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ORIGINAL PAPER

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Short-term comparison of the 660 and 830 nm laser in the treatment of temporomandibular dysfunction – a randomized clinical trial

Physiotherapy Course of the Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil

ABSTRACT

Introduction. The objective of this study was to compare the effects of low-level laser therapy (LLLT), 660nm laser with 830nm, in temporomandibular dysfunction (TMD).

Aim. To compare the effect of LLLT 660 nm and 830 nm in treatment of TMD.

Material and methods. This is a randomized clinical study, composed of 30 volunteers with TMDs selected and divided into three groups: LLLT 660nm, LLLT 830nm and Sham. After the intervention, the results were reevaluated with the Fonseca anamnestic questionnaire (FAQ), American Academy of Orofacial Pain Questionnaire (AAOPQ), McGill Pain Questionnaire and Visual Analog Scale.

Results. Analysis of the results showed that, although all groups had reduced values in the FAQ, only the laser groups presented alterations in the level of classification; for AAOPQ, only the treatment groups had a reduction in the positive responses, variables, the reduction was similar for all groups.

Conclusion. LLLT produced a reduction in severity of symptoms but was like the sham for pain.

Keywords. low-level light therapy, physical therapy modalities, temporomandibular joint

Introduction

Temporomandibular dysfunction (TMD) is a set of disorders that encompass: masticatory muscles, temporomandibular joint (TMJ) and other associated structures. Posterior, postural, psychological and neuromuscular anatomical imbalances may cause changes in TMJ, resulting in clinical manifestations such as palpation pain, orofacial pain, stress and altered joint mobility.[1]

Physiotherapeutic treatment is presented as an alternative for the relief of TMD symptoms. Resources such as electrothermotherapy, manual therapy, and kinesiotherapy stand out in an attempt to reduce muscle pain that

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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mainly affects the masseter, lateral pterygoid and temporal muscles.[2] In the relief of symptomatology and in the reestablishment of TMJ function, the low-level laser therapy (LLLT) is established as an efficacious and low cost option. Its effect of analgesia and muscle relaxation is caused by factors such as increased pain threshold and the production of endorphins, and through the electrolyte blocking mechanism of nerve fibers, in addition to reducing the inflammatory process.[3,4]due to its impact on biological processes, especially inflammation, considered as an adjuvant treatment modality in TMD cases. Materials and methods: All original articles related to PBMT for TMDs in EMBASE, MEDLINE (NCBI PubMed and PMC Laser is a non-ionizing electromagnetic radiation, in which all the waves that make up the beam have the exact same wavelength, and thus are monochromatic.[5]

The most used lasers for the therapy are those that act between the red light range (630nm to 700nm) and infrared (700nm to 904nm).[6,7] In the absorption aspect, the red light, due to its lower wavelength, has a lower penetration power in the tissue and is indicated for superficial lesions, whereas the infrared due to its high penetration power reaches deeper structures.[3,8,9]

Aim

Considering that these two types of lasers present different wavelengths, with possible different receptors, but with similar applicability, it is interesting to compare the clinical effects among them, even though there are divergences in the use of this resource in the treatment of TMD due to the diversity of parameters, this research had the objective of analyzing the effect of LLLT 660 nm compared to 830 nm in TMD.

Material and methods

It is a randomized clinical trial. It was composed of 30 volunteers, who were referred by the Clínica de Odontologia of the Universidade Estadual do Oeste do Paraná (UNIOESTE), who, through a dental screening, selected volunteers with TMDs, of which 27 women and 3 men, the average age being 21.5 years, university students. After screening, the selected volunteers were invited to perform the second part of the evaluation and started the research activities after signing the Free and Informed Consent Form (TCLE), approved by the Ethics Committee of Unioeste under number 2,356,498.

We included volunteers who presented TMDs with painful processes (regardless of the source of the pain), of both sexes and any age group. Among the exclusion criteria were: individuals with and/or presenting with central or peripheral neurological disorders, systemic inflammatory disease, malignant tumors, individuals who were unable to respond to questionnaires, and those who left the therapy during the period of physical therapy treatment.

As assessment instruments were used: the Visual Analogue Pain Scale (EVA), which scores the intensity of pain, in a range from no pain (zero) to maximum pain (10 points); the QAF – Fonseca Anamnestic Questionnaire assessing the severity of TMD symptoms, classifying the patients in: without TMD (0 to 15 points), mild TMD (20 to 45 points), moderate TMD (50 to 65) and severe TMD (70 to 100 points), are 10 questions, scored according to the yes - 10, no - 0 and sometimes - 5; the American Academy of Orofacial Pain Questionnaire (AAOPQ) also presents ten specific TMD-related questions, with the dichotomous answers, yes or no, that lead to deviations from organic normality if answered positively; the McGill Pain Questionnaire presents a series of questions that indicate to the patient words that resemble their pain, which aims to quantitatively measure and associate sensory (42 descriptors), affective (14 descriptors) and evaluative (5 descriptors) and pain, in a total of 78 descriptors (17 belong to the miscellaneous subgroup).[10-12] The evaluation was performed before (EV1) and after the intervention (EV2), which lasted two consecutive weeks, for two days each week in the Centro de Reabilitação Física (CRF) of Unioeste - Cascavel campus.

According to the inclusion criteria, the volunteers were divided into three groups with a simple draw. The first group (G1) received LLLT treatment with a wavelength of 660nm, with intensity of 4 J/cm² (per point), at three points in the masseter muscle and three in the temporal bilaterally, with a power of 30 mW, with the incidence of a 90° bundle with respect to the tissue (fig.1).

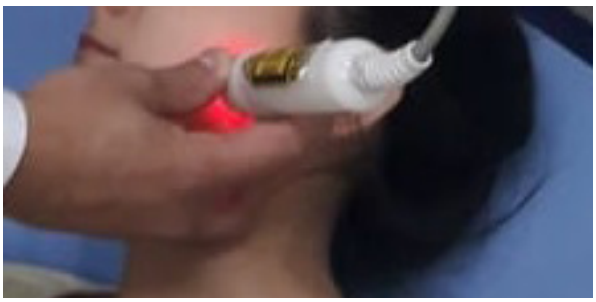

Fig. 1. Application of radiation in masseter region

The second group (G2) received the LLLT 830nm, with the same criteria as the first one. The third (G3) received sham treatment (fig.2), in that, all steps was followed by the above groups, however, there was no escape of radiation. The application was carried out taking all safety measures, such as the use of goggles for both therapists and patients, in addition, hygiene and asepsis standards were included in the pre-treatment to decrease the tissue impedance.

The sample size was calculated based on the evaluation of EVA, by the difference between the means and standard deviation of 1.5, power of 80% and sig-

nificance level of 5%, being found 10 individuals per group. The data were verified and analyzed by descriptive and inferential statistics, and the SPSS 20.0 program was used, by the Generalized Mixed Model test and after the LSD test, the accepted significance level was 5%. Also, the effect size was calculated by Hedges' g (https://www.estimationstats.com/#/), with the following interpretation: insignificant <0.19; small 0.20 – 0.49; medium 0.50 - 0.79; large 0.80 – 1.29; very large > 1.30.[13]2010

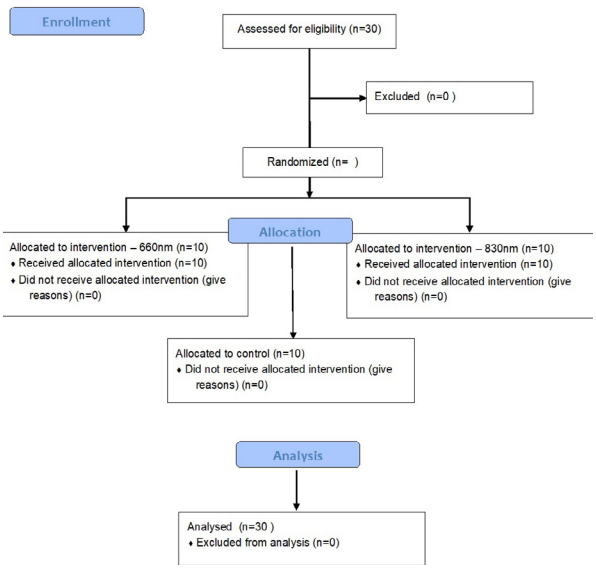

Fig. 2. Consort based flowchart

Results

When analyzing VAS data, there were different between evaluations (p<0.001), but not between groups (p=0.117) or interaction (p=0.266). When effect sizes were analyzed, before and after therapy, very large sizes were noted. The Fonseca questionnaire showed differences between the evaluations (p<0.001) and between the groups (p=0.034), but no interaction (p=0.414). The comparison between G1 and G3 was different (p=0.010), with the G1 is presenting higher values. The effect sizes were very considerable for G1 and G2, but small for G3 (table 1).

The American Academy of Orofacial Pain Questionnaire was evaluated for the rate of positive responses in pre- and post-intervention, one can notice differences between the evaluations (p=0.006), but not between groups (p=0.105) and interaction (p=0.504). Effect sizes were: large for G1 and medium for G2 and G3. Finally, for McGill's questionnaire, the differences occurred between the evaluations (p=0.039), but not between the groups (p=0.356) or interaction (p=0.724); and the effect sizes were: large for G1, medium for G2 and small to G3 (table 1).

Discussion

Literature describes TMDs as functional and often disabling problems, generating pain and difficulties in speech and chewing.[1] Therefore, important studies have been conducted evaluating therapeutics for such dysfunction, and the present study sought to compare two forms of low-level laser application in patients with painful TMD, in order to analyze pain and oral function. The groups were composed of a sample composed basically of young women referred by the Dentistry Clinic of the Unioeste, and the literature presents these characteristics as the population with the highest incidence of TMD.[14,15]

The Fonseca index was used in this study in order to identify the degree of TMD severity, both in pre-intervention and post-intervention, thus being able to identify the presence of variation in the level of TMD, and it is possible to observe in all groups, even in the placebo, significant differences indicating a reduction in severity, when analyzing by the criteria of the G1 and G2 index the volunteers were classified as severe and evolved to moderate, while G3 showed moderate classification even with the average values having decreased.[11] Thus, even if small, the clinical effect of low power laser therapy can be glimpsed by the effect sizes presented, which according to Fikácková et al. can be explained by stabilizing the membrane potential, directly interfering with the neural trans-

Table 1. Data presented in mean and standard deviation, for the different forms of evaluation, of the patients of G1, G2 and G3, with the respective effect sizes (ES)

	G1		G2		G3	
	AV1	**AV2**	**AV1**	**AV2**	**AV1**	**AV2**
VAS	6.0±1.9	2.6±0.7	6.9±1.0	2.2±1.0	5.4±1.9	1.9±1.3
ES	-2.29		-4.44		-2.03	
QAF	73.5±9.4	55.0±14.5	69.5±17.9	47.5±13.8	54.5±19.8	46.0±21.0
ES	-1.45		-1.32		-0.40	
QADOF	7.0±2.7	4.4±2.5	5.5±2.0	3.9±2.3	4.6±2.0	3.7±2.1
ES	-0.96		-0.70		0.79	
McGill	24.5±11.3	14.4±10.3	22.4±11.1	16.0±12.3	16.3±14.1	12.5±14.6
ES	-0.89		-0.52		-0.25	

mission of pain, which may result from the formation of varicosities in neurons Aδ and C neurons.[16,17]

Regarding the evaluation by the American Academy of Orofacial Pain Questionnaire, it was analyzed only according to the rates of positive responses in the pre- and post-treatment, since they indicate orofacial disorders, but it must be taken into account that this is an instrument indicated for screening patients.[11,12] When the groups were analyzed, there were larger effect sizes of G1 compared to the others, indicating a greater reduction of the former. Regardless of the wavelength, the literature points out that the LLLT favors increased blood flow and elimination of algogenic substances, thus having anti-inflammatory effects, as considerably as the output of endorphins that work right away on pain control.[18-23]

Several studies describe the use of LLLT in TMD patients, such as Catão et al. who compared the 830nm laser with the 660nm, 4 J/cm^2, in 3 points, using VAS and palpation of pain points, reported that there was a reduction of pain in both groups, with advantages for the first, however, did not use control group or placebo.[8] Borges et al. evaluating dose-response of 830 nm LLLT, in 44 individuals with TMD, distributed in 4 groups (8 Jcm^2, 60 J/cm^2, 105 J/cm^2 and control) that the LLLT of 830 nm reduced the pain, but only the first 8 J/cm2 showed functional improvements.[24] Frare and Nicolau used the 904 nm LLLT or placebo over 4 weeks (2 weekly sessions), at a dose of 6 J/cm^2 at 5 points (preauricular region and external auditory meatus), and observed reduction of the pain in the treated group.[25] The present study, differently from the ones mentioned above, used the laser at 6 points, including points in the temporal muscle region, with higher energy density and the presence of a placebo group. In the Nadershah et al. study, the treatment was performed with a 940 nm laser in regions of the temporal and masseter muscles, in addition to the pre-auricular and mastoid regions in individuals with TMD with myofascial pain, reporting the effectiveness of the treatment.[26]despite the lack of understanding of its exact mechanism. The aim of this study is to examine the effectiveness of photobiomodulation in the treatment of myofascial type TMD. Methods: Patients with unilateral TMJ and masticatory muscles pain during function were recruited and divided into two groups: a control group that received a sham laser treatment every 48 h for 10 days and a test group that received the same frequency of treatment to deliver a dose of 257 J per treatment and a total dose of 1285 J for the entire treatment. Pain was assessed using the visual analog scale (VAS It can be observed that the absence of an accurate diagnosis of the TMD origin in the present study is also a limitation. However, the laser has shown positive effects, regardless of the origin of the pain, as presented by Madani et al. in which they used both LLLT (810 nm, 21 J/cm^2, 6 J) on mandibular condyles, acoustic meatus and tendon points, or on acupuncture points (ST6, ST7 and LI4), with significant pain reduction.[27] Oliveira et al. comparing the irradiation of red (660 nm) with infrared (790 nm) by 3 therapies (at 48-hour intervals), in trigger points (8 J/cm^2) and TMJ (4 J/cm^2), observed a reduction in TMD symptoms, but the effects dissipated over time.[28]

In this study for specific pain evaluation, the McGill questionnaire was used to analyze the multidimensional aspect, and in this aspect all groups presented a reduction of scores, but the effect sizes indicated that the best results were in the treated groups, mainly at 660 nm.[10] For the intensity of the pain, evaluated by VAS, again, in general, there was a reduction in the pain, but the effect size presented greater values when the laser was used at a wavelength of 830 nm. It must be taken into consideration that pain arises not only from injured peripheral tissues, but also as an emotional experience capable of modulating the nociceptive input, thus, as the patients did not present important functional improvements for the simulacrum group, one can imagine that the placebo effect occurred, which would be the response to an inert form of treatment, dependent on various sensory and social stimuli related to the entire therapeutic act, modulated by the endogenous opioid, dopaminergic and serotoninergic system, which is influenced by anxiety, since the population of this study was composed basically of university students with TMD, which generally has high levels of anxiety.[15,29-32] This presents one more limitation of the present study, which was the lack of a control group only, which would not receive the simulation of laser use. Another limitation pointed out is that the groups initially showed themselves to be different for some variables, with the simulacrum group being less severe, thus suggesting that future studies may stratify the functional and pain variables prior to the experiment and the use of larger sample sizes.

Conclusion

It was observed that LLLT produced a decrease in the severity of the symptoms, regardless of the wavelength used, but was not different from the sham group in relation to the pain.

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ORIGINAL PAPER

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The predictive value of fetal middle cerebral artery/descending aorta ratio doppler parameter in the evaluation of perinatal results of intrauterine growth restriction

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ABSTRACT

Introduction. Although there are various reasons for intrauterine growth restriction (IUGR), the main cause is inadequate utero-placental and feto-placental circulation.

Aim. To determine the predictive values of fetal middle cerebral artery/descending aorta (MCA/DA) Doppler parameter in the evaluation of perinatal outcomes in pregnancies with IUGR.

Material and methods. 15 with IUGR and 35 normal newborn, who were born at the 34th gestational week or over included into the study. Doppler ultrasonography (US) measurements were performed. The ratio of pulsatility index/resistive index (PI/RI) from MCA, umbilical artery (Umb), DA was determined. Neonatal characteristics such as Apgar scores, neonatal intensive care unit (NICU) requirement, weight and sex were also recorded.

Results. In the IUGR group, mean MCA/DA RI-PI, MCA/UmB RI-PI were 0.88±0.19, 0.86±0.28, 1.22±0.18 and 1.55±0.39, respectively. In the control group, mean MCA/DA RI-PI, MCA/UmB RI-PI were 1.15±0.13, 1.09±0.41, 1.37±0.35 and 1.82±0.44, respectively. There were statistically significant relationship between MCA/DA PI with cord blood pH value and NICU requirement, age with gravida, parity, MCA/UmB RI, MCA/UmB PI; gravida with age and parity; parity with age, gravida, weight, MCA/DA RI, PI ratios.

Conclusion. Intrauterine MCA and DA Doppler US parameters of IUGR can be used safely in predicting perinatal outcomes in pregnancies with IUGR over 34 weeks.

Keywords. fetal MCA/DA Doppler parameters, IUGR, perinatal outcomes

Introduction

Although there are various reasons for intrauterine growth restriction (IUGR), the main cause is inadequate utero-placental and feto-placental circulation. IUGR is a fetal development disorder in which the expected fetus weight for gestational age is below the 10th percentile.[1] The fetus progressively deviates from the normal growth curve. Among the maternal, utero-placental and fetal causes that adversely affect the growth potential of the fetus, most cases of IUGR are caused by primary

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or secondary utero-placental circulatory failure. Inadequate nutrient and oxygen transfer from the placenta to the fetus, chromosomal abnormalities and intrauterine infections may cause the fetus to be small.[2-4] The risk of perinatal morbidity and mortality was increased in all IUGR patients, regardless of etiologic causes. Mortality rates are 4-8 times higher than in non-IUGR fetuses. Half of the infants with growth restriction have serious short-term and long-term morbidities such as meconium aspiration, pneumonia and metabolic disorders.[5,6] IUGR is present in 3-10% of all pregnancies and approximately 20% of stillbirths. In the presence of maternal hypertension or previous history of IUGR in pregnant women, the risk of IUGR is reported to be 25% or more. Low birth weight associated with IUGR is associated with at least 60% of neonatal deaths worldwide.[2,7] Doppler ultrasonography (US) is used to detect placental deficiencies. Placental deficiencies may cause perinatal death, intrauterine growth restriction (IUGR) and preeclampsia. Doppler US applications are important in the management of these cases.[8,9] To demonstrate systemic arterial features of the uterine arteries to the placenta, middle cerebral artery (MCA) and descending aorta (IA), venous examination is required for detailed cardiovascular status and respiratory system evaluation.[10] Middle cerebral artery (MCA) Doppler flow indicates cerebral resistance. Ductus venosus (DV) shunt with umbilical venous blood passes through the liver and leads to the heart and brain. Cardiac afterload changes due to parallel regulation of fetal circulation determine how this increased blood volume is distributed to the circulation. Increase in right ventricular afterload or decrease in left ventricular afterload leads to direct cardiac output to the left ventricle and thus to the coronary circulation and brain.[11] Diastolic velocity increases in MCA, which is a brain-protective effect. Decrease in MCA pulsatility index (PI) is a direct evidence of brain sparing effect. The brain protective effect of placental respiratory function impairment is due to hypoxia-induced cerebrovascular dilatation. These compensatory changes usually occur in the presence of sufficient cardiac output. When placental insufficiency enters the terminal period, insufficient cardiac function results in a decrease in MCA flow, which is called normalization.[12] In the last stage of placental insufficiency, cardiac output is reduced as a result of hypoxic or ischemic myocardial dysfunction and coronary vasodilation occurs. Cardiac dysfunction becomes serious if this does not adequately support myocardial nutrition.[13] Increases cardiac dysfunction and high afterlood central venous pressure. Increased central venous pressure leads to pulsations of the venous system and umbilical vein.[14] Used to determine appropriate cordocentesis and transfusion time in anemic fetuses.[15] Normal blood in the fetal descending aorta (FDA) shows a severe pulsatility with minimal diastolic component. The descending part of the aorta provides fetal abdominal organs, umbilical-placental circulation and perfusion of the lower extremities. As pregnancy progresses, fetal aortic diameter increases, peripheral resistance decreases and as a result, diastolic flow increases in the aorta. However, there is no significant decrease in the systolic/diastolic (S/D) ratio of FDA. Elevated S/D ratio, resistive index (RI) and PI ratios in FDA are considered to herald that poor perinatal outcomes such as IUGR and perinatal mortality may occur.[16]

Aim

To determine the predictive values of fetal MCA/DA Doppler parameter in the evaluation of perinatal outcomes in pregnancies with IUGR.

Material and methods

The study was performed as a retrospective cohort. A total of 50 newborns, including 15 patients with IUGR and 35 normal newborn, who were born at the 34th gestational week or over between January 2017-December 2018 at the Obstetrics and Gynecology Clinic, University of Health Sciences Adana City Training and Research Hospital were included in this study. Doppler US was performed by a specialist physician using the Samsung Medison H60 ultrasound device with 3.5 MHz convex transducer considering fetal movements and Doppler waveforms were obtained. Measurements were performed in the absence of fetal movement and during the apnea period, the angle of insonation is as close to 0 as possible (<20) and cover the sample volume vessel. PI, RI ratios of MCA, UmB, DA (just below renal bifurcation) were calculated in two measurements. At the time of sonographic examination, pregnant women were at the 26-28th weeks of pregnancy. There was mean 11 weeks between Doppler examination and delivery. Deliveries were carried out when pregnants' labour started. In addition, newborn characteristics such as 1st and 5th minute Apgar scores, NICU needs, weight and sex were also recorded. SNAPPE-2 scoring was used to evaluate patients who needed NICU.

Statistical analysis

SPSS 23.0 program was used for statistical analysis. In the evaluation, numerical data were expressed as mean ± standard deviation (ss), median, distribution range (min-max), and categorical data were expressed as percentage (%).The normality of distribution for the variables was evaluated using the Shapiro-Wilk test. Independent test and Mann-Whitney test were used to evaluate the differences between the groups. To analyze intraobserver reproducibility, the evaluation was repeated twice at 5 minute intervals. The 95% confidence interval for the area under the curve (AUC) and the de-

Table 1. Maternal Demographic Datas

	IUGR Group (n:15)		Control Group (n:35)		p value
	Min-Max	Mean±Sd	Min-Max	Mean±Sd	
Age (years)	19-39	26.32±4.27	21-44	27.32±4.85	0.674
Height (cm)	150-171	158.05±7.01	151-176	163.44±7.19	0.018*
Weight (kg)	52-102	71.18±10.54	56-146	75.63±14.58	0.012*
BMI (kg/m^2)	23.39-38.15	26.12±4.57	21.30-48.50	31.25±5.36	0.369
Time of birth (week)	35-39	37.33±1.36	36-40	38.41±1.22	0.035*

Table 2. Gravida and Parity Numbers of Groups

		IUGR Group (n:15)		Control Group (n:35)		p value
		n	%	n	%	
Gravida	1	6	12.0	6	12.0	0.004*
	2	6	12.0	7	14.0	
	3	2	4.0	13	26.0	
	4	1	2.0	4	8.0	
	5	0	0.0	2	4.0	
	6	0	0.0	2	4.0	
	7	0	0.0	1	2.0	
Parity	0	8	16.0	9	18.0	0.035*
	1	5	10.0	15	30.0	
	2	1	2.0	6	12.0	
	3	1	2.0	3	6.0	
	4	0	0.0	2	4.0	

Table 3. Mean values of MCA, DA and Umb RI and PI ratios

	IUGR Group (n:15)			Control Group (n:35)			p value
	Min-Max	Ort ±Std	Patient number with RI<1	Min-Max	Ort ±Std	Patient number with RI<1	
MCA RI/DA RI	0.65-1.22	0.88±0.19	6	0.85-1.32	1.15±0.13	9	0.771
MCA PI/DA PI	0.34-1.41	0.86±0.28		0.72-1.93	1.09±0.41		0.283
MCA RI/Umb RI	0.98-1.77	1.22±0.18	3	1.06-2.21	1.37±0.35	0	0.865
MCA PI/UmB PI	0.71-2.55	1.55±0.39		1.29-2.91	1.82±0.44		0.547

fined variables was estimated. p<0.05 was considered significant.

Results

The maternal demographic data of the groups included in the study are shown in Table 1.

62% of the newborns in the IUGR group were female and 38% were male. 54% of the newborns in the control group were female and 46% were male. Mean maternal age of the infants was 26.32±4.27 years and 27.32±4.85 years in the patient and control groups, respectively. Mean delivery time of IUGR and patient group was 37.33±1.36 and 38.41±1.22 week, respectively.

Table 2 shows the gravida and parity values of both groups. Accordingly, a statistically significant relationship was found between the two groups in terms of gravida and parity (p<0.05).

In the IUGR group, mean MCA RI/DA RI was 0.88±0.19, mean MCA PI/DA PI was 0.86±0.28, mean MCA RI/UmB RI was 1.22±0.18 and mean MCA PI/UmB PI 1.55±0.39. The number of patients whose MCA/DA RI ratio was less than 1 was 6 and the number of patients whose MCA/Umb RI ratio was less than 1 was 3. In the control group, mean MCA RI/DA RI was 1.15±0.13, mean MCA PI/DA PI was 1.09±0.41, mean MCA RI/Umb RI was 1.37±0.35 and MCA PI/UmB PI was 1.82±0.44. No statistically significant relationship was found between all groups (p>0.05).

Table 4. Evaluation of MCA, DA and Umb RI and PI ratios below 1 for the diagnosis of IUGR

	RI	PI
Sensitivity	88 %	70 %
Specificity	96.7 %	91.3 %
Positive Predictive Value	81 %	76 %
Negative Predictive Value	79 %	71 %
Precision	96.7 %	91.3 %

MCA, DA and UmB RI and PI values below 1 is important for the diagnosis of IUGR. Table 4 shows that

Table 5. Mean Weight and Cord Blood pH Values

		IUGR Group (n:15)		Control Group (n:35)		p value
		Min-Max	Mean±Sd	Min-Max	Mean±Sd	
Weight	Girl	1663-2752	2189.9 ±244.3	2350-4150	3421.3 ±423.1	0.115
	Boy	1748-2580	2210.1 ±307.8	2650-4320	3314.6 ±447.6	
	Total	1696-2632	2242.5± 296.7	2370-4255	3364.5± 421.5	
Cord Blood pH Values	Girl	7.24-7.41	7.32±0.044	7.22-7.39	7.32±0.037	0.742
	Boy	7.22-7.40	7.30±0.041	7.33-7.40	7.31±0.044	
	Total	7.22-7.41	7.32±0.05	7.22-7.40	7.32±0.038	

Table 6. Datas of 1st and 5th Minute Apgar Scores and Neonatal Intensive Care Requirement of Newborns

		IUGR Group (n:15)		Control Group (n:35)		p value
		n	%	n	%	
Apgar Scores 1st Minute	4	1	2.0	-	-	0.654
	5	-	-	-	-	
	6	1	2.0	4	8.0	
	7	6	12.0	6	12.0	
	8	5	10.0	14	28.0	
	9	2	4.0	8	16.0	
	10	-	-	3	6.0	
Apgar Scores 5st Minute	6	1	2.0	-	-	0.857
	7	-	-	-	-	
	8	4	8.0	8	16.0	
	9	7	14.0	16	32.0	
	10	3	6.0	11	22.0	
NICU requirement	Evet	6	40.0	3	8.57	0.789
	Hayır	9	60.0	32	91.43	

Table 7. The Relationship Between MCA PI / DA PI Value with Cord pH and NICU Requirement

		MCA PI/DA PI	Cord Blood pH Value	NICU requirement
MCA PI/DA PI	r	1	-0.013	-0.509*
	p		0.956	0.022
Cord Blood pH Value	r		1	-0.137
	p			0.004
NICU requirement	r			1
	p			

Table 8. Comparison of age, birth week, gravida, weight, BMI and parity values with MCA, DA and Umb RI and PI values in IUGR group

		Age	Time of birth (week)	Gravi-da	Parity	Weight	BMI	MCA RI/ DA RI	MCA PI/ DA PI	MCA RI/ Umb RI	MCA PI/ UmB PI
Age	r	1	-.038	.651**	.523*	-.164	-.273	-.381	-.355	-.437	-.453*
	p		.872	.002	.018	.490	.245	.098	.125	.044	.045
Time of birth (week)	r		1	-.021	-.031	-.248	-.325	-.234	-.176	.050	-.005
	p			.928	.898	.291	.162	.320	.459	.834	.983
Gravida	r			1	.781**	-.306	-.135	-.324	-.333	-.304	-.318
	p				.000	.189	.569	.163	.152	.193	.171
Parity	r				1	-.607**	-.391	-.439	-.419	-.378	-.378
	p					.005	.089	.043	.036	.101	.101
Weight	r					1	.720**	.042	-.047	.146	.031
	p					.	.000	.828	.812	.538	.897
BMI	r							1	-.215	-.154	-.189
	p								.363	.517	.425

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

the MCA, DA and UmB RI and PI ratio below 1 is determinative for the diagnosis of IUGR.

There was no statistically significant relationship between the two groups in terms of weight and cord blood pH values of the newborns (p>0.05) (Table 5).

There was no statistically significant relationship between the groups in terms of 1st and 5th minute apgar scores and NICU needs of newborns (p>0.05) (Table 6).

A statistically significant relationship was found between MCA PI/DA PI value with cord blood pH value and NICU requirement (p<0.05) (Table 7).

Regression analysis was used to compare the age, time of birth, gravida, weight, BMI and parity values of the IUGR group with MCA, IA and Umb RI and PI values. Accordingly, statistically significant results were found between age with gravida, parity, MCA RI/Umb RI and MCA PI/UmB PI, gravida with age and parity, parity with age, gravida, weight, MCA RI/DA RI and MCA PI/DA PI, weight with BMI (Table 8).

Discussion

IUGR is a fetal development disorder in which the expected fetus weight for gestational age is below the 10th percentile.[17] Most cases of IUGR are caused by primary or secondary utero-placental circulation failure. Inadequate nutrient and oxygen transfer from the placenta to the fetus, chromosomal abnormalities and intrauterine infections cause small fetal size.[18]

Doppler US is currently used routinely in the diagnosis and follow-up of fetal and maternal vessels. Growth and development in the fetus is ensured by normal fetomaternal circulation which provides sufficient oxygen and nutrient intake as a result of maternal circulation. Doppler US provides rapid and reliable noninvasive evaluation of physiopathological changes in fetomaternal circulation.[19,20]

There are some maternal demographic factors affecting IUGR formation. Physiological variables including maternal height, weight, parity, ethnicity, fetal gender and gestational age are known to affect fetal growth. One of the most important of these is maternal age. Maternal age is too young or too old to be a high risk factor for IUGR.[21,22] Similarly, advanced maternal age was associated with low birth weight.[21] In our study, mean maternal age of the patients in the IUGR group was 28.15±5.19 years and the mean maternal age of the patients in the control group was 28.91±5.31 years. In addition, a significant relationship was found between maternal age and gravida, parity, MCA RI/Umb RI and MCA PI/UmB PI.

Maternal weight at birth, low weight before pregnancy and poor weight gain during pregnancy are associated with IUGR.[23] In our study, the mean maternal weight of IUGR group was 70.11±9.75 kg and the mean maternal weight of control group was 79.57±16.14 kg. A statistically significant relationship was found between the groups in terms of maternal weight (p<0.05).

MCA is preferred in the evaluation of fetal cerebral circulation because it carries more than 80% of cerebral blood flow and is the most easily visualized sonographic vascular structure. Doppler flow sampling should be done from the proximal segment close to the Willis polygon. Middle cerebral artery PI reference values vary according to gestational week.[24] Under normal conditions, high resistance current wave pattern is observed in fetal cerebral circulation. In the presence of fetal hypoxia, peripheral blood circulation decreases and brain blood flow increases. In this case known as brain-protective effect, the diastolic flow rate increases and the PI value decreases. Presence of brain protective effect is determined by cerebroplacental ratio expressed as MCA PI/UmB PI ratio. If this ratio is below the 5th percentile according to gestational age, it shows that there is brain protective effect in fetus.[25] In our study, no statistically significant relationship was found between the groups in terms of MCA RI/AO, MCA PI/AO PI, MCA RI/Umb RI and MCA PI/UmB PI (p>0.05).

Blood perfusion of fetoplacental unit is evaluated by UmB Doppler sampling. End-diastolic flow is usually absent in the first trimester. As the gestational week progresses, placental vascular resistance decreases, the diastolic component increases, and from the 14th week, low-resistance current wave begins to be seen in UmB. The UmB current is best analyzed at the level of the free-floating umbilical cord. Quantitative Doppler US parameters such as S / D ratio, PI and RI are sufficient in the evaluation and follow-up of most cases with IUGR. The use of Umb Doppler US examination in the second trimester in the follow-up of high-risk cases significantly reduces the risk of perinatal death.[26] Diastolic flow loss in UmB reflects increased placental resistance from obliteration of placental villi, and reverse flow in Doppler US can be seen after 70% of placental villi occlusion.[27] Progressive hypoxemia may cause redistribution of blood flow to the brain, heart, and adrenals, and leads to increased diastolic flow in MCA.[28,29] DV Doppler US waveform may reflect diastolic pressure due to increased cardiac output in the right ventricle.[5] In our study, the mean MCA RI/Umb RI was 1.25± 0.19 and the MCA PI/UmB PI was 1.61±0.49 in the IUGR group, and the mean MCA RI/Umb RI was 1.32±0.22 and the mean MCA PI/UmB was 1.84±0.54 in the control group. There was no significant relationship between the groups (p>0.05). In the study of Seyam et al., 100 pregnant women in 28-41 week with IUGR fetus were followed up, serial Doppler US was performed and were examined in 3 groups. Patients with normal Umb Doppler US findings were classified as group 1 (16%), group 2 (77%) with diastolic flow loss, and group 3 (7%) with reverse flow. From group 1 to 3, gestational age and birth

weight at birth were significantly lower, and oligohydramnios and NICU hospitalization were higher. There was no perinatal death in Group 1. From group 1 to 3, the rate of cesarean section due to fetal stress increased and the need for mechanical ventilation increased.[30]

In our study, cord blood pH was 7.32±0.06 and 7.33±0.041 in IUGR and control groups, respectively. Yoon et al. used UmB Doppler US and biophysical profile (BPP) to determine fetal acid-base status in 24 fetuses with IUGR.[31]They found that Doppler US and BPP correlated with the degree of fetal acidemia, and Doppler US was more effective than BPP in detecting fetal acidemia.

Fetal PI is calculated sonographically using EFW and FL measurement. This index is more useful for diagnosing IUGR in the newborn. However, the correlations between fetal PI and neonatal PI are weak. Therefore, PI provides limited benefit during pregnancy.[32,33]Another parameter is the transcerebellar diameter (TCD) which is not affected by IUGR and can therefore be used independently.[34] It has been shown that the TCD/AC ratio predicts IUGR if the mean is above 2 SD, but this ratio is not routinely used until further studies have proven it.

Sensitivity of the relationship between abnormal PI and IUGR in UmB Doppler is around 12% in the first trimester.[34] Cnossen et al. examined datas in 79547 pregnant with PE and 41131 fetuses with IUGR.[35] They reported that, UmB Doppler US performed in the second trimester shows a higher estimate than it performed in first trimester, and increased PI findings are more accurate. In addition, PI increase is the best predictor of general and severe IUGR for low-risk patients. In our study, no statistically significant relationship was found between the groups in terms of MCA PI/AO PI and MCA PI/UmB PI ($p>0.05$).

Conclusion

MCA/AO Doppler US parameter has a positive predictive value in predicting neonatal outcomes in patients with IUGR born at 34 weeks of gestation or over. In addition, significant correlations were found between MCA PI/DO PI with cord blood pH and NICU requirement. Therefore, both Doppler US findings and the patient's general condition and additional clinical findings should be taken into consideration when making a delivery decision. Doppler US plays an important role in accurate timing of delivery and predicting neonatal outcomes.

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ORIGINAL PAPER

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Comparative efficacy of kneading massage and pulsed mode ultrasound in the management of chronic knee osteoarthritis

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ABSTRACT

Introduction. Osteoarthritis of the knee is the most common presentation of osteoarthritis with prevalence between 12% and 35% of general population and is considered the leading cause of musculoskeletal disability in the elderly population worldwide.

Aim. The study compared efficacy of kneading massage and pulsed ultrasound on pain, joint stiffness and difficulty in knee osteoarthritis (KOA).

Material and methods. Fifty subjects with radiological evidence of KOA participated in the study. They were randomly allocated into kneading massage group (KMG) (25) and Ultrasound group (USG) (25). KMG received kneading massage for 7 minutes while USG received pulsed mode ultrasound for 15 minutes. Treatment was twice in a week for six weeks. Pain intensity (PI), joint stiffness and difficulty were assessed pre, 3rd and 6th weeks of treatment session with semantic differential scale and WOMAC. The data were analyzed using descriptive and inferential statistics, alpha level was set at 0.05

Results. There was a significant difference in present PI (F=11.45,P=0.001) and stiffness (F= 11.32, P=0.003) in USG. There was a significant reduction in PI (F=7.95, P=0.001) and joint stiffness (F=8.86, P=0.003) in KMG. At the 6th week, there was a significant differences in PI (t=12.23,P=0.000) and stiffness (t=8.08, P=0.000) when USG (3.00$\pm$0.4, vs 7.14$\pm$ 1.49) was compared with KMG (3.16$\pm$0.5 vs 7.50$\pm$1.5).

Conclusion. Ultrasound (US) and kneading massage (KM) reduced PI and joint stiffness of KOA effectively; however US reduced PI than KM while KM reduces joint stiffness than US.

Keywords. kneading massage, knee osteoarthritis, pulsed ultrasounds

Introduction

Osteoarthritis (OA) of the knee is the most common presentation of osteoarthritis with prevalence between 12% and 35% of general population and is considered the leading cause of musculoskeletal disability in the elderly population worldwide.[1,2] A study conducted by Deshpande reported that about fourteen million persons had symptomatic knee OA, with advanced OA comprising over half of those cases.[3] The study mentioned further that this includes over three million African American, Hispanic, and other racial/ethnic minorities and over half of all persons with symptomatic knee OA are younger than 65 years of age. Kevin et al reported that chronic knee OA is characterized by an insidious onset of pain and progres-

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sive limited range of motion with the symptoms worsen over time, typically in a stepwise fashion.[4] Patients note symptoms with ambulation, transfers, and ascending stairs. Knee stiffness or "locking" secondary to the formation of loose bodies, degenerative meniscal tears, and osteophyte formation may occur.[5] These can significantly increase pain severity, hinder activities of daily living, and reduce quality of life.[4]

Globally approximately 250 million people have osteoarthritis of the knee equivalent to 3.6% of population.[3] Symptomatic knee OA occur in 10% men and 13% in women age 60years or older.[6] There is also an evidence that it is an inevitable part of ageing.[7] Moreover community-based prevalence estimation of OA in Nigeria is not readily available for referencing. A study conducted by Akinpelu et al. investigated the prevalence and pattern of knee OA in Igbo-Ora a rural community in southwestern Nigeria.[8] They found out that the prevalence of knee OA among people of Igbo-Ora was 21.4% among female and 17.5% among male giving a female a bias ratio of 1.2:1.They concluded that one out of five adult age 40years and above in the Nigeria rural community has symptomatic knee OA with female preponderance in the ratio of 1.2:1. Ojoawo et al., also documented that prevalence of knee OA is high in urban community and with female more affected than male.[9]

Physiotherapy is recommended in the management of symptomatic knee OA and therapeutic ultrasound is one of the most physical modality used in many countries.[10,11] Therapeutic ultrasound is based on the application of high frequency sound wave to the tissue of the body in order to generate mechanical and thermal effect.[12]This effect is aimed to enhance soft tissue healing, decrease inflammatory response, increase blood flow, metabolism and decrease pain.[13] Therapeutic ultrasound has frequency range of 0.8-3.0 MHz with most machines set at frequency of 1-3MHz. Low frequency ultrasound waves have greater depth penetration but are less focused. Ultrasound at frequency of 1MHz is absorbed primarily by tissue at a depth of 3-5cm and is recommended in deeper injuries and patients with more subcutaneous fat.[14] A frequency of 3MHz is recommended for more superficial lesion at the depth of 1-2cm.[15] A typical ultrasound treatment takes 3-5 mins depending on the size of the area under treatment. Because of low quality of evidence, the magnitude effect of therapeutic ultrasound on pain relief and physical function is uncertain, however it is widely used for it potential benefits of both knee pain and function.[16]

Massage therapy is another physical modality used in physiotherapy and has become one of most complementary use health in United State.[17] Massage is an age-old process that involves stimulation of tissue by rhythmically applying both stretching and pressure.[18] Massage are of different types and each type is indicated for a particular condition, this include efflurage, frictional, patrissage and percussion. Petrissage massage includes the following categories: wringing, rolling, kneading etc.[19] Kneading massage is indicated in topical drugs delivery. It is done by pulling the muscle from the bone and release it intermittently.[20]

Therapeutic ultrasound is widely used in clinical setting for the management of various ailments including chronic knee OA.[21] The usage of kneading massage especially for deep seated joints has been a major practice for almost all patients that presented with chronic pain among physiotherapists. However the efficacy of kneading massage on pain, joint stiffness and physical function has not been well investigated especially among patients with knee osteoarthritis. Hence this study compared the efficacy of kneading massage and pulse mode ultrasound on pain, joint stiffness and physical function in chronic knee OA.

Aim

The study compared the effects of US and Kneading massage on pain intensity, stiffness, difficulty of patients with knee osteoarthritis

Material and methods

Subjects

The approval of the Research and Ethic Committee from Institute of Public Health, Obafemi Awolowo University, Ile-Ife Nigeria was obtained for this study (HREC No: IPHOAU/12/780). The pretest and post-test Quasi experimental design recruited patients with chronic knee osteoarthritis attending Out-patient Physiotherapy Clinic at the Department of Physiotherapy of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife. They were referred from the Orthopaedic clinic of the same hospital. Each subject had symptoms and radiographic evidences of osteoarthritis of the knee. The inclusion criterial includes patients with chronic knee osteoarthritis (with duration of onset more than 3 months), with radiological report confirming knee OA and having or combination of these signs: pain, early morning stiffness, reduced range of motion, muscle weakness, cracking and creaking with Kellgren Lawrence classification ranging from II to III. Subjects with acute inflammation, metallic implant, septic arthritis, infection and malignancy were excluded from the study. Purposive sample technique was used to recruit 50 patients with chronic knee OA using this formula for a study comparing two means according to Eng (25 participants for group US and 25 participants for group KM).[22]

Research protocol

Subjects' age, sex, weight height and body mass index were recorded before the treatment. The purpose and the procedure of the research was explained to each

subject and their consent was obtained before they participated in the research. The instruments used for the study includes therapeutic ultrasound machine (US); Sonopuls 490s made in china with an output for either continuous or pulsated mode and frequencies of 0.7-3.3MHz. It has heads of various sizes but the 5cm^2 head was used for the pulsed mode. The pulse ratio and the modulation of the equipment was 1:4 and 10% respectively according to the manufacturer). Semantic differential scale (10 point scale): This scale has a range from 0-10. Zero (0) indicates the absence of pain, 5 indicates moderate pain level while 10 indicates the presence of highest level of pain. This was used for the study examine the pain intensity of the patients

Another instrument was WOMAC (Western Ontario Mcmaster Osteoarthritis Index): A widely used, proprietary questionnaires used by health professionals to evaluate the condition of patients with osteoarthritis of the knee and hip, including pain, stiffness and difficulty. The WOMAC measures five items for pain (score range 0-20), two for stiffness (score range 0-8), and 17 for difficulty (score range 0-68).[23] Difficulty questions cover everyday activities such as stair use, standing up from sitting or lying position, standing, bending, walking, getting in and out of a car, shopping, putting on or taking off socks, lying in bed, getting in and out of birth, sitting.[24]

Subjects were randomized into two groups of USG and KMG, using simple randomization of fish and bowl method - an envelope which contains 50 small papers was made. Twenty five was inscribed with kneading massage group and 25 with ultrasound group. Each participant was asked to pick a paper from the envelope, and all participants that picked kneading massage group was assigned as experimental group while participants that picked ultrasound was grouped as control group (Fig 1). The intervention was carried out twice in a week for six weeks making 12 sessions of treatment for each subject.

Kneading massage group received kneading massage with cold topical gel. Kneading massage was administered as follows: Muscles of the quadriceps and patellar tendon were held, lifted up, rolled and squeezed in a compressive action using cold topical gel as coupling medium. The techniques was applied to the muscles of the popliteal region as well. The underline muscles were well compressed with deep pressure. Each maneuver took 7 minutes. It was repeated twice with a resting period of 3 minutes for a session.

The USG received pulsed mode ultrasound machine (Sonopuls 490s), with frequency of 1MHz and intensity of 1.5w/cm for 15mins according to Ebadi et al.[25] Each participant in this group was treated in prone position with rhythmic circular movement of transducer over the knee at popliteal region. Ultrasonic gel (cold topical gel) was used as medium to prevent friction and to enhance transmission of sound wave.

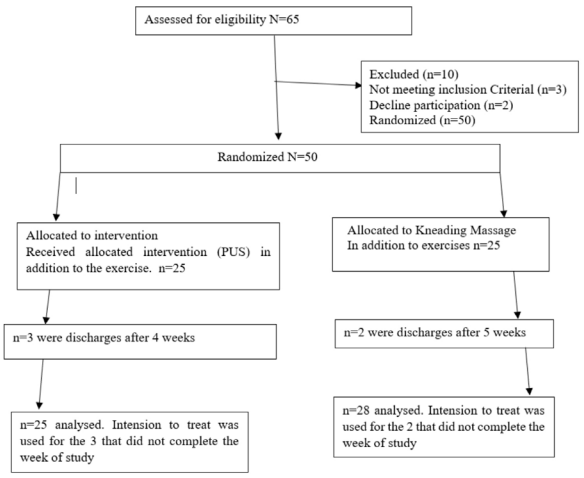

Fig. 1. Consort diagram of random allocation of subjects into 2 groups

As a base line intervention, subjects in both groups performed isometric muscle contraction with principle of overloading according to ACSM in order to strengthen the quadriceps muscles.[26] Treatment was performed twice in a week for six weeks.

Post Intervention assessment

Pain (pain during activity and present pain intensity), joint stiffness and difficulty was recorded at pre-treatment, 3rd and 6th week. All outcome measures were assessed by the researcher before the treatment and every week of treatment. Values of outcome measures for pre-treatment, 3rd and 6th week were used for the analysis. Present pain intensity of participants in both groups was rated on a 10 points semantic differential scale where patients touched the level of his/her pain on the scale. WOMAC was used to measure pain during activity, joint stiffness and difficulty of the knee joint. Each subjects was given a copy of WOMAC to mark the corresponding degree of pain during activity, joint stiffness and difficulty during physical activities. The level of pain, stiffness and difficulty before the treatment, 3rd week and 6th week of the treatment were recorded.

Data Analysis

Statistical Package for Social Sciences (SPSS 17) was used to analyze the data. Descriptive statistics was used to summarize the data while inferential statistics were used to determine the significance. Repeated measure ANOVA was used to compare the mean values of pre-treatment, 3rd week and 6th week treatment of present pain intensity, pain during physical activity, stiffness and difficulty within the two groups. Independent-t-test was used to compare the 6th week treatment of present pain intensity, pain during physical activity, stiffness and difficulty scores of US group and KM group. An alpha level of <0.05 is set as significant level.

Results

Physical characteristics of all the subjects for the two groups were shown in Figure 2. The mean age for USG is 64.14±8.3years and the mean age for KMG is 64.83±8.8years while the mean age for the total subjects is 64.46±8.2 years in the two groups.

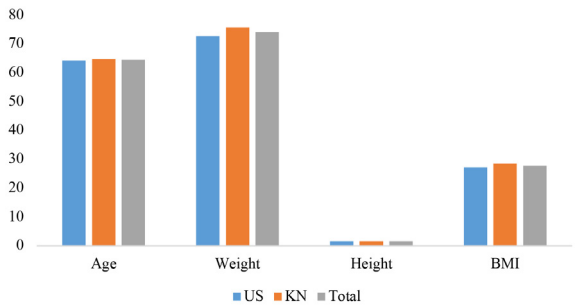

Fig. 2. Physical characteristics of participants

Revealed in the table 1 is the summary of Repeated Measure ANOVA comparing outcome measures for pretreatment, 3rd week and 6th week of USG.

Table 1. Summary of repeated measured of ANOVA comparing pre-treatment, 3rd week and 6th week of outcome measures using USG 25

Variables	US GROUP Mean±SD	F	P
PI			
PRE	11.28±1.9		
3rd	8.00±1.7	11.10	0.001
6th	7.14±1.4		
Sti			
PRE	5.00±1.0		
3rd	3.71±0.7	11.32	0.001
6th	3.00±0.5		
Diff			
PRE	31.71±7.2		
3rd	26.28±6.6	2.55	0.106
6th	23.57±6.7		
Tw			
PRE	48.00±9.2		
3rd	37.71±7.5	5.73	0.012
6th	33.71±7.4		
PP			
PRE	5.85±0.6		
3rd	4.28±0.7	20.11	0.000
6th	3.71±0.4		

Key: PI=Pain during activities, Sti=Stiffness, Diff=Difficulty, Tw = Total WOMAC, PP=Present pain intensity, USG=Ultrasound

It was observed that there were significant difference in pain during activities (F=11.10, P=0.001), stiffness (F=11.32, P=0.001), present pain intensity (F=20.11, P=0.00) and total WOMAC (F=5.73, P=0.012) but there was no significant difference in difficulty (F=2.55, P=0.106). It was observed that there were significant difference in pain during activities (F=8.67, P=0.003), stiffness (F=8.86, P=0.003), present pain intensity (F=11.41, P=0.001) and total WOMAC (F=3.89, P=0.043) but there was no significant difference in difficulty (F=1.54, P=0.245). This was shown in table 2.

Table 2. Summary of repeated measured of ANOVA comparing pre-treatment, 3rd week and 6th week of outcome measures using kneading massage

Variables	KMG Mean±SD	F	P
PI			
PRE	11.33±2.1		
3rd	8.33±1.2	8.67	0.003
6th	7.50±1.5		
Sti			
PRE	5.16±0.9		
3rd	3.66±1.0	8.86	0.003
6th	3.16±0.4		
Diff			
PRE	32.16±7.7		
3rd	27.66±6.3	1.54	0.24
6th	25.16±6.7		
Tw			
PRE	48.66±9.9		
3rd	39.33±6.8	3.89	0.043
6th	36.33±6.6		
PP			
PRE	5.83±0.7		
3rd	4.00±0.8	11.45	0.001
6th	4.00±0.6		

Key: PI=pain during activities, Sti=Stiffness, Diff=Difficulty, Tw = Total WOMAC, massage PP=present pain intensity, KMG=Kneading

Table 3. Summary of Independent t-test comparing the 6th week of outcome measures between USG and KMG

Variables	US GROUP Mean±SD	K.M GROUP Mean±SD	t	P
PI	7.14±1.4	7.50±1.5	7.95	0.000
Sti	3.16±0.5	3.00±0.4	8.08	0.000
Diff	23.57±6.7	25.16±6.7	1.54	0.245
Tw	33.71±7.4	36.33±6.6 e	3.94	0.007
PP	3.71±0.4	4.00±0.6	12.23	0.000

Key: PI=Pain during activities ,Sti=Stiffness, Diff=Difficulty, Tw = Total WOMAC, PP=Present pain intensity, KMG =Kneading massage, USG=Ultrasound

There were significant differences in pain during activities (F=7.95, P=0.000), stiffness (F=8.08, P=0.000), total WOMAC (F=3.94, P=0.007), and present pain intensity (F=12.23,P=0.000) but there was no significant difference in difficulty (F=1.54, P=0.245). when the 6th week values of outcome measures were compared between US group and KM group as presented in table 3.

Discussion

This study was conducted to compare the efficacy of ultrasound and kneading massage in the management of present pain intensity, joint stiffness and difficulