the role of new biomarkers for the diagnosis and treatment of colon cancer.

Analysis of the literature. A review is discussed the role of new biomarkers for the diagnosis and treatment of colon cancer using current literature.

Conclusion. The screening tests based on diagnostic new biomarkers may cause faster detection of cancer and risk factors, and provide prognostic information in order to adjust individual therapy.

Keywords. colon cancer, diagnosis, treatment

Introduction

Nowadays colon cancer takes a third position among neoplastic disease in the USA. The overall 5-year survival rate is approximately 65%, higher for localized disease (90%) than metastatic disease (14%).[1] Morbidity is predicted to increase in incidence by 60% by 2030.[2] American Cancer Society recommend commencement screening for all average-risk adults at 45 years old. Family history, other cancers, and advanced colon polyps are strong risk factors that must take into account earlier diagnostic.[3] Conventional colonoscopy contains about 25% of false-negative results.[4] Colorectal carcinogenesis is long-term process. Tumor arise from adenomatous polyps that gradually progress to dysplasia and eventually to carcinoma about 5-15 years.[5] By auto- and paracrine secretion of cytokines, chemokines, proteins

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and growth factors, cells create optimal conditions for progression and act as a suppressor on immune mechanisms. Thus, in order to assess the cause of expansive tumor growth and spread, it is necessary to determine cancer biomarkers, which are a more sensitive marker than traditional clinical-histopathological grading.[6,7]

Aim

We investigated the feasibility of an increase in research towards the better understanding of the role of new biomarkers for the diagnosis and treatment of colon cancer.

Material and methods

All materials are based on data base such as Pubmed, Science Direct and Medline.

Analysis of literature

NAA10

NAA10 is a orthologous gene of arrest-defective 1 (ARD1), which was first identified in *Saccharomyces cerevisiae* 30 years ago. NAA10 is located on chromosome Xq28 and composed of eight exons, protein consists of 235 amino acid in humans.[8] Ren et al. proved increased mRNA and protein expressions of NAA10 in colon cancer. Also Jiang et al. and Yang et al. reported high expression levels of NAA10 in patients with this tumor, which might suggest the potential role of NAA10 as a prognostic biomarker for colon cancer. Moreover Jiang group demonstrated that, of 106 patients with high level of NAA10, 74 died of cancer.[9] The NAA10 analysis also concerned other, neoplasms such as breast, lung and bone cancer.[10]

Transmembrane 4 L six family 1 (TM4SF1)

Transmembrane 4 L six family 1 (L6-Ag, TAL6, L6) and was a highly expressed surface protein of colon, lung, breast tumors. human lung, breast, colon, and ovarian carcinomas, discovered in the 90s. The TM4SF1 gene is located on chromosome 3. Many studies have shown that TM4SF1 plays an indispensable role in promoting cancer cell proliferation and migration through a series of signaling pathways.[11] Otsuka et al. proved that TM4SF1 expression was higher in metastatic cancer-derived tumors than in primary tumor-derived cells from a single colorectal cancer patient.[12] Park et al. found that TM4SF1 was upregulated in colon cancer tissues and cell lines and was positively correlated with lymph node metastases.[13]

S100 protein

All chemokines, chemokines and growth factors, demonstrating pleiotropic effects, affect almost every stage of the creating and spread of colorectal cancer. S100 is a Ca2 + ion-binding protein, having EF-hand motifs (regulatory domain, hand-domain), a factor involved in the processes of translating changes in Ca2 + ion levels into a specific cellular response by binding to specific proteins - annexin, a cytosolic phospholipase A2, endoplasmic reticulum proteins and myosin.[14] Many proteins of the S100 family have been identified, which are involved in growth and progression (S100Ab and S100A9). S100A1B and S100BB are present in melanoma, thyroid cancer, clear cell kidney cancer and colon diseases.[15] The expression is low in benign polyps while the S100 increases in the inflammatory tissue from which the tumor grows.[16] Scientist proved that S100 secretion in colon cancer positively correlates with the clinical stage of the disease, progression, metastasis effect and evidence of early or late relapse. Zeng et al. showed a high S100A10 levels was associated with advanced-stage colon cancer. Also noticed that high expression of S100A1 was correlated with poorer overall survival and disease-free survival and that overexpression of S100A2 and S100A11 was associated with weak colon cancer disease-free survival, indicating that S100A1, S100A2 and S100A11 are potential prognostic markers.[17]

Cyclin A2

Cyclins are proteins that regulate the cell division cycle by binding and activating cyclin-dependent kinases. Cyclin A2 is an established regulator of cell proliferation and has been used for molecular diagnostics as a proliferation marker. A number of studies have investigated the role of cyclin A2 on cancer development in vitro and in vivo. Guo et al. generated mice deficient for cyclin A2 in colonic epithelial cells. Colons of these animals showed severe inflammation and mucosal remodeling leading to low- and high-grade dysplasia. In result cyclin A2 deletion promoted the development of dysplasia and adenocarcinomas in a murine colitis-associated cancer model. Adenocarcinomas were only detectable in cyclin A2–deleted mice, but not in controls. In next steps researchers explored the status of cyclin A2 expression in clinical samples at the mRNA and protein levels and found higher expression in tumors of patients with stage 1 or 2 compared with those of patients with stage 3 or 4 colon cancer. High *level of cyclin 2* is associated with a better prognosis in patients. Based on the analysis, a conclusion can be drawn that cyclin A2 is a candidate for a prognostic marker for cancer.[18] Li et al. proved that cyclin A2 and Cyclin B1 were also expressed higher in adenocarcinoma t. Cyclin genes were highly related to the drug sensitivity of some drugs, which might provide guidance for clinical treatment. In conclusion, cyclin genes are new a and unusually promising biomarkers for the diagnosis and prognosis of colon cancer.[19]

IL-6

It has also been shown that IL-6 plays a key role in the development of tissue neoplasms due to their chronic

inflammation, being the basic link between inflammation and carcinogenesis with TNF-α and the nuclear factor NF-κB.[20,21] A strong release of IL-6 occurs in cases of uterine, lung, colorectal, kidney, breast, pancreatic and ovarian cancer.[22,23] The correlation between the concentration of this cytokine has been proven, and unfavorable prognosis - serum levels increase in proportion to the cancer stage, malignancy and tumor mass, and is also associated with shorter survival times.[24,25] The relationship of IL-6 with colorectal cancer has also been demonstrated, in which the concentration of this cytokine increases with the stage of advancement, low cell differentiation as well as tumor infiltration and progression, correlating with the concentration of the carcinoembryonic antigen CEA.[26] and with the survival time of patients.[27] Recent studies have reported an increase in interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R) levels in the sera of patients affected by colon cancer that correlate with the tumor size, suggesting a potential role for IL-6 in colon cancer progression.

NAP1L1

Qeiroz et al. and Aydin et al. utility of nucleosome assembly protein 1-like 1 (NAP1L1), in animal models and colon cancer patients. Adenomatous polyposis coli (*APC*) inactivating mutations are the earliest and most common genetic alterations in the colon cancer. Queiroz et al. of analyzed mouse models of *Apc* deletion and tried to discover new colon cancer biomarkers. Scientist decided to research Nap1L1 expression in Apc deficient mice. *NAP1L1* expression is increased in the mouse small intestine following *Apc* inactivation and its expression is also altered in human with colon cancer and correlated with overall survival in a patient.[28] Aydin et al. conducted research on 95 patients with colon cancer and 50 healthy people. Serum NAP1L1 levels were higher in colon cancer patients as compared with control. This makes a NAP1L1 promising biomarker in the diagnosis and prognosis of colorectal cancer.[29]

MUC1

The normal surface of the colon is lined with various types of mucins, secreted by specialized epithelial cells that protect the lining of the epithelium against pathogens. Some mucins, such as MUC1 and MUC13, act as oncogenes, whereas others: MUC2, MUC6 are suppressors.[30] The immunohistochemical analysis of colon cancer tissues from 45 patients revealed positive expression of MUC1 (in 55.6%) and totally negatively of nontumor tissue.[31] In another histopathological study of tissues removed from 381 patients with colon cancer, it was discovered that MUC1 is expressed in 64%.[32] Zhang et al. demonstrated that MUC1 has a pro-tumor role in immune-competent mice.[33]

Conclusion

That predictive and prognostic biomarkers for colon cancer has become a vast and modern field. Widely used carcinoembryonic antigen (CEA) is also observed in inflammatory bowel disease reducing its utility as a single marker for early cancer. Recent advances in molecular technologies have led to the discovery of multiple biomarkers that might facilitate early detection of colorectal lesions.

Declarations

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Author contributions

Conceptualization, A.C.C., D.B.A., A.K.K., D.A., M.K.O., W.L. and G.C.; Formal Analysis, A.C.C., D.B.A., A.K.K., D.A., M.K.O., W.L. and G.C.; Writing – Review & Editing, A.C.C., D.B.A., G.C., D.A., M.K.O., W.L. and A.K.K.

Conflicts of interest

The authors declare no conflict of interest.

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CASUISTIC PAPER

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A very rare complication of frontal sinusitis: Pott's puffy tumor

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ABSTRACT

Introduction. Pott's puffy tumor is a very rare clinical condition characterized by subperiosteal abscess, a complication of frontal sinusitis, or as a result of head trauma.

Aim. Early diagnosis of this condition is significantly important to prevent sequelae and severe neurological complications. This phenomenon, which is generally seen in children, can rarely be encountered in adults.

Description of the case. In this report, we share a rare case of Pott's puffy tumor in an adult patient.

Conclusion. Pott's puffy tumor, a rare complication of frontal sinusitis, should be considered to prevent neurological and intracranial complications.

Keywords. emergency, headache, Pott's puffy tumor

Introduction

Pott's puffy tumor is a rare complication of sinusitis, mastoiditis, malignancy, or insect bites.[1] It's characterized by a subperiosteal abscess with associated osteomyelitis originating from the frontal sinus. In the late 18th century, Sir Percival Pott described this phenomenon as forehead trauma and early sinusitis.[2] Usually, it's seen in the pediatric age group, but there are few cases reported in the adult population.[3] It has a high risk of intracranial complications such as meningitis or neurological symptoms like seizures if not recognized treated early. Computed tomography (CT) scan is successfully able to diagnose this condition and detect progression into intracranial complications such as meningitis and focal abscess.[4] Plain and contrast-enhanced cranial CT scans are accepted as the most reliable investigations, but magnetic resonance imaging, technetium-99m, and gallium-67 scans are also useful. However, these may still not show intracranial involvement precisely.[5]

Aim

Early diagnosis of sinusitis is very important to prevent sequelae and severe neurological complications. Pott's puffy tumor, a rare complication of frontal sinusitis, should be considered to prevent neurological and intracranial complications.

Description of the case

A 22-year-old man presented to the emergency department with complaints of swelling on the forehead and headache for one week. On physical examination; vitals were stable, Glasgow Coma Scala was 15 (E4, V5, M6), an 8cm x 8cm fluctuant, tender, warm swelling on the forehead was palpated (Fig. 1).

The rest of the systemic examination was unremarkable. The CT scan of the patient was reported as pansinusitis, a bone defect in the anterior wall of the right frontal sinus, and there were collection areas under the skin (Fig. 2).

Full blood count, urea and electrolytes, viral markers, glucose level, C reactive protein, and kidney function

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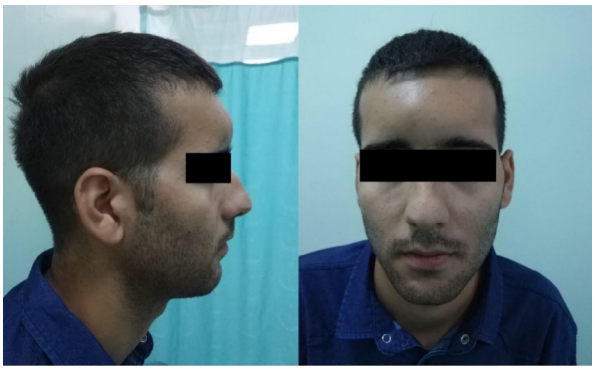

Fig. 1. An 8 cm x 8 cm fluctuant, tender, warm swelling over the forehead

tests were all within the normal range. Pott's puffy tumor was explained as a rare complication of current clinical frontal sinusitis. The patient was referred to the otolaryngology clinic for further examination and treatment. The patient was examined by the otolaryngology clinic with a diagnostic rhinoscopy. Rhinoscopy showed bilateral hyperemic mucosa and non-obstructive turbinates. No purulent discharge was observed. The abscess was drained by an external coronal incision and the culture was taken. Empyrical therapeuticsiv 2 gr ceftriaxone was given daily by the otolaryngology clinic. The patient wanted to leave the hospital on the 2nd day of his hospitalization without waiting for the culture result. At the end of the second day, the general condition of the patient was good. The patient was started orally empiric therapeutic amoxicillin-clavulanic acid 2g/day and metronidazole 2g/day. The culture of the pus revealed *Streptococcus pneumoniae*. After 4-weeks, the patient

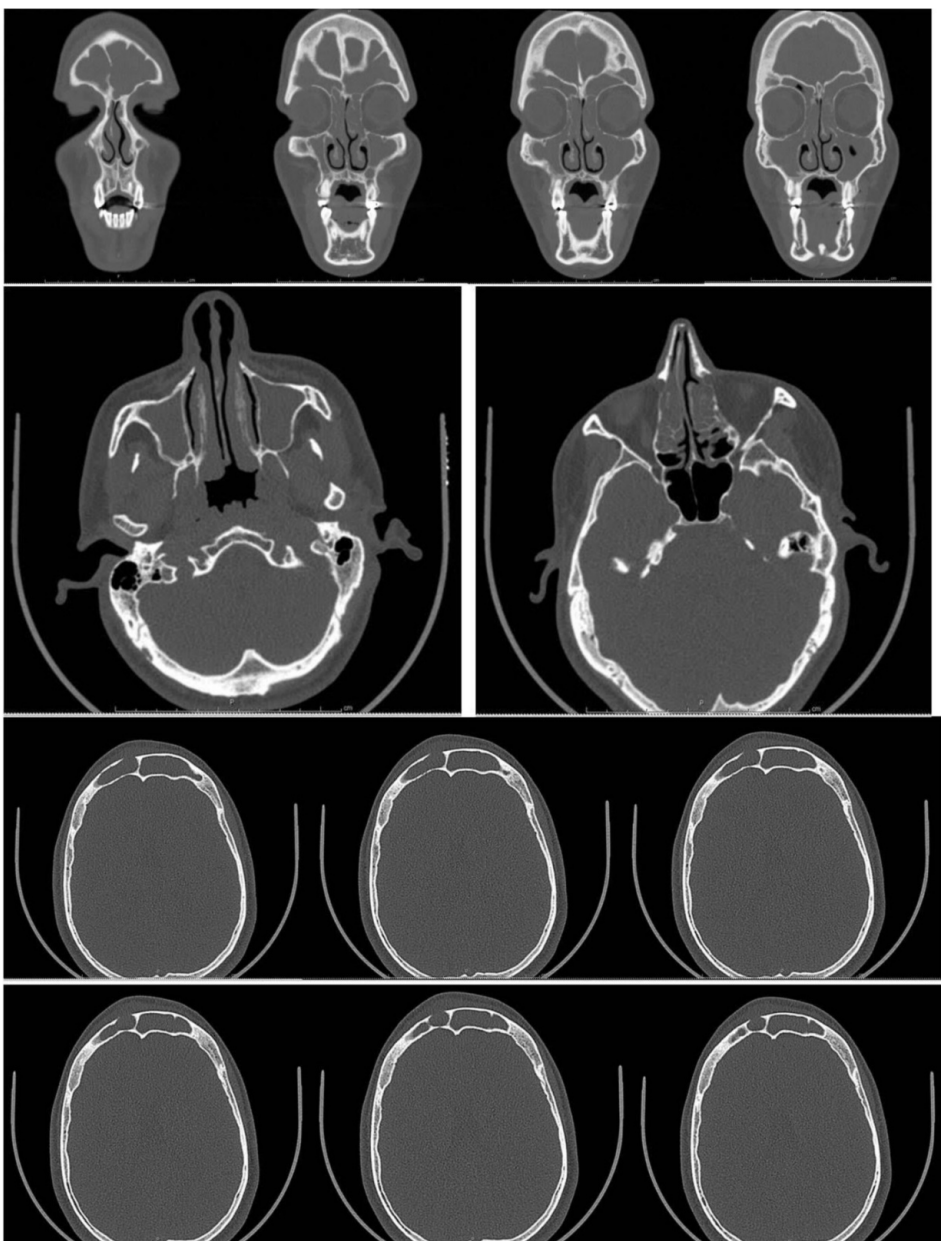

Fig. 2. Pansinusitis, bone defect in the anterior wall of the right frontal sinus, and collection are as under the skin wereseen

came for control. The patient had no complaints. Systemic examination was unremarkable. There is no need for functional endoscopic sinus surgery.The patient was treated without the need for an operation and did not suffer from neurological complications.

Discussion

Rare complications of sinusitis should also be considered in the differential diagnosis of headache, which is one of the common presentations to the emergency department.[1] Recognition of Pott's puffy tumor is very important to prevent future complications.[6] Osteomyelitis of the frontal bone and the resulting subperiosteal abscess leads to the characteristic features of Pott's puffy tumor.[7] The management involves surgical drainage of the abscess and commencement of the appropriate antibiotics.[8] The most common infectious agents seen in Pott's puffy tumor are *Staphylococcus*, non-enterococcal streptococci, and anaerobes that colonize the upper respiratory tract. When this condition is not treated promptly, it can lead to neurological complications such as meningitis, epidural, or subdural abscess and neurological symptoms like seizures.[9] Sinus surgery may be required later in untreated frontal sinusitis, as in the treatment of congenital hypoplastic sinus cases or sinusoid malignancies.[10,11] It is vital to recognize this false tumor in the emergency department to prevent unnecessary intracranial operations, long-term antibiotic use and to reduce the length of hospital stay.[12]

Conclusion

Patients frequently apply to the emergency department with the complaint of a swelling on the forehead and associated headache. Therefore, Pott's puffy tumor, a rare complication of frontal sinusitis, should be considered to prevent neurological and intracranial complications such as abscesses, meningitis, and neurological symptoms like seizures.

Declarations

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Author contributions

Conceptualization, B.C. and F.P.; Methodology, B.C. and F.P.; Validation, O.U., A.C. and F.P.; Formal Analysis, B.B.; Investigation, B.B. and B.C.; Resources, F.P.; Data Curation, A.C.; Writing – Original Draft Preparation, F.P. and O.U.; Writing – Review & Editing, F.P., B.C. and B.B.; Visualization, F.P.; Supervision, B.C.; Project Administration, F.P.

Conflicts of interest

There is no conflicts of interest.

Data availability

This case is obtained in Bagcilar Training and Research Hospital.

Ethics approval

The informed consent was obtained from patient.

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CASUISTIC PAPER

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Primary leiomyosarcoma of bones – a rare entity in two different presentations

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ABSTRACT

Introduction. Leiomyosarcomas (LMS) originate from smooth muscle cells. They are very rare malignant neoplasms. Bony Leiomyosarcoma is a variant of spindle cell sarcoma, primarily affecting long bones, predominantly the distal femur and the proximal tibia followed by craniofacial skeleton.

Aim. To describe clinical presentation and diagnostic approach of primary leiomyosarcoma of bones in two different patients.

Description of the cases.

Case 1. A 64-year-old male with a fracture of left distal femur after a fall was investigated and found to have a pathological fracture. An open biopsy of the fracture site confirms leiomyosarcoma.

Case 2. A 58-year-old previously healthy female presented with a swelling on right side mandibular region. Orthopantomogram radiograph (OPG) of mandible and Cone beam CT (CBCT) mandible was taken initially and revealed a large area of bone destruction of the right side of the mandible associated with a soft tissue mass. Initial incisional biopsy made the diagnosis of spindle cell sarcoma followed by excisional biopsy, which confirms the diagnosis of moderately differentiated leiomyosarcoma.

Conclusion. Primary leiomyosarcoma of bones is very rare. Imaging features are helpful in the evaluation of such conditions, but final diagnosis should be based on histopathologic and immunohistochemical features.

Keyword. bones, leiomyosarcoma, malignant neoplasms

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Introduction

Primary malignant bone tumours are rare. Majority (>90%) of them are osteosarcomas, Ewing sarcomas and chondrosarcomas. Clinical presentations, diagnostic approaches and treatment options of these tumours are well established. There are other rare primary malignant bone sarcomas (RPMBS) that are even rarer with lack of specific clinical and radiological characteristics. RPMBS comprise of wide variety of tumours showing complex histopathological features requiring ancillary studies such as immunohistochemistry and molecular genetic studies to arrive at a definitive diagnosis. Therefore, specific diagnosis of such a RPMBS is a challenging task for clinicians.[1,2] Although it is rare, awareness of this condition may help clinicians to consider in their differential diagnoses list, when a patient with a bone tumour presenting with non-specific clinical and radiological features.

Histopathologically, RPMBS show differentiation towards variety of cell types giving rise to architectural patterns which can be used to narrow down differential diagnoses. Those entities include sarcomas comprising spindle cells, round cells and vascular spaces lined by endothelial cells. Some of those are biphasic in nature.[1]

Primary intraosseous leiomyosarcoma is a malignant neoplasm showing smooth muscle differentiation without the production of osteoid. They primarily affect long bones, predominantly the distal femur and the proximal tibia. The second most common site affected is the craniofacial skeleton.[3] Spinal involvement is another reported site.[4]

In the initial periods these tumours grow slowly without characteristic clinical features. Most frequent clinical symptom is pain. Sometimes, pathological fractures can be seen.[4] However oral/maxillofacial involvement of lesions may manifest in the form of a significantly malignant tumour with rapid growth, needing radical surgical management and adjuvant radiotherapy.[5]

Aim

Here we present two cases of primary leiomyosarcoma of the bone, affecting the femur in a male patient and the mandible in a female patient.

Description of the cases

Case 1.

A previously healthy,64-year-old male presented to an orthopaedic unit at the National Hospital of Sri Lanka, with a fracture of left distal femur after a fall on the floor (Fig. 1).

He had intermittent pain in left thigh, which exacerbated at night in preceding 2 months of the fracture. Previously he never had trauma or radiation therapy to that site. There were no symptoms pointing towards a systemic pathology such as recent loss of appetite or loss of weight. Family history was insignificant.

Fig. 1. Initial X ray of left distal femur

On examination the fracture site was swollen and tender. Distal neurovascular status was normal. Abdominal and per rectal examination as well as other systemic examinations did not reveal any significant findings.

Diagnosis of a pathological fracture was made considering the age, site and low energy that caused the fracture and investigation were carried out. His blood investigations results are summarized in Table 1.

Table 1. Summary of biochemical results of case 1

Investigation	Results
White blood cells	7.3×10^9/l Neutrophils – 63%, Lymphocytes – 15%, Eosinophils – 14%
Erythrocyte sedimentation rate (ESR)	70 mm/1hr
C-reactive protein (CRP)	73 mg/l
Liver function tests	AST – 34 U/l, ALT – 21 U/l, Total bilirubin – 1.1mg/dl
Serum Creatinine	1.0 mg/dl
Serum Electrolytes	Na^+ – 140 mmol/l, K^+ – 4.1mmol/l, Cl^- – 100 mmol/l
Serum calcium	8.8 mg/dL
Thyroid stimulating hormone (TSH)	1.43 mU/l
Prostate specific antigen (PSA)	0.89 ng/ml

Patient underwent MRI scan of the affected femur, which revealed heterogeneously low signal intensity lesion in T1W and T2W images with heterogeneous post contrast enhancement (Fig. 2-5).

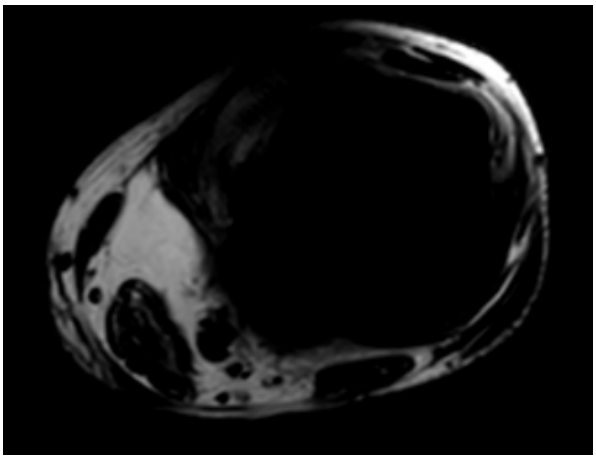

Fig. 2. MRI-T1W axial image

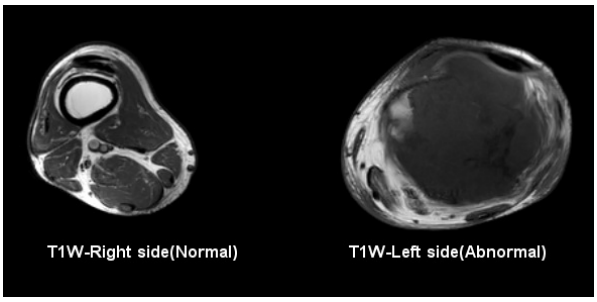

Fig. 3. MRI- T1W axial image

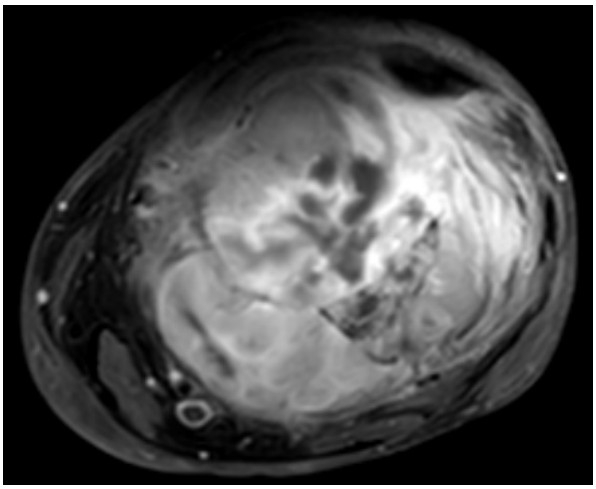

Fig. 4. MRI-Post contrast T1W axal

An open biopsy was taken from the fracture site and skeletal traction was applied using a Steinmann pin through the proximal tibia as the initial management.

Histopathological examination of the biopsy sample revealed malignant spindle cell tumour composed of long intersecting fascicles. The tumour cells showed highly pleomorphic, vesicular nuclei with blunt ends and small nucleoli. The cytoplasm was eosinophilic and abundant. Scattered multinucleate giant cells were also noted. The mitotic activity was brisk (12 per 10 high power fields) with abnormal forms. Areas of myxoid stroma and foci of necrosis were evident. There were no malignant osteoid or chondroid areas within the examined specimen. Numerous thin-walled capillaries, nuclear hyperchromasia and diffuse infiltration of skeletal muscle fibers are not seen.

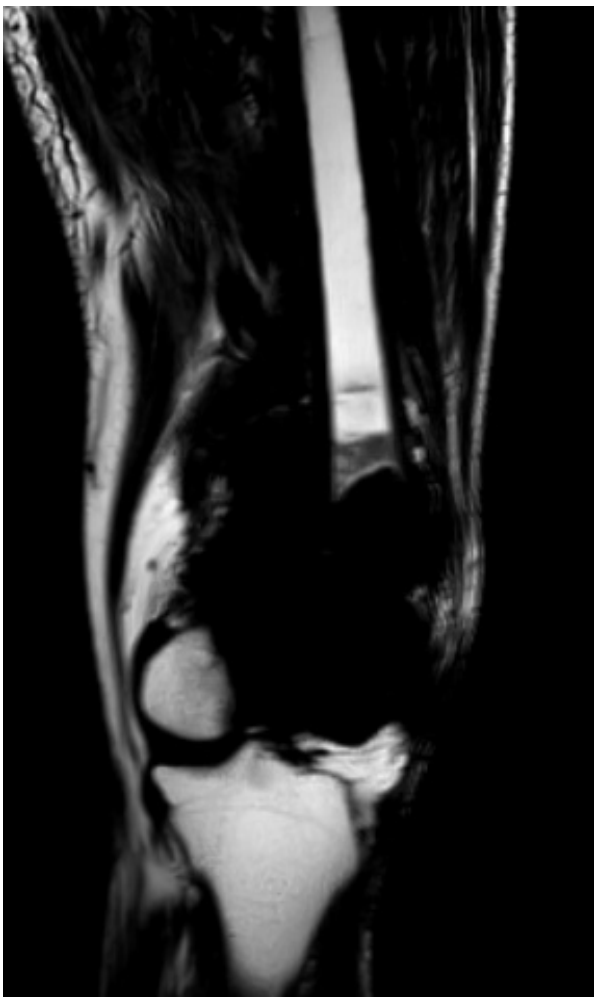

Fig. 5. MRI-T2W m DIXON coronal image

Immunohistochemical (IHC) assay showed diffuse and strong cytoplasmic positivity of tumour cells to smooth muscle actin (SMA). IHC for desmin, S-100, CD31, MyoD1, AE1/AE3 and CD117 were negative. Although two smooth muscle cell markers are required for a definitive diagnosis of a leiomyosarcoma, facilities to perform IHC for additional, novel smooth muscle markers such as h-caldesmon are not available in our institution. Therefore, based on the histomorphology which is highly characteristic of a leiomyosarcoma and on the strong and diffuse cytoplasmic positivity for SMA, a diagnosis of a leiomyosarcoma was made. It has been reported in the literature that loss of one or more markers of myogenic differentiation can be observed in leiomyosarcomas when they acquire poorly differentiated areas. It also mentioned that such loss may have an impact on the prognosis as well.[6]

When considering the differential diagnoses, features suggestive of a low grade myofibroblastic sarcoma such as numerous thin-walled capillaries, nuclear hyperchromasia and diffuse infiltration of skeletal muscle fibres were not seen in the biopsy specimen. The spindle cells in a fibroblastic osteosarcoma are arranged in a storiform pattern rather than a fascicular pattern and usually contain (at least focally) malignant osteoid. In addition, the age and the immunohistochemistry profile do not keep in with the diagnosis of an osteosarcoma. Therefore, the histomorphology and immunohistochemistry findings were used in the diagnosis of leiomyosarcoma.

After a multidisciplinary team discussion, the patient was transferred for specific oncology unit for chemotherapy. Traction with the Steinmann pin was continued.

Case 2.

A 58-year-old female presented to oral and maxillofacial unit at the National Dental Hospital (Teaching), complaining of a gradually increasing lump on the right mandibular region of the face for last two months. However, the patient did not have significant pain in the region except for mild difficulty in opening of the mouth. She denied numbness in the region or in the lower lip as well as otalgia.

On examination, a significantly large swelling (8cm x 6cm) was noted in the right mandibular region which involves angle and the ramus of the mandible. It was a well circumscribed hard swelling with clear margins, with no surface changes, skin tethering or discharging fluids and was deeply fixed but was not tender. There were enlarged level II lymph nodes on the right side.

Table 2. Summary of biochemical results of case 2

Investigation	Results
White blood cells	6.2×10^9/l Neutrophils – 70%, Lymphocytes – 25%, Eosinophils – 3%
Erythrocyte sedimentation rate (ESR)	52 mm/1hr
C-reactive protein (CRP)	45 mg/l
Liver function tests	AST – 28 U/l, ALT – 25 U/l, Total bilirubin – 1.0mg/dl
Serum creatinine	0.97 mg/dl
Serum electrolytes	Na^+ – 138 mmol/l, K^+ – 4.0mmol/l, Cl^- – 101mmol/l

There were no restrictions to shoulder movements. Maximum mouth opening measured between central incisors was 30mm.

Intra oral examination revealed poor oral hygiene with multiple carious teeth. The right-side lower quadrant was partially edentulous, which she had undergone extractions long time ago.

There was no significant lingual expansion of right-side mandible and there was no taste disturbance or altered sensation in right side of the tongue.

The lesion was not extending posteriorly beyond the anterior pillar fauces or towards the maxillary tuberosity along the pterygo-mandibular raphe area.

Her blood investigations results are summarized in table 2.

A orthopantomogram radiograph (OPG) and cone beam CT (CBCT) of the mandible was performed initially. The OPG image revealed a large area of bone destruction of the right side of the mandible involving the coronoid and condylar processes and proximal most part of the angle of the mandible (Fig. 6). The CT scan of the mandible demonstrated a soft tissue density enhancing lesion in relation to the bone destruction (Fig. 7, 8).

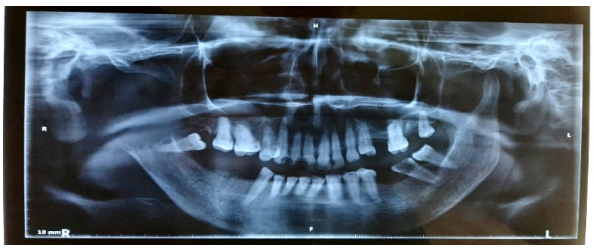

Fig. 6. OPG image

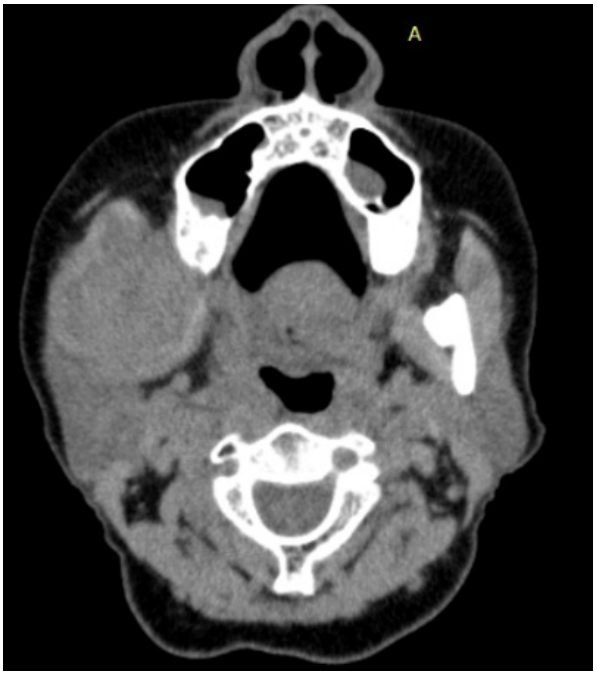

Fig. 7. CT axial image-Soft tissue window

Clinically, differential diagnoses were bone sarcoma or an odontogenic malignant tumor. With the provi-

sional clinical diagnosis of a malignancy, an incisional biopsy was performed under general anesthesia. The histopathological diagnosis of spindle cell sarcoma was made and was advised to excise.

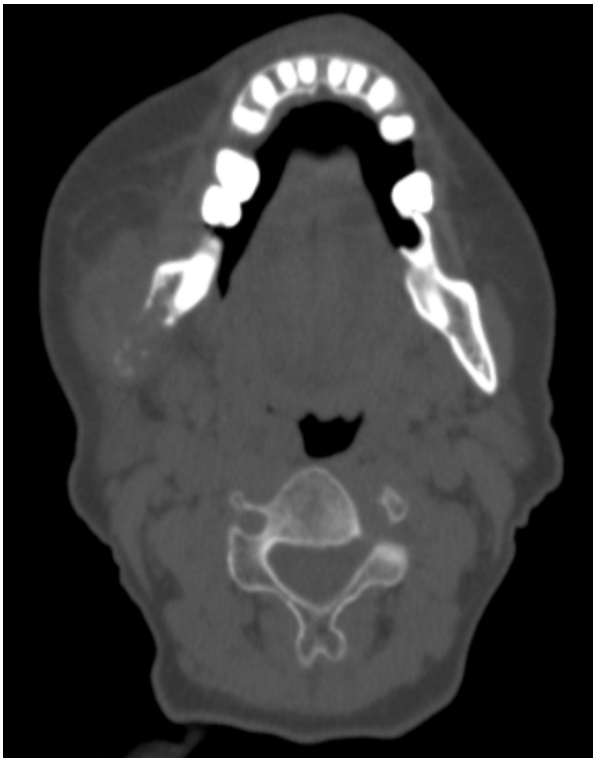

Fig. 8. CT axial image-Bone window

The ultrasound scan of the neck showed few prominent lymph nodes in the right-side level II with preserved fatty hilum and the largest lymph node was 6mm x 17mm in size. Subsequently, patient underwent chest radiography, ultrasound scan of abdomen and CT scan of the chest and abdomen but none of them showed any abnormalities.

After a multidisciplinary meeting with the oncologist and the oral pathologist, it was decided to treat the patient with curative intent, with surgical ablation followed by adjuvant chemo-radiation. As the lesion was centered in the angle and ramus of mandible a right side hemi mandibulectomy was planned in view of resecting with adequate margins.

Histological evaluation of the post-surgical sample showed unencapsulated tumour comprising spindle cells arranged in long interlacing fascicles in most areas. Cells show eosinophilic cytoplasm with indistinct cell borders. In most areas, cells show elongated, centrally placed and blunt ended nuclei whilst round to oval nuclei are seen in others. Moderate nuclear atypia was present in most cells with cells showing marked nuclear atypia in cellular areas. Focal areas showed epithelioid morphology. There was brisk mitotic activity amounting to 14 in 10 high power fields. There was evidence of tumour necrosis in cellular areas (less than 10%.). Scattered foci showed background myxoid stroma. Tumour cells invaded into adjacent skeletal muscles and adipose tissue (Fig. 9).

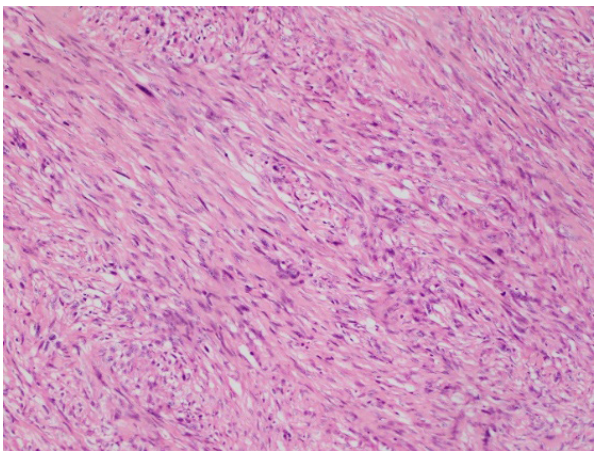

Fig. 9. Tumour cells (H&E, 20x)

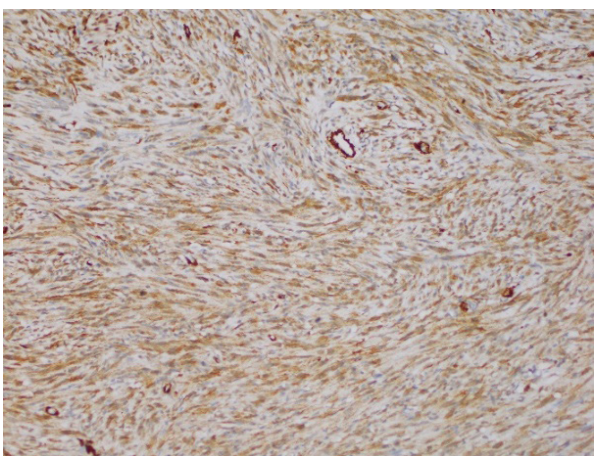

Fig. 10. Smooth muscle actin (IHC, 20x)

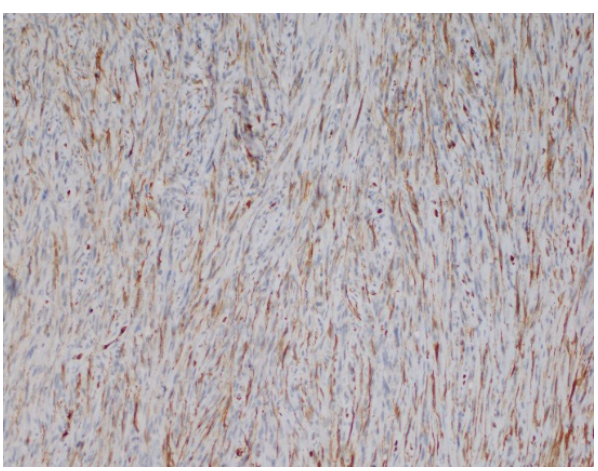

Fig. 11. Desmin (IHC, 20x)

Immunohistochemical studies (IHC) with smooth muscle actin showed strong cytoplasmic positivity in tumour cells (Fig. 10). Focal areas showed cytoplasmic

positivity for desmin (Fig. 11). Scattered nonspecific staining was present with S-100. These histopathological features together with IHC findings were consistent with a moderately differentiated leiomyosarcoma (G2). Histological evaluation of submandibular lymph nodes showed reactive lymphoid hyperplasia without evidence of metastatic disease.

Patient was subsequently transferred to the National Cancer Institute for specific treatments including chemotherapy.

Discussion

According to the recent review by Emanuela Palmerini et al., there is lack of knowledge on the RPMBS due to rarity of these tumours. Furthermore, there are very limited studies currently available in the literature on RPMBS.[1] Therefore, we considered sharing these two cases to increase the awareness among clinicians who are involving in the process of clinical, radiological and histological diagnosis of primary leiomyosarcoma of bones.

When considering the RPMBS, the differential diagnoses that should be kept in mind, include metastatic leiomyosarcoma from another site, primary undifferentiated pleomorphic sarcoma of bone, fibroblastic osteosarcoma, and metastatic sarcomatoid carcinoma.[7] Bone lymphoma may present with similar imaging findings to leiomyosarcomas. Therefore, bone lymphoma is one of the differential diagnoses considering the imaging findings alone. Further, lytic lesions with nonspecific imaging features of primary or secondary bone lesions may be considered as differential diagnoses on individual basis.[4] Metastatic leiomyosarcoma, especially from genitourinary tract or bowel need to be ruled out during the investigation process. In these two cases, possible primary sites of leiomyosarcoma were excluded with imaging modalities including CT scan of the chest and abdomen.

In the first patient, the bone lesion was seen in the left distal femur, who presented with a pathological fracture in the absence of a significant trauma to the affected limb. The plain X ray of the affected bone showed a lytic lesion with minimal expansion involving the distal meta-diaphyseal region of the left femur with extension in to the epiphysis, associated with a soft tissue component and an aggressive type of periosteal reaction. In a retrospective study done in Brazil, the most commonly affected bone site was the distal femur (31.7% cases), followed by the proximal femur (27.3% cases), proximal humerus (13.7% cases) and distal ulna (13.7% cases), proximal tibia (9.1% cases) and pelvis (4.5% of cases).[8]

In one case series, the metaphysis was involved in all cases with extension into epiphysis, diaphysis or both. In the same study, all long bone lesions showed osteolysis associated with variable degree of aggressive features including endosteal scalloping, permeation, ill-defined margins and lack of sclerosis. None of the lesions measured less than 7 cm in length, with average length being 11cm.[9] Longitudinal/elongated type of growth of long bone leiomyosarcoma also observed in other studies.[10] According to E. Santini-Araujo et al. these lesions showed high preference to grow in the longitudinal length relative to the medio-lateral expansion.[4] Most of these X ray features were comparable with our patient's X ray findings and the length of the lesion was approximately 10cm.

On MR images, most typical osteolytic lesions of long bones showed predominantly iso- or hyperintensity on T2-weighted spin echo sequences rather than heterogeneity associated with areas of iso- and hypointensity, in relation to fat. However, one study concluded that the MR imaging features of bone leiomyosarcoma are of T2 shortening in relation to the fat on conventional and fast spin echo sequences, manifesting as hypointensity. In that study, bone lesions show T2-weighted heterogenicity associated with areas of iso- and hypointensity in relation to fat.[9] Jiufa Cui et al. also mentioned that the lesions are not very hyperintense on T2-weighted images.[10] Similar signal heterogenicity was observed on T2-weighted sequences in our patient with distal femoral lesion. One case of bone leiomyosarcoma revealed fluid-fluid levels on MR images according to López Soriano et al. Further, it was the first case report mentioned about fluid-fluid levels on MRI in bone leiomyosarcoma. Therefore, the differential diagnoses for the presence of fluid-fluid levels in a bone tumour should include leiomyosarcoma.[11] However, this rare MRI appearance was not seen in our patient.

A study done in China revealed an epiphyseal involvement with subchondral bony extension in all cases of long bone leiomyosarcomas in their study. Other tumour characteristics include aggressiveness and extensive soft tissue oedema in relation to the primary lesion. This type of imaging features can be aided in the diagnosis of leiomyosarcoma of bones.[10] In our patient, the lesion was an expansile lobulated lesion with an epiphyseal extension. There was a cortical disruption with an extra osseous component as well. In addition, there was subcortical extension with knee joint involvement along the medial femoral condyle without knee joint effusion.

Due to relative paucity of smooth muscles in the maxillofacial skeleton, primary leiomyosarcomas in this area are exceedingly rare. There are very few reported cases of primary leiomyosarcoma arising in the mandible.[12]

LMSs of the oral cavity are usually painless lesions, presenting as swelling of the affected area leading to diagnostic difficulties as they mimic other common oral pathologies.[12] Absence of characteristic symptoms leading to challenges in the diagnosis and therapeutic management in maxillofacial leiomyosarcomas.[5] In one case series published by H. Amarapala et al, mod-

erately larger lesions mimicked an ameloblastoma or myxoma.[13]

In early stages, the mandibular leiomyosarcomas can be presented commonly with symptoms such as loss or mobility of teeth and gingival swelling or increase in volume.[14] Our patient with the mandibular lesion, had cheek swelling which gradually increased over the time. The orthopantomogram findings include a well-defined expansile lytic lesion with cortical destruction involving the mandible associated with complete destruction of the condyle on the right side. However, usual plain X ray features are of poorly defined lytic lesion with cortical destruction. The final diagnosis should be based on histopathologic and immunohistochemical features.[14]

As Dunfee et al. mentioned, specific diagnosis is not necessarily provided by radiological imaging, though, imaging should help to narrow-down the differential diagnoses of mandibular LMSs.[15]

Chemotherapy is one of the major treatment options for bone leiomyosarcomas. Palliative therapy is mainly reserved for patients with metastatic disease.[16-18] Our two patients were transferred for specialized cancer management center and started on chemotherapy. Recurrence rate and metastasis rate are directly correlate with the histological grade of LMSs while prognosis may be better than for other primary bone sarcomas of the same grade in non-metastatic patients.[4]

Both two patients were transferred to a cancer specific treatment institution after diagnosis of the disease condition. Therefore, we have limitations of the follow up of these two patients, with regard to their specific management aspects.

Conclusion

Primary leiomyosarcoma of bones is very rare. Imaging features are helpful in the evaluation of such conditions. However, final diagnosis should be based on histopathologic and immunohistochemical features.

Declarations

Funding

This research received no external funding.

Author contributions

Conceptualization, W.D.D.; Methodology, W.D.D.; Formal Analysis, W.D.D., D.R.S. and A.A.G.H.A.; Investigation, W.D.D., D.R.S., A.A.G.H.A., R.L.P.R.L. and I.W.G.A.L.M.; Resources, R.S., P.A.G.N. and R.M.A.S.R.; Data Curation, W.D.D., D.R.S. and A.A.G.H.A.; Writing – Original Draft Preparation, W.D.D.; Writing – Review & Editing, W.D.D., D.R.S., A.A.G.H.A., P.L.P.R.L. and I.W.G.A.L.M.; Visualization, W.D.D.; Supervision, R.S., P.A.G.N. and R.M.A.S.R.; Project Administration, W.D.D.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Data is available with the corresponding author on reasonable request.

Ethical consideration

Informed consent was taken from the patients and data was used anonymously.

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CASUISTIC PAPER

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A 16-year-old patient with Charcot Marie Tooth disease in variant c.217G>C of the *INF2* gene and focal glomerulosclerosis – a case report

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ABSTRACT

Introduction. Charcot Marie Tooth disease (CMT) is currently one of the most commonly diagnosed and commonly hereditary sensorimotor neuropathies. Concluding from the literature, this is the first study describing the case of a patient with CMT disease in the c.217G> C variant of the INF2 gene and focal segmental glomerulosclerosis.

Aim. To present a case of a 16-year-old patient suffering from CMT disease in variant c.217G> C of the INF2 gene and focal glomerulosclerosis.

Description of the case. The text describes the CMT disease in a patient who underwent the WES / WGS-NGS genetic test and found a mutation within the INF2 gene at the chromosomal position hg38 14: 104701582-G> C, cDNA level c.217 G> C , notation at the p protein level (Gly73Arg). Genotype record according to Human Genome Variation Society: NM_022489.4: c. [217G> C]; [217 =]. The publication includes data on genetics, molecular mechanisms of the disease, diagnostic methods, rehabilitation and surgical treatment.

Conclusion. CMT disease is a heterogeneous group of diseases caused by mutations in various genes. The incidence of this pathology has increased significantly in the last century. Currently, there are no treatments available to combat this disease, and symptomatic treatment is the only treatment available.

Keywords. exome sequencing, neuropathy, nephropathy

Introduction

Charcot Marie Tooth disease (CMT) is currently one of the most commonly diagnosed and commonly hereditary sensorimotor neuropathies.[1] It is a heterogeneous group of genetic disorders characterized by dysfunction of peripheral nerves. It leads to limitations of motor activity, reduction of muscle tone, loss of tendon reflexes, progressive symmetrical atrophy of the distal muscles of

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the lower limbs and feet. Sequentially it affects the upper limbs, ultimately causing atrophy of the optic nerve.[2-5] The observations so far indicate a positive correlation between the length of the nerve fiber and the sequence of its involvement, leading to the involvement of the longest nerve fibers in the first place.[1] The development of the disease is associated with many other complications leading to limit independent activity and reduce fitness, both in terms of movement and sociology. The clinical features of CMT are pain, deformity, and disability, however, these are dependent on the type of CMT.[6] There is no approved pharmacological treatment at the current stage of research.[7]

CMT classification is based on the average nerve conduction velocity and is divided into three types: CMT type 1 also known as demyelinating, intermediate CMT and CMT type 2, which is dominated by axonal injuries.[5-8] CMT type 1 is characterized by significantly reduced speeds of the motor nerve (below 38 m/s) as well as segmental demyelination and remyelination with bulbous formations visible in the histological image. histological image.[9] In contrast, in the intermediate CMT, the velocity is 25-45 m/s.[8] Currently, there are three types of inheritance: autosomal dominant, autosomal recessive and X-linked.[10] with autosomal DI-CMT, while GJB1 is associated with X-linked DI-CMT.[11,12]

Formins are a family of proteins whose main task is to create linear actin polymers. Formin INF2 and its mutations have been found to be a major factor in the development of focal segmental enamelware (FSGS), which leads to glomerular degeneration, resulting in end-stage chronic kidney disease.[13] The INF2 gene encodes formin, which reacts, among others, Rho-GTPase CDC42 and the MAL protein responsible for the structure of lymphocytes as well as myelination and its proper maintenance.[14] Various actin binding processes are modulated by proteins, including formin. Forming use, among others FH2 (formin homology 2) being the domain of creating new filaments that remain on the hooked end until the elongation process is complete.[15,16] FH1 (formin homology 1) accelerates elongation through its interaction with profilin.[17]

Concluding from the literature, this is the first study describing the case of a patient with Charcot Marie Tooth disease in the c.217G> C variant of the INF2 gene and focal segmental glomerulosclerosis.

Aim

To present a case of a 16-year-old patient suffering from CMT disease in variant c.217G> C of the INF2 gene and focal glomerulosclerosis.

Description of the case

This paper describes the case of a 16-year-old female patient, white. The second pregnancy was normal, terminated by caesarean section, with a birth weight of 3250 g, the second birth, the newborn scored 9 points on the Apgar scale. Development in the neonatal period was normal. At the age of 5 months, an ultrasound examination was performed, which gave the following results: heart positioned correctly, venous inflow and the size of the heart cavities normal, atrioventricular closed joints, continuous interatrial septum, normal thickness and systolic function of the left ventricular muscle, arterial openings normal and their diameter and flow velocities remained normal, normal left-sided aortic arch, normal flow in the abdominal aorta, echo from the endocardium and pericardium was normal. Based on the

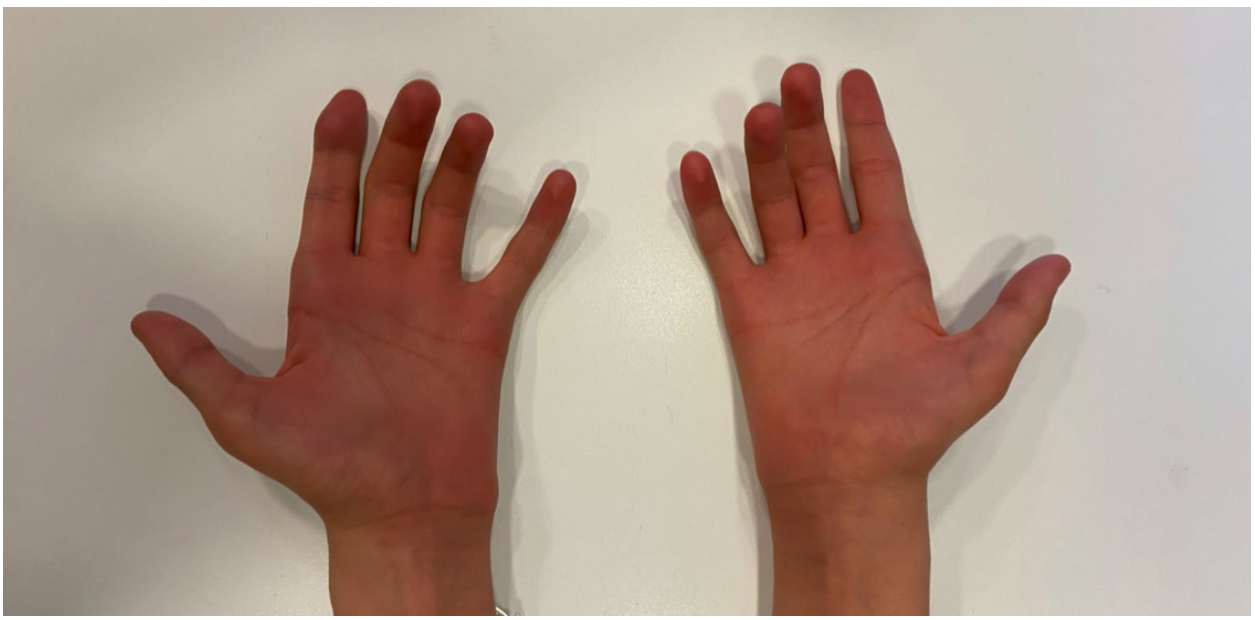

Fig. 1. Deformed, shrunken fingers

above-mentioned description, no anatomical and functional abnormalities were found. Additionally, the examination did not reveal any abnormalities in the structure of the kidneys. The child walked a year ago, developing according to the norms. From about the second/third year of life she showed a tendency to walk on tiptoes, this symptom developed through foot drop, weakening of the distal muscles, especially of the lower limbs, until the onset of contractures. As the disease progressed, the patient developed a wading gait.

At the age of seven, during hospitalization, an EMG examination was performed, which showed the features of mixed, axonal-demyelinating neuropathy, then he was diagnosed with congenital motor-sensory neuropathy, the speed of conduction in the motor fibers of the median nerve was 29.3 m/s, in the ulnar nerve, conduction at the speed of 30.7 m/s, the peroneal nerve responded only to the tibial nerve, no sensory responses from the median, ulnar and sural nerves, and a record from the tibial muscle with features of chronic reinnervation. Additional tests revealed an increased TSH value (12.6 IU/ml) and an endocrinological consultation was recommended. Rehabilitation began.

At the age of 14, ultrasound of the thyroid gland and abdominal cavity was performed, which gave the following results: thyroid located in a typical site, nodule and both lobes of homogeneous echogenicity, in the left lobe and nodule without focal changes, a focal change visible in the right lobe. A 3.5 mm thick wick left and right lobes with the correct dimensions. In the middle part of the right lobe, near the posterior contour, there is an oval focal lesion with reduced echogenicity compared to the thyroid gland, with a diameter of 7 mm vascularized from the periphery. Cervical lymph nodes without signs of enlargement. Liver not enlarged, without echogenicity disturbances, thin-walled gallbladder without deposits present, common bile duct and portal vein not dilated. The area of the pancreas of homogeneous echogenicity, not dilated, without focal changes. The abdominal aorta is normal, the peri-aortic and retroperitoneal spaces are free. Both kidneys have the correct position, size, improper differentiation of the cortical spinal cord, no clear boundaries between the cortical and medullary parts with the hyperechoic cortical part, no focal changes.

The right kidney measures 107 mm/42 mm, the left one measures 98 mm/50 mm. Adrenal glands free. Spleen of homogeneous echogenicity and not enlarged, smooth-walled bladder.

In October 2019, during hospitalization due to incorrect values of outpatient tests of kidney function parameters, slight swelling of the face was found on physical examination. In a history of several months, periodic swelling of the eye area, the tests performed confirmed the high values of urea, creatinine and potassium. Moreover, anemia and biochemical indicators of disturbances in the functioning of calcium and phosphate metabolism were found. Hemodialysis was performed. The treatment was prescribed furosemide, calcium preparations and active vitamin D3. Due to the high blood pressure, amlodipine was administered. During hospitalization, the patient required regular hemodialysis treatments. Despite the implementation of erythropoietin and iron, there was a need for transfusion of the erythrocyte mass twice. Urine red blood cells and urine proteins (3.6 g/day) were present in the urine all the time. With a slightly reduced level of protein and albumin in the blood. During the same hospitalization, a biopsy was also performed, the complication of which was an ultrasound-controlled retroperitoneal hematoma. Before the biopsy results were obtained,

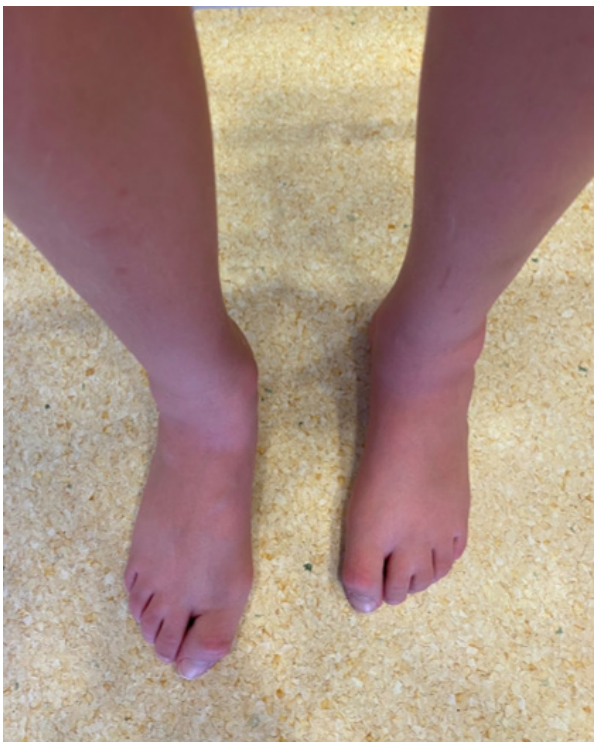

Fig. 2. Deformed toes

immunosuppressive treatment was attempted, unfortunately without any therapeutic effect. The biopsy results confirmed the irreversible nature of the kidney damage: the biopsy covers the cortical and medullary parts of the kidney, contains up to seven glomeruli, almost all of them completely or almost completely sclerotized. Remains of postcapillary hyperplasia were found within the glomeruli. The dilated, fibrotic stroma contains chronic inflammatory infiltrates, numerous atrophic tubules, and numerous vitreous cylinders. Negative amyloid reaction. Immunofluorescent moderately abundant peripheral IgG deposits, C3c irregular deposits, copious irregular IgM deposits, trace fibrinogen deposits. Ultrastructurally enhanced glaz-

ing features. The changes present in the biopsy correspond to chronic advanced nephropathy, possibly crescent-forming glomerulopathy. A cardiological consultation was also performed, during which secondary left ventricular hypertrophy was found. During the neurological consultation, it was found that it was impossible to induce deep reflexes from the lower limbs and a weakly expressed deep reflex from the upper limbs, as well as hollow feet, deformed toes, drooping feet, and stork gait. After discharge, hemodialysis continued and genetic testing was suggested. At a later stage, a permanent catheter was also inserted.

In September 2020, the patient was admitted to a genetic clinic. WES/WGS-NGS genetic test was performed. The probation's parents and sister were also examined. The test sample showed a mutation within the INF2 gene at the chromosomal position hg38 14: 104701582-G> C, cDNA level c.217 G> C, and p. Protein (Gly73Arg). Genotype record according to Human Genome Variation Society: NM_022489.4: c. [217G> C]; [217 =]. The above-mentioned variant in the INF2 gene was not found in the father and sister. Two different nucleotides in the c.217G> C position of the INF2 gene were found in the patient's mother, which suggests a possible low-percentage mosaic pattern for the variant studied. Pathogenic INF gene variants are responsible for Charcot Marie Tooth diseases and/or focal segmental glomerulosclerosis in an autosomal dominant model of inheritance.

Discussion

Molecular understanding of disease is very difficult, but medicine is advancing, making the possibility of targeted therapy emerging, but it is an extraordinary challenge.[18,19]

Charcot-Marie-Tooth disease (CMT) indicates a genetically heterogeneous group of primary genetic neuropathies classically with sensory and motor involvement, referred to as hereditary sensory and motor neuropathy (HSMN).[18] CMT is the most common hereditary disease of the peripheral nerves in the world with a frequency of 1:2500.[18]

Pain in CMT patients is frequent, frequent, and has a strong impact on patients, however, it is difficult to classify as the literature data are inconclusive, suggesting either a biomechanical or neuropathic pathomechanism.[19,20] Early treatment in physical medicine and rehabilitation of patients with Charcot-Marie-Tooth disease is essential to reduce the sequelae of the disease and slow its progression.[21-23]

Knowledge of Charcot-Marie-Tooth disease (CMT) has grown significantly in recent years. It is an increasingly common disease as the spread of defective genes associated with it increases among the population (around 10-28/100,000). Due to the development of new studies on this disease, more and more genes are identified with it, which significantly hinders its classification.

One of the publications of Spanish researchers from the La Fe University Hospital in Valencia describes a case of surgical treatment of CMT symptoms. The study included 16 patients, mainly women (62.5%) with the CMT1A phenotype (62.5%) with an average age of 39.5 years. In 13 patients, Achilles tendons were surgically lengthened, interphalangeal arthrodesis and plantar fascia dissection were performed. Two of them required additional ankle arthrodesis (due to its persistent varus) and extension of the long toe extensor. Patients were followed for an average of 42 months. 75% of patients assessed the effect of the surgery as "excellent" or "good". This study showed that the above-mentioned surgical techniques show high therapeutic efficacy and a high level of patient satisfaction.[24]

Another study was to test the effectiveness and safety of suppression of the PMP22 gene, duplication of which in Schwann cells is one of the causes of Charcot-Marie-Tooth 1A disease (CMT1A). For this, a recombinant AAV serotype 9 (AAV2/9) vector was used, which introduces GFP and shRNAs (targeting Pmp-22mRNA) and causes their expression in the recipient organism. This vector was injected into the sciatic nerve of animal models of the disease (mice, rats, and non-human primates). This treatment resulted in the recovery of the expression level of the PMP22 gene to normal, which resulted in increased myelination of peripheral nerves. This effect prevents motor and sensory impairment in rat CMT1A models.[25]

Based on research from the Department of Neuro-Orthopedic Rehabilitation of the Rothschild Hospital, Assistance Publique-Hôpitaux de Paris, it has been found that early treatment and rehabilitation of patients with Charcot-Marie-Tooth disease is essential to reduce symptoms and slow the progression of the disease. Before starting therapy, the extent of neurological disorders, deficits in muscle tissue and joints should be clinically assessed, and then an individual rehabilitation program should be established for each patient. The condition of patients with this condition can be assessed through a number of different tests, including balance assessment on a stabilometric platform and gait assessment. In the rehabilitation of such people, e.g. cycloergometers (for lower limb exercises), treadmills (gait estimation, fatigue calibration and endurance training), isokinetic machines (training quadriceps muscles, hamstrings, extensors and spinal flexors), orthoses for gripping and training hand muscles. Such therapy enables patients to maintain physical and manual fitness and ensures independence in everyday life, which significantly improves their comfort and quality of life.[21]

CMT is associated with kidney disease in many sources, so a study was conducted to test the hypothesis

that mutations in the INF2 gene (indirectly involved in nerve myelination) may be responsible for Charcot-Marie-Tooth neuropathy in the course of focal segmental glomerulosclerosis renal function (FSGS). For this purpose, direct INF2 genotyping was performed in sixteen CMT and FSGS patients who had no mutations in the PMP22 and MPZ genes. Of these individuals, 12 had mutations in exons 2 and 3 coding for INF2. During immunohistochemical analysis, strong expression of this gene in Schwann cells was demonstrated. Mutant INF2 genes disrupted the INF2-MAL-CDC42 pathway involved in important steps in myelination. These results suggested that INF2 may be responsible for diseases of the glomeruli and the peripheral nervous system.[14]

Despite the holistic approach offered by the study of the clinical exome, the molecular tools available today guarantee the possibility of analyzing about 80% of target genes. Additional analyzes are necessary to verify the nature of the identified lesions, in this case the study of mosaicism in the patient's mother and the detection of genetic variants that could explain the cause of the observed disease.

Charcot-Marie Tooth disease is associated with a wide spectrum of different peripheral neuropathies. They affect the sensory and motor nerves, they cause muscle atrophy. At the moment, patients with this disease can be treated with rehabilitation and corrective surgery. Research is carried out on the mouse CMT-1A model to check the effectiveness of ascorbic acid. Research has shown that ascorbic acid decreased PMP22 expression to a level below what is needed to induce disease. As ascorbic acid has shown therapeutic efficacy for patients with Charcot-Marie Tooth disease, it has been approved by the FDA.[26] Different muscles are attacked at different stages of disease progression. When the activity of one muscle weakens, the antagonist defeats it and, as a consequence, deforms. The main purpose is to reduce the action of the forces that cause the deformation. Minimally invasive procedures include plantar fasiotomy, Achilles tendon lengthening, transfer of the long fibula to the fifth metatarsal bone.[27] To improve the functions of the hand areas, a tendon transfer procedure is used in clinical practice.[28] To prevent joint contracture, use orthoses and rehabilitation that will improve the functioning of the patient. In clinical treatment, bone operations are also performed, e.g. osteotomies and joint fusions.[29,30]

Conclusions

Charcot Marie Tooth disease is a heterogeneous group of conditions caused by mutations in different genes. The course of the disease is variable due to genotypic and phenotypic heterogeneity. The incidence of this pathology has increased significantly in the last century, which is related to the dynamic and rapid development of medicine in the field of clinical genetics. Currently, there is no pharmacotherapy for Charcot-Marie-Tooth disease, and the only treatments available are rehabilitation and surgery for skeletal deformities, although best practices have not been identified. However, patients in Poland can increasingly count on the help of specialists involved in the treatment of diseases. related to the human genome. This commitment gives hope for the improvement of patients' clinical condition and psychomotor and social functioning.

Declarations

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Author contributions

Conceptualization, M.P., D.M., M.J., A.S., K.R., J.W., A.P.; Methodology, M.P., D.M., M.J., A.S., K.R., J.W., M.R., J.K., R.P., D.A., A.P.; Validation, M.P., D.M., M.J., A.S., K.R., J.W., M.R., J.K., R.P., A.P., D.A.; Formal Analysis, M.P., D.M., M.J., A.S., K.R., J.W., M.R., J.K., R.P., D.A. A.P.; Investigation, M.P., D.M., M.J., A.S., K.R., J.W., M.R., J.K., R.P., D.A., A.P.; Resources M.P., D.M., M.J., A.S., K.R., M.R., J.K., R.P., D.A., A.P.; Data Curation, M.P., D.M., M.J., A.S., K.R., M.R., J.K., R.P., D.A., A.P.; Writing – Original Draft Preparation, D.M., M.J., M.P., A.S., K.R., J.W., D.A.; Writing – Review & Editing, D.M., M.J., M.P., A.S., K.R.; Visualization, M.P., D.M., M.J., A.S., K.R., J.W., M.R., J.K., R.P., D.A. A.P.; Supervision, A.P. and D.A.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

Further enquiries can be directed to the corresponding author.

Ethics approval

Informed consent was taken from the patients.

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CASUISTIC PAPER

Berrin Erok [1], Kemal Harmancı [1], Ferdi Aksaray [2], Nazmi Uğur Unlu [3], Seckin Aydın [3]

A long clinical course with late distant metastases from follicular thyroid carcinoma

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ABSTRACT

Introduction. Follicular thyroid carcinoma (FTC) accounts for 10-20% of the differentiated thyroid carcinomas (DTCs), and it is the second most common thyroid malignancy after papillary thyroid carcinoma (PTC). FTC is typically more common in women and in older age group than PTC. Unlike PTC, FTC metastases late to the lymph nodes, with only up to 10-20% of the patients having nodal metastases at the time of diagnosis.On the other hand, distant metastasis via hematogenous spread is more likely in patients with FTC due to the invasion of blood vessels. Prognosis depends on the extent of the distant metastasis which drop 10-year survival significantly.

Aim. Although DTCs have usually favorable prognosis, metastatic disease in these patients has a long clinical course. Cranial imaging in these patients should be performed during the follow-up after the treatment of FTC with thyroidectomy and RAI.

Description of the case. We report a case of late onset but catastrophic hematogenous distant metastases beginning 8 years after the diagnosis & treatment of FTC and becommimg widespread during the following 10 years with a long clinical course in a 60 year old female patient.

Conclusion. In the RAI refractory metastatic lesions SRS and surgical resections should be conserned as the first management approach to improve survival.

Keywords. follicular thyroid carcinoma, brain metastasis, skull metastasis

Introduction

Follicular thyroid carcinoma (FTC) accounts for 10-20% of the differentiated thyroid carcinomas (DTCs), with higher prevelance in iodine deficient areas.[1,2] It is the second most common thyroid malignancy after papillary thyroid carcinoma (PTC). FTC is typically more common in women and in older age group than PTC, presenting at about 40-60 years of age.[3] It is the neoplasm of differentiated follicular cells, just like the follicular adenoma (FA). It is differentiated from FA by the presence of capsular and/or vascular invasion and from the PTC by the absence of characteristic nuclear features.[4] Surgical resection is needed for accurate diagnosis. Unlike PTC, FTC metastases late to the lymph nodes, with only up to 10-20% of the patients having nodal metastases at the time of diagnosis. On the other hand, distant metastasis via hematogenous spread is more likely in patients with FTC due to the invasion of blood vessels.[5] Prognosis depends on the extent of the distant metastasis which drop 10-year survival significantly.

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Aim

We report a case of late onset but catastrophic hematogenous distant metastases beginning 8 years after the diagnosis & treatment of FTC and becommimg widespread during the following 10 years with a long clinical course in a 60 year old female patient.

Description of the case

In 2003, when the patient was 42-year old, she was diagnosed with FTC and treated with total thyroidectomy and radioiodine (RAI). In 2011 (8 years after the treatment of FTC), her serum thyroglobulin (Tg) level started to be increased and the first distant metastasis was established on the left femur. She was treated with total hip arthroplasty and started to be followed up more closely. In 2012, she came to our hospital and examinations revealed multiple lung (Fig. 1) and right clavicle metastases (Fig. 2).

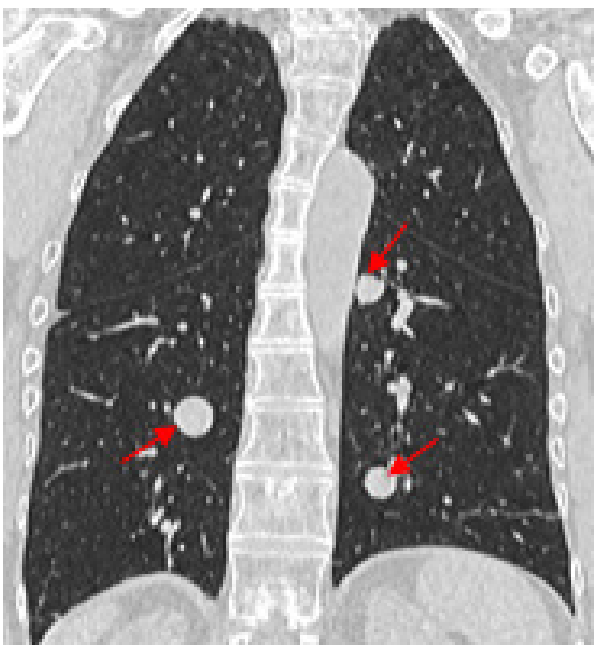

Fig. 1. Coronal pulmonary CT image showing multiple well defined lung metastasis from FTC (arrows)

She was admitted for treatment with RAI and radiotherapy. However, since she became refractory to RAI, Lu-177 treatment was applied. She was also under the treatment of a thyrosine kinase inhibitor (TKI) at that time. In 2014, she presented with a left frontoparietal skull metastasis. Brain magnetic resonance imaging (MRI) revealed a 55x35x25 mm sized metastatic lesion involving the epidural space and adjacent dura, characterized with heterogenous signal intensity on T2w images and hemorrhagic components on T1w and gradient echo (GRE) images. Prominent contrast enhancement was present after gadolinium (Fig. 3).

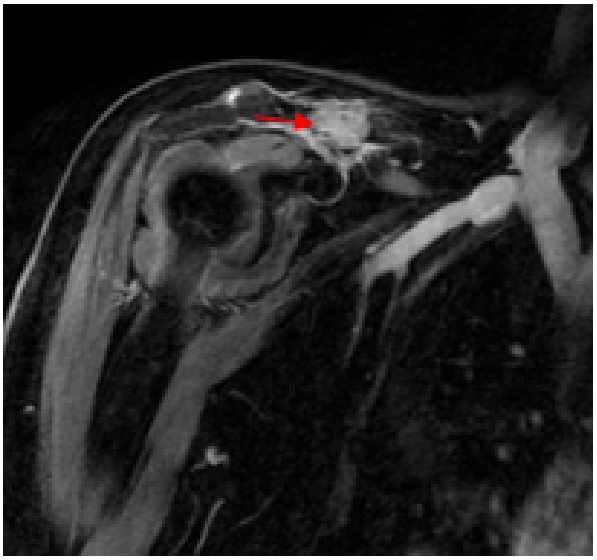

Fig. 2. Coronal postcontrast T1w shoulder MRI showing obviously enhancing matastatic lesion of the right clavicle (arrow)

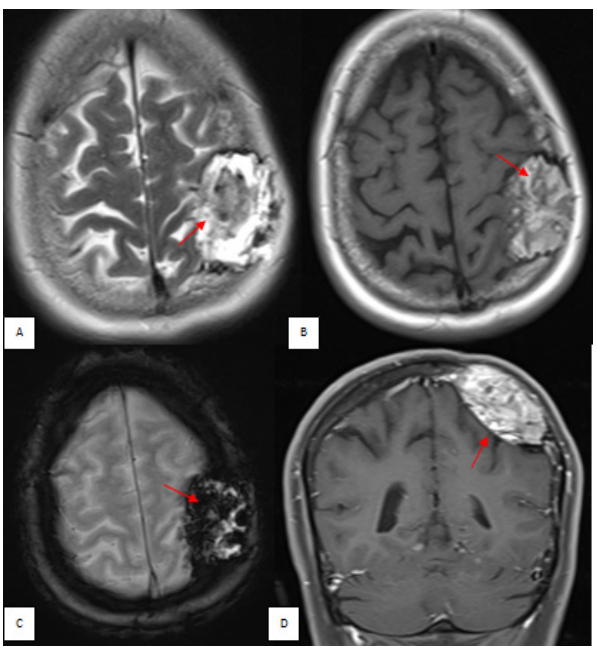

Fig. 3. MRI of the brain showing a 55x35x25 mm sized left frontoparietal skull metastasis having heterogenously high signal intensity on axial T2w (A, arrow), spontaneous hemorrhagic hyperintensities shown on T1w (B, arrow) and signal void areas corresponding to the hemorrhagic components on GRE images (C, arrow). Prominent enhancement of the mass is demonstrated on coronal pstcontrast T1w image (D, arrow). Note the epidural extention of the lesion with compression of the neural parenchyma without invasion

At that time, due to the development of myelodisplastic syndrome, surgical removal of the metastatic lesions had to be started. Between the years 2017–2021, right clavicle, left frontoparietal and left mandibular

bone metastases was surgically treated. After 1 year another skull metastasis appeared at the posterior parietal region invading the superior sagittal sinus and then another at the left parietal bone. Both were also surgically removed (Fig 4).

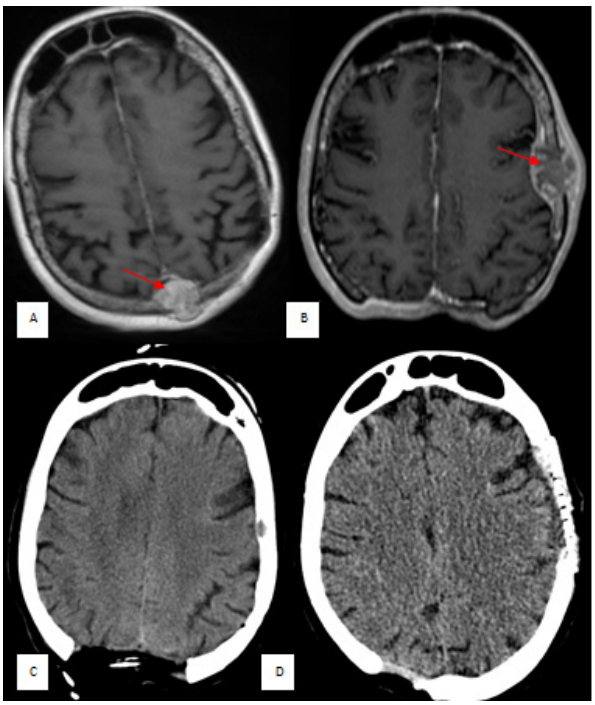

Fig. 4. (A, B) Axial postcontrast T1w MRI of the brain showing 2 cm sized midline posterior parietal bone metastasis invading the superior sagittal sinus (A, arrow) and 35x25 mm sized left parietal bone metastasis (B, arrow) involving to the adjacent skalp and dura with epidural extention. Surgical removal of both lesions is seen on axial CT images (C, D)

Then the disease progressed rapidly with newly appeared multiple skull metastases in addition to the first cerebral metastasis in the right occipital lobe (Fig. 5). Since the radioiodine scan was negative, GA-63 DOTA-TATE PET-CT was performed to demonstrate all the metastatic lesions (Fig. 6)

Discussion

In DTCs the most common sites of distant meastasis are lung and extracranial bones.[6] Skull bones are rare sites and when occured there are usually lung and other extracranial bone metastases. Metastatic tumors to the skull are most often from lung, breast, and prostate malignancies.[7] Involvement of the dura and scalp may occur in these cases, as in our patient. In a study including 473 patients with all types of thyroid cancers conducted by Negamine et al. skull metastasis was established only in 12 (2.5%) of the cases.[3] The average period from diagnosis of the thyroid cancer until the appearance of the skull metastasis was 23.3 years. In our patient the first distant metastases occured 8 years after the tretment of FTC. The appearance of the first skull metastasis occured 11 years and the brain matastasis occurred 18 years after the diagnosis. Brain metastasis from DTCs is much more rare with reported prevelance between 0.15% to 1.4% of the cases in different studies.[8]

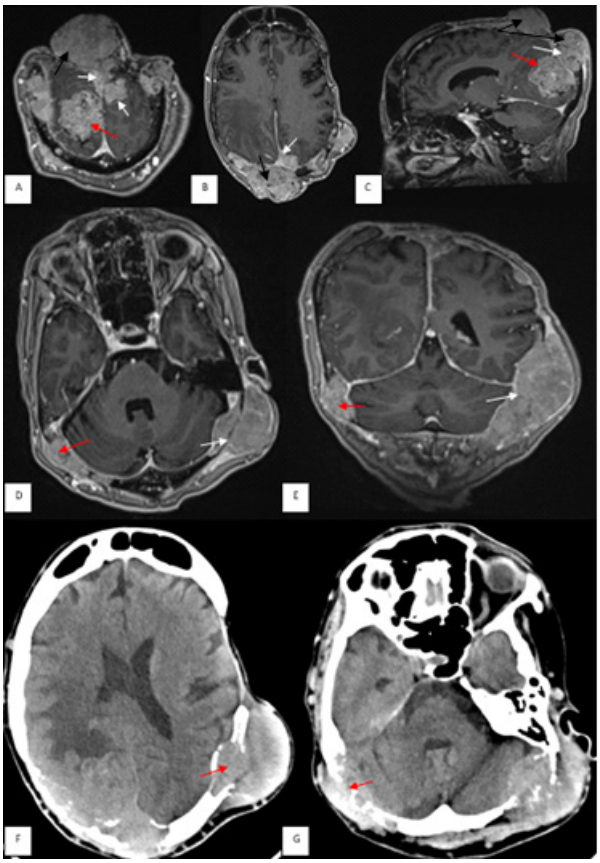

Fig. 5. (A-E) The last follow-up MR images demonstrating multiple obviously enhancing skull bone and cerebral metastases. Coronal (A), axial (B) and sagittal (C) postcontrast MR images showing the right occipital parenchymal metastasis which is 4 cm in diameter (A, C; red arrows). Multiple extraaxial dural based metastasis are shown (A, B, C; white arrows). 51x37 mm and 55x34 mm sized posterior parietal (A, B, C; black arrows), 45x20 mm sized right occipital (D, E; red arrows) and 95x50 mm sized left occipital (D, E; white arrows) matastases invading the transverse sinuses are shown. F, G) Axial CT images showing lytic and expansile behaviour of the skull metastases (F, G; arrows)

Metastatic tumors to the brain are most often from lung cancer, breast carcinoma, renal cell carcinoma, melanoma and gastrointestinal tract adenocarcinomas.[7] In the literature, regarding the histopathological subtypes, among the brain metastasis from DTCs, there is a predominance of PTC which is most probably due to its much more higher prevelance than FTC.[9] Although, in most of the reported cases, the appearance of distant

metastasis occur long after the initial diagnosis as in our case, distant metastasis at the time of diagnosis of FTC may also occur in up to 20% of the cases.[6,7] The metastatic masses of FTC are highly vascular lesions showing prominent contrast enhancement on imaging studies and having expansive-lytic appearance when involved to the bones. Prognosis in patients with distant metastasis is poor and its poorer in case of skull metastasis. In the study of Nagemina et al. mean survival in patients with skull metastasis was reported as only 4.5 years.[3] The prognosis is even worse with reported mean survival time of 12.4 months when brain metastasis occurred from DTC in a study including 32 patients.[10] When the radioiodine scans are negative, 18F-FDG PET/CT can be used for detection of recurrent and metastatic lesions in follow up of patients with increased Tg level. However, it may also not detect the tumor in all patients, as in our case. In such cases PET-CT imaging with Ga-68-labelled DOTA-somatostatin analogues is useful to demonstrate metastatic masses.[11] We used octreotate (Ga-68-DOTA-TATE) in our patient.

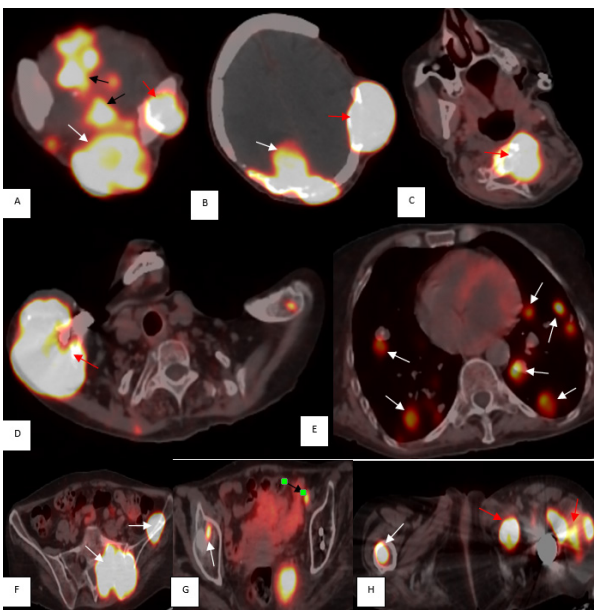

Fig. 6. Whole body DOTA-TATE PET-CT images showing right occipital cerebral metastasis (A, B; white arrow), multiple extraaxial dural based metastases invading the superior sagittal sinus (A, black arrows) and skull bone metastases (A, B; red arrows), metastatic masses involving the cervical vertebrae (C, arrow), the distal part of the right clavicle (D, arrow), multiple pulmonary nodular metastases (E, arrows), the right sacroiliac metastases (F, arrows), the right acetabular (G, white arrow) and the right femoral head (H, white arrow) metastases and the multiple metastatic masses involving the muscle groups of the left femoral region (H, red arrows). Note the small external iliac lymph node metastasis (G, black arrow)

In the treatment of metastatic disease, iodine concentrating tumors can still be treated with RAI, however when the recurrent or metastatic masses from DTC lose their ability to concentrate RAI they poorly respond with a low remission rate.[12] In the 2015 American Thyroid Association guidelines for the brain metastases from DTC surgical resection and stereotactic radiosurgery (SRS) are recommended as the mainstray of therapy.[13] In one study the median survival time in patients treated with surgery and/or SRS was reported as 11.9 months in contrast to the median survival time of 7.1 months in patients who were not treated with surgery and/or SRS.[8] The median survival time of 19 months was reported in patients with RAI-refractory DTC when TKIs were combined with local treatment.[14] Number of surgical resections of extracranial and skull bone metastases were performed in addition to SRA in our patient in addition to the treatment with a TKI.

Conclusion

Although DTCs have usually favorable prognosis, distant hematogenous metastasis is an important consern after the treatment of FTC with thyroidectomy and RAI. Cranial imaging in these patients should be performed during the follow-up. The metastatic disease in these patients has a long clinical course and should be managed appropriatelly. In the RAI refractory metastatic lesions SRS and surgical resections should be conserned as the first management approach to improve survival.

Declarations

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Author contributions

Conceptualization, B.E.; Methodology, B.E. and K.H.; Validation, B.E., K.H. and F.A.; Formal Analysis, B.E. and K.H.; Investigation, B.E.; Data Curation, B.E. and S.A.; Writing – Original Draft Preparation, B.E.; Writing – Review & Editing, B.E., F.A., N.U.U., and S.A.; Supervision, B.E., F.A. and N.U.U.

Conflicts of interest

The authors declare no conflict 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

Informed consent was taken from the patients.

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CASUISTIC PAPER

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Mirror aneurysm of ICA terminus associated with adult polycystic kidney disease

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ABSTRACT

Introduction. Bilatereal saccular cerebral aneurysms (SCAs) that developed symmetrically on the same named vessels are defined as mirror aneurysms and account for a small subset of multiple cerebral aneurysms. The internal carotid artery (ICA) bifurcation is a rare location for mirror aneurysms.

Aim. We aimed to present the importance of risk status assessment for SCAs and screening in all ADPKD patients for timely detection and managementof SCAs before catastrophic complications occur

Description of the case. We present mirror aneurysms of bilateral ICA bifurcation that appear like a couple of dancing men on coronal computed tomography angiography (CTA) images, which were successfully treated with single stage coil embolization in a 45 year old female patient with medical history of autosomal dominant policystic kidney disease (ADPKD).

Conclusion. SCAs are more frequent in patients with ADPKD than in general population and also these aneurysms are more likely to rupture at earlier ages**.** Mirror aneurysms of ICA terminus can be treated effectively and safely by single stage coil embolization.

Keywords. autosomal dominant polycystic kidney disease, ICA terminus aneurysm, mirror aneurysm

Introduction

Saccular cerebral aneurysms (SCAs) also called Berry aneurysms account for the majority of intracranial aneurysms with a reported wide range of prevelance of 0.2-8.9% in asymptomatic population. Most of them occur typically at the branching points of larger vessels, with 90% occuring in the anterior circulation.[1] Bilateral SCAs that developed symmetrically on the same named vessels in the form of mirror image of each other are defined as 'mirror aneurysms' and account for a small subset of multiple cerebral aneurysms. In the study conducted to evaluate predictors of future hemorrhage in patients who had unruptured mirror aneurysms performed by the International Study of Unruptured Intracranial Aneurysms (ISUIA) investigators, the prevelance of mirror aneurysms was 12% (376 of 3120 patients). They were more prevelant in women and usually tend to be larger. While they were found to be common in patients with family history of subarachnoid hemorrhage (SAH), they were not found to be an independent precursor of SAH in the future.[2,3]

Aim

We present an unruptured mirror aneurysms of internal carotid artery (ICA) terminus established on computed tomography angiograhy (CTA) in a 45 year old

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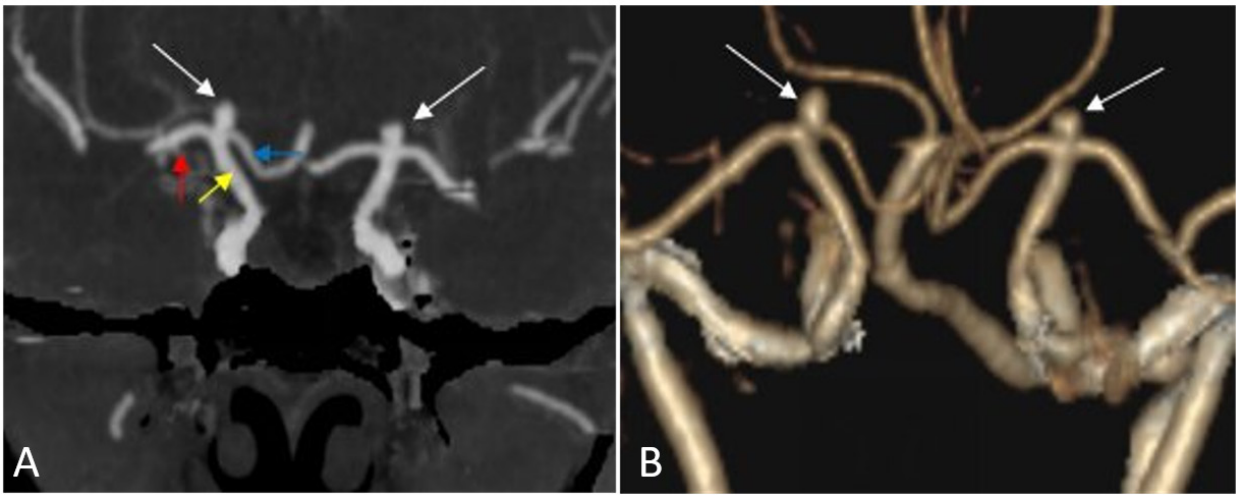

Fig. 1. CTA of the brain; maximum intensity projection image (A) and three-dimensional reformatted image (B) show mirror saccular aneurysms projecting antero-superiorly from the bifurcation point of bilateral ICA terminus, appearing like 'dancing men (white arrows), (yellow arrow: ICA, red arrow: MCA-M1 segment, blue arrow: ACA-A1 segment)

female patient with medical history of autosomal dominant polycystic kidney disease (ADPKD).

Description of the case

A 45 year old female patient presented with the complaint of longlasting intermittant headache. Her medical history was remarkable with the presence of ADPKD and a family history of subarachnoid hemorrhage in her brother years before. Due to the family history and the presence of PCKD both of which are associated with increased incidence of cerebral aneurysm, CTA of brain was performed. A paired symmetrical unruptured aneurysms in bilateral ICA bifurcation that mirror each other and appear like 'dancing men' on coronal images were revealed. In addition to being located symetrically on the same named vessels, they were also both projecting anterosuperiorly and had the same size of 4x3.5 mm. Both of them showed uniform, bright enhancement without thrombosis or calcification (Fig. 1). There were no branches taking off from or in the vicinity of the aneurysms. The right posterior communicating artery (PCoA) was hypoplasic with a diameter of less than 0.5 mm, but the rest of the circle of Willis was unremarkable (Fig. 2). The mirror aneurysms were succesfully treated with single-stage bilateral coil embolization (Fig.3).

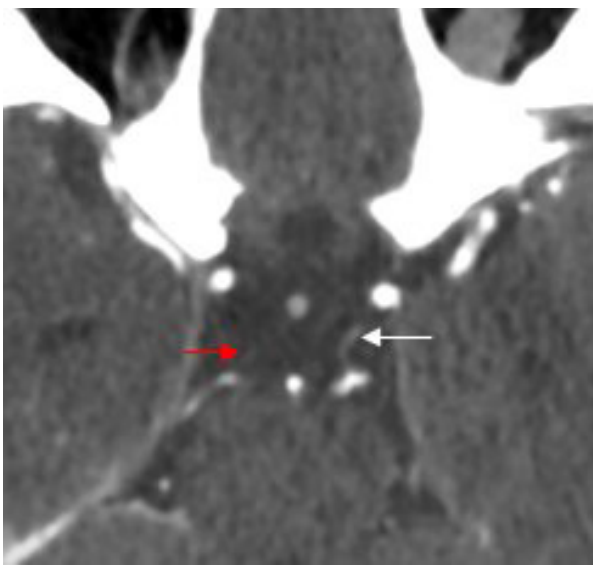

Fig. 2. Axial image of the CTA of the brain showing right hypoplasic PCoA (red arrow). The left PCoA was normal in caliber (white arrow) and the remaining of the circle of Willis was unremarkable

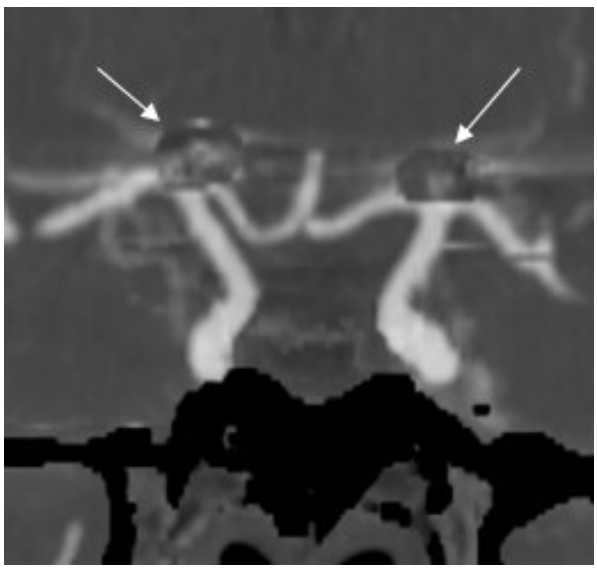

Fig. 3. Maximum intensity projection image of CTA of the brain showing the artefacts associated with the coil embolization of the mirror aneurysm (white arrows)

Discussion

SCAs have a well recognized association with ADPKD.[2] As in general population familial predisposition for the formation of SCAs is apperant among the patients with ADPKD in whom the SCAs are found in up to 16% of patients with a family history of aneurysms [1,4] . In addition, the risk of rupture of these cerebral aneurysms at an earlier age is higher in familial patients than those having nonfamilial, sporadic ones.[4,5] SCAs are multiple in approximately 15% to 45% of the cases of cerebral aneurysms.[6] However, occuring in the form of mirror aneurysms symmetrically at the same named vessels are not frequent. In addition, the ICA bifurcation is a rare location for these mirror aneurysms. In the ISUIA study the most common location of mirror aneurysms was the middle cerebral artery (MCA) which was involved in 126 patients (34%).[2] In the retrospective review of 172 cases treated for 344 mirror aneurysms performed by Choi et al., the ICA bifurcation was reported only in 4 patients (2.3%). In this study, the MCA bifurcation was seen in 83 patients (48.2%) and was reported as the most common site of involvement.[7] In a review of ADPKD patients with ruptured cerebral aneurysm it was revealed that most of ADPKD patients with ruptured cerebral aneurysms were young, female patients having small aneurysms located in the anterior circulation.[8] In a single center cohort of 495 consecutive patients with ADPKD submitted to targeted cerebral aneurysm screening, it was concluded that the intracranial aneurysm rupture rate is high in ADPKD despite targeted screening, and involves mostly patients without familial risk factors for cerebral aneurysm.[9] Current American Heart Association/American Stroke Association (AHA/ASA) guidelines recommend noninvasive screening to all patients with ADPKD.[10] On the other hand, the Kidney Health Australia – Caring for Australasians with Renal Impairment (KHA-CARI) 2015 guideline suggest screening for intracranial aneurysms in high-risk individuals with ADPKD that is those with a positive family history of subarachnoid hemorrhage, intracerebral hemorrhage, and/or unruptured intracranial aneurysm in at least one affected first-degree relative.[11] Similarly, Kidney Disease Improving Global Outcomes (KDIGO) experts recommended only targeted- selective screening based on the presence of other risk factors like family history of intracranial aneurysm or subarachnoid hemorrhage, previous intracranial aneurysm rupture, high-risk professions such as airline pilots and patient anxiety despite adequate information.[12] In the management of detected, unruptured mirror aneurysms, as in all aneurysms, some nonspecific measures are recommended to all patients like blood pressure control, smoking cessation. The treatment decision must be carefully made in an individualized manner. Endovascular or neurosurgical treatment should be considered in patients with high rupture risk aneurysms or growing aneurysms.[13] Single stage treatment is preferred, if applicable, in order to prevent further delay that can be associated with the rupture of one of the aneurysms and to avoid second general anesthesia. In their retrospective review, Choi et al. suggested that when applicable, single-stage coil embolization should be considered as a reasonable treatment option for mirror aneurysms.[7] Our patient has also be successfully treated with single stage coil embolization. The aneurysms considered at low rupture risk can be followed by serial imaging to avoid possible treatment related complications. Repeat screening every 5 years with MR angiography after a negative initial study and annual surveillance MR angiography in patients with detected, incidental intracranial aneurysm have been showed as cost effective.[13]

Conclusion

Since SCAs are more frequent in patients with ADPKD than in general population and also these aneurysms are more likely to rupture at earlier ages. Risk status for intracranial aneurysms should be carefully assessed in all ADPKD patients and screening should be conserned in these patients for timely detection and management before catastrophic complications occur. Management should be performed in an individualized manner and the aneurysms with high rupture risk should be appropriately managed before catastrophic complications occur. Mirror aneurysms of ICA terminus can be treated effectively and safely by single stage coil embolization.

Declarations

Funding

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Author contributions

Conceptualization, B.E. and N.N.W.; Methodology, B.E. and O.C.; Formal Analysis, B.E. and M.O.C.; Investigation, B.E., O.C. and N.N.W.; Data Curation, B.E. and N.N.W.; Writing – Original Draft Preparation, B.E. and O.C.; Writing – Review & Editing, B.E., O.C. and M.O.C.; Supervision, B.E. and N.N.W.

Conflicts of interest

The authors declare no conflict 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

Informed consent was taken from the patients.

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LETTER TO THE EDITOR

Artur Palak

William Harvey, discovery and life

Student's Scientific Club "URCell" at the Medical College of Rzeszow University, Rzeszow, Poland
Supervisors: Dorota Bartusik-Aebisher, Sabina Galiniak

Dear Editor,

William Harvey was an English biologist and physician living at the turn of the 16th and 17th centuries (born April 1, 1578, died June 3, 1657). He was born in Folkestone (a city on the south-east coast of Great Britain), the son of a farmer, Thomas Harvey. He had numerous siblings, including seven brothers and two sisters.[1] William Harvey began his education at the age of 10 at King's School in Canterbury, and the next stages of his medical education were Gonville and Cauis Collage. In 1599, he graduated from Cambridge University, moving to the University of Padua, which at that time was a leading center when it comes to medical science. His mentor was Hieronymus Fabricius ab Aquapendente, who gave a detailed description of the crescent-shaped valves (in his work "On the Valves of the Veins"), which was the basis for Harvey's later discoveries. On April 25, 1602, he obtained his doctorate and then returned to England to practice his profession. Two years later he married Elizabeth Browne, the daughter of the London physician Launcelot Browne, who served as King James I's physician. his daughter) and raising his social status opened the way for him to salons.[1] In 1607 he joined the Royal College of Physicians, which was an organization treating patients from the highest society (he made an unsuccessful attempt to join in 1604 shortly after the wedding). Harvey was active in it until the end of his days. In 1609, he began working at St. Bartholomew's Hospital, London. In 1616, he received the Lambley Lecturer status in the Royal Collage of Physicans (which he kept until 1656), which meant that he was to teach anatomy to both doctors and surgeons (an interesting fact is that at that time both professions were separated from each other). However, he did not do it in the way it has been done so far. He presented anatomy on bodies during non-book dissections, which became the standard until the end of the 17th century, and the model of the practical study of anatomy has remained unchanged to this day.[1] From that moment he started climbing the career ladder to obtain the status of the Extraordinary Physician of the King at the court of James I on February 3, 1618, and then of his son, Charles I (here he had already taken the position of the Ordinary Physician, i.e. the king's chief physician). The relationship with the second of the aforementioned monarchs was reportedly very friendly, which resulted in permission to experiment on deer that were owned by the royal family. Harvey was also asked by the king to present him with medically interesting cases, which indicates his strong interest in medicine. We do not know much about his private medical practice (due to the later events related to the loss of his works and documents), and quite interesting information is that his patient was Francis Bacon – one of the greatest authors of empiricism.[1-3] The most important and groundbreaking for all medicine is the description of the movement of blood and the functioning of the heart, in the work "Anatomical Exercise on the Motion of the Heart and Blood in Animals" (today known as "De Motu Cordis"), which is his peculiar magnum opus. [Z] The field of his interest was also the biology of animals and their reproduction, which resulted in the publication of "Exercises on the Generation of Animals" dealing with the physiology of animals, in particular about reproduction.[4]

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Unfortunately, however, his wide field of interest and numerous discoveries have not been presented or have been published by other people due to the looting of his collections. It happened in 1642 in Whitehall, where Harvey had his home. He lost, among other things, a book containing numerous species of insects, notes with reported cases of his patients, descriptions of reports from post-mortem sections of people and animals, and many other significant notes. Harvey himself used to call the loss of so much of the collection "the greatest crucifixion". It is possible that this was a very strong component of his suicide attempt. [4] The Great Fire of London of 1666 also contributed to the loss of his legacy, which consumed the literature he had helped to gather for the Royal College of Physicians.[4]

It is also worth mentioning here that Harvey had his role in the famous witch hunt. However, it was not a negative role, because he approached women tried for witchcraft objectively and found them innocent. He also made expeditions alongside the king during the English Civil Wars, and participated in diplomatic missions. [4] William Harvey died in London of a stroke. Before his death, he suffered from insomnia caused by his work, gout and kidney stones. While still alive, he was recognized by the environment for his groundbreaking discoveries. It is one of the few cases where a groundbreaking explorer can enjoy fame while still in existence. [1] As mentioned above, his life's work is "Anatomical Exercise on the Motion of the Heart and Blood in Animals". It is a 70-page book published in Latin in 1628 and in English in 1653 dealing with the movement of blood and the role of the heart in that movement. She presented the groundbreaking statement that the heart acts as a suction pump, and blood circulates in the body. To better present how groundbreaking this discovery was for those times, we should briefly mention the beliefs about blood, heart and liver at the turn of the 16th and 17th centuries. The theories of the doctors of that time were still based on the works of Galen, a Roman physician who lived around 100 AD. He assumed that clear blood was flowing in the arteries, transmitting substances that it bound in the lungs (no molecules, especially oxygen, were known at that time) and transferred it to organs such as the brain, muscles or pancreas with the help of the heart, which was supposed to pump only "lung blood". Dark blood was supposed to flow in the veins, carrying nutrients bound in the liver. As we know today, Galen was not mistaken much in his assumptions when it comes to the generalized properties of blood. However, he additionally postulated that blood is consumed by the organs to which it goes and then reconstituted (by the liver), which was the basis for such blood circulation. It was also the basis for the use of phlebotomy, which was to restore the balance of the body's fluids, because disease was then associated with this imbalance.[5] Harvey during the lessons he gave (Lambley Lecturer) was able to closely observe and examine the human body, which allowed him to question Galen's postulates about blood and heart. Probably around 1618 he came up with the concept of the heart as a pump. He began his deliberations by asking himself questions that Galen and his theory of anatomy and physiology could not answer, eg. Why do valves, despite the same structure, fulfill different functions? Why is the pulmonary artery so large compared to the other "nourishing vessels" if (according to Galen) it was intended only to nourish the lungs? And why would blood flow into one ventricle and drain out of the other if they both contract at the same time?.[1] With these questions, Harvey questioned the whole philosophy of the Roman genius's understanding of the heart and started a period of theory based on experience and facts without authority. I profess to learn and to teach anatomy not from books but from dissections, not from the tenets of Philosophers but from the fabric of Nature - William Harvey.[2]

While observing the capacity of the vessels, counting the amount of blood that was in the body and estimating the amount of blood pumped by the heart per minute (today we know this value as cardiac output, it is 5-6 liters), Harvey concluded that such a large amount was impossible to achieve. resynthesized by the liver in such a short time and thus has to constantly circulate. However, he did not know how it happened, but his subsequent experiences were to shed light on it.[4] Harvey based his further experiments on studying the veins and valves in them. They consisted in ligating the subject's hand above the forearm or other examined parts of the body and forcing the person to exercise in order to fill the veins with blood. On the subject's hand, there were clearly visible superficial veins with valves. The researcher then pressed a finger against the vein to block the flow of blood in it. t turned out then that the blood "blocked" on the valves as if it were reflecting on them. If the blood in the veins was flowing around the perimeter as Galen assumed, the veins should collapse below the finger position, not above which meant that blood was flowing inside the cage. Harvey repeated this experiment on other parts of the body and determined that the valves were arranged around a centrally located heart.[4]

To answer the question about the role of contraction and relaxation of the heart, Harvey chose cold-blooded animals (then referred to as cold-blooded), whose hearts beat much slower than human ones, which facilitated the observation of changes occurring during the heartbeat. An English physician noticed that during contraction, the aorta fills with blood similar to the pulmonary artery, which was already the basis for refuting Galen's assumptions. Further observations allowed Harvey to distinguish systolic and diastolic pulses, which he

himself did not know, and the term was used by medicine years later. Harvey, on the basis of the above-mentioned (and other not mentioned in his book) theories and observations, presented a comprehensive model of blood circulation in the body and the role of the heart in this movement. The heart was supposed to pump blood with a vital particle taken from the lungs (today we know it as oxygen) around the periphery to all organs. From them, blood was to be collected by less flexible venous vessels and pushed by the muscles towards the heart into the right atrium (from today's perspective, we know that the main force driving venous return is the pressure difference between the left and right atrium, but the mechanism presented by Harvey also plays big role in this movement). Then the blood was sent to the lungs by the pulmonary artery, where it was supplied with the above-mentioned substance and returned to the left atrium and then to the ventricle, which was a condition for the closure of the entire cycle.[1] It is absolutely necessary to conclude that the blood is in a state of ceaseless motion; that this is the function which the heart performs by means of its pulse; and that this is the sole and only end of the motion and contraction of the heart. - William Harvey Harvey's groundbreaking theory caused the expected uproar among his colleagues, but it was mostly negative despite the positive reception of King Charles I. use of laxatives.[5] Despite general rejection from the community, Harvey continued teaching as Lambley Lecturer, which helped train a new generation of physicians to base their therapies and research on facts and experiences. Additionally, to counter criticism by Jean Riolan, the English anatomist published "Two Anatomical Exercises on the Circulation of the Blood" in 1649.[2-4]

Despite such extensive knowledge and numerous attempts, Harvey did not prove the existence of a connection between the veins and arteries that would complement his theory, but Marcello Malpighi did it with a microscope, which during the English researcher's lifetime was not developed enough to be able to show images at a magnification sufficient for observation of capillaries.[5] His impact on medicine, as well as on the history of the world, is placed on the 55th place of the hundred most important people in the history of the world according to Michael H. Hart. [3] Another significant event showing William Harvey's influence on medical science is the exhibition dedicated to him at the opening of the 500th anniversary of the Royal College of Physicians, a member of which he was a member practically throughout his professional life. [6] Many centers named after him were named in honor of the genius explorer, e.g. the residential wing of the Royal College of Physicians, the hospital in Ashford in Great Britain named after him (it is a town 34 km away from Folkestone), The Harvey School which is an institution for children from 6th-12th grade located in New York, William Harvey Research Institute / Heart Center under the jurisdiction of Quinn Mary University of London dealing with biomechanics and biochemistry of the heart.[5-6]

Declarations

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Author contributions

Conceptualization, A.P.; Writing – Original Draft Preparation, A.P.; Writing – Review & Editing, A.P.

Conflicts of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR

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Contiguous diploic veins and ıntraosseous arachnoid granulations: can they function more than necessary?

Dear Editor,

Cerebrospinal fluid (CSF) is produced within the ventricular system by the choroid plexus and flows from the lateral ventricles to the third and then fourth ventricle and eventually reaches the subarachnoid space around the brain and spinal cord. Then the majority of CSF is reabsorbed from the subarachnoid space into the venous system by the arachnoid granulations (AGs), which are projections of the arachnoid membrane into the dural venous sinuses, particularly the superior sagittal sinus and the transverse sinuses. Up to 15% of these AGs are also found within the perineural spaces of the cranial and spianal nerve sheaths to provide CSF drainage into the lymphatics.[1]

Additionally, diploic veins (DVs) which are present throughout the cranium and provide an important connection between the extracranial and intracranial venous systems was previously suggested as an alternative drainage pathway for intracranial CSF via communication with intraosseous projections of AGs.[2,3] What if these contiguous diploic veins make any sense when they are dilated? Adjacent dural venous sinuses are expected to be atretic. Can they also cause excessive CSF reabsorption? We thinkt that, the contiguous dilated DVs in relation with intraosseous AGs may not only offer a simple alternative pathway, but may also cause an undesirable rate of CSF reabsorption which may result in compansatory CSF overproduction and ultimately a communicative hydrocephalus. We examined this probability on neuroimaging studies of a 77-year old female patient presented with recently started decline in cognitive functions (dementia) and ataxic gait. Brain magnetic resonance imaging (MRI) findings included ventriculomegaly with Evan's index of 0.38 and transependymal edema in addition to disproportionate changes in subarachnoid spaces characterized with minimal cortical atrophy with narrow convexity-parafalcine sulci in contrast to dilated sylvian fissures compatible with hydrocephalus in addition to moderate age related cerebral atrophy (Fig. 1).[4,5]

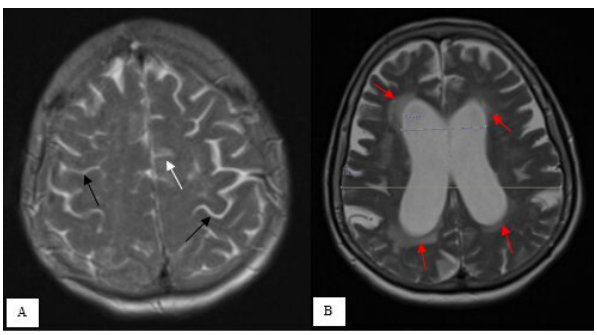

Fig. 1. The brain axial T2w MRI showing disproportionate changes in the subarachnoid spaec. The relatively narrow convexity (A, black arrows) and parafalcine (A, white arrow) sulci in contrast to more prominent ventriculomegaly with Evan' index of 0,38 (50/130) and associated transependymal edema (B, red arrows)

In addition, on the head computed tomography (CT) performed two days previously when she pre-

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sented to the emergency department with similar complaints, intraosseous AGs in the right side of the occipital bone and dilated diploic veins throughout the cranium but more prominent on the right side were noted. As expected, the right sided sigmoid sinus and the jugular bulb were atretic (Fig. 2).

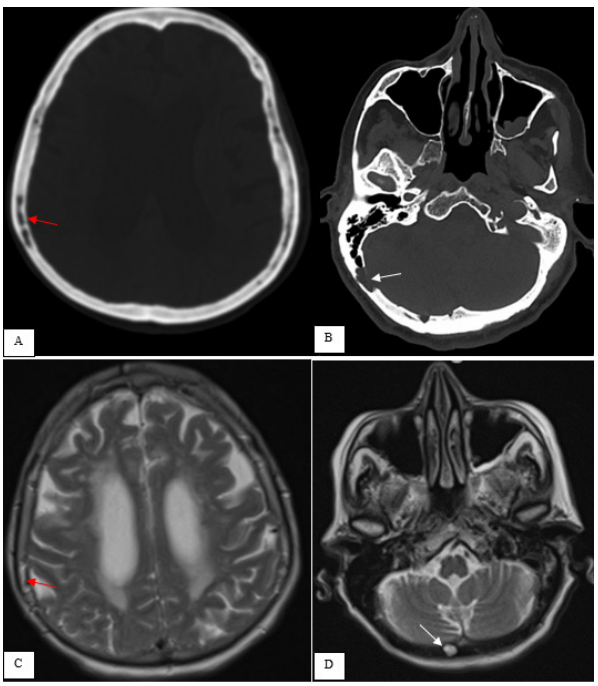

Fig. 2. Head CT scan axial images Show the right sided atretic sigmoid sinüs (A, red arrow) and judular bulb (B, white arrow). Compare these with the normal sized left sigmoid sinüs (C, red arrow) and jugular bulb (C, white arrow)

Hyperintensity compatible with CSF signal within these dilated varicose DVs was suggestive of CSF reabsorption via intraosseous AGs (Fig. 3).

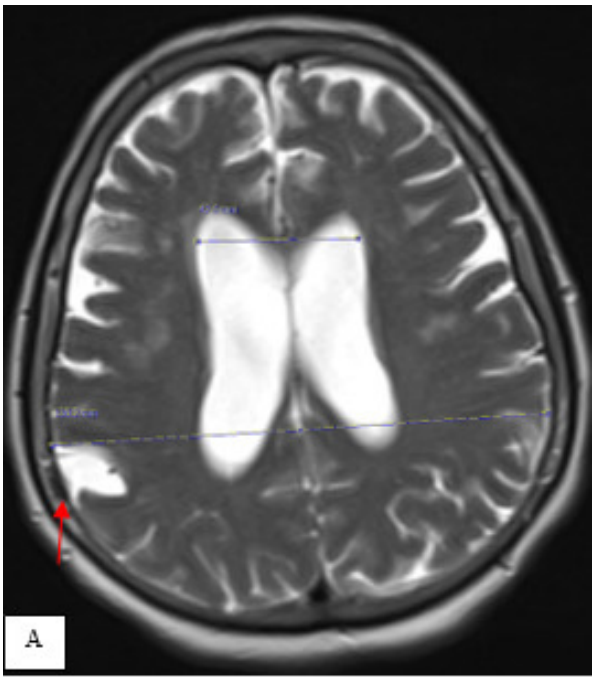

Fig. 3. (A, B) Axial Bone window images of the head CT demonstrating dilated diploic veins throughout the cranium, more prominent at the right side (A, red arrow). An intraosseous AG is shown (B, white arrows).C,D) Axial T2w MRI showing the CSF signal intensity within the same DV (C, red arrow) and another intraosseous AG projecting into the occipital bone with characteristic CSF signal on T2w images (D, white arrow)

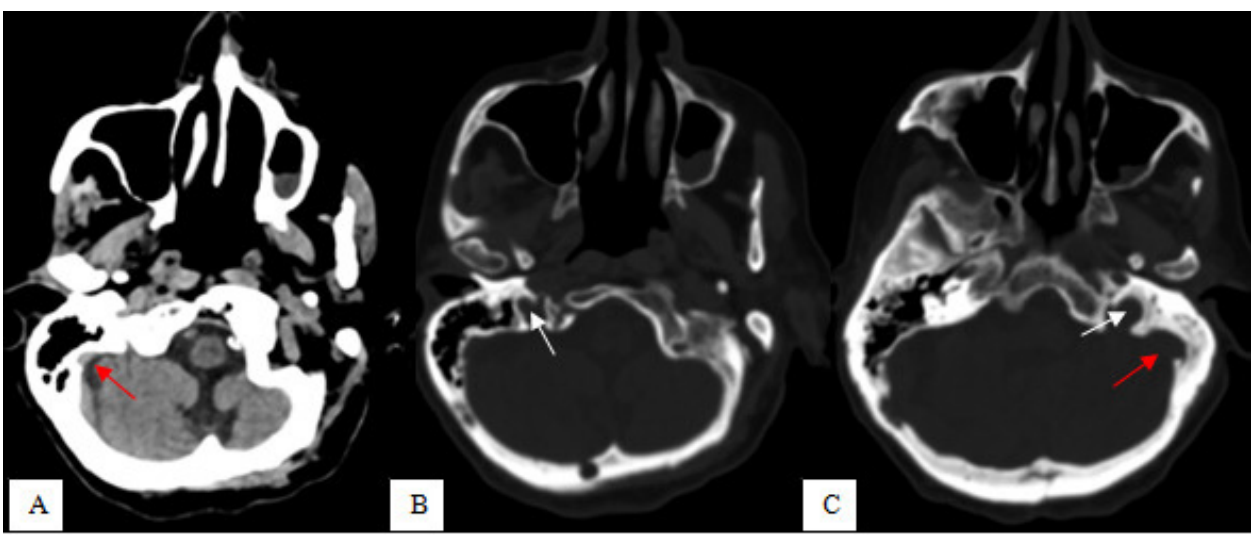

Fig. 4. Axial T2w MR image performed 5 years ago at the same level with figure 1B showing less ventriculomegaly (Evan's index of 0.30) without transependymal edema, compatible with age related dilatation of the ventricular system. Please note the absence of CSF signal witihin the same DV as shown in figüre 2C. Instead of CSF signal flow void is present (red arrow)

After examining the patient's previous neuroimaging studies, we realized that three years ago while the hydrocephalus (Evan's index of 0.32) was not so obvious, the signal intensity of the DVs was not the same as CSF, on the contrary, there was a flow void on T2w images (Fig. 4). As in our patient, overfunctioning of dilated DVs in association with intraosseous AGs may be the underlying cause for development of normal pressure hydrocephalus (NPH).

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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
Informed consent was taken from the patients.

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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 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.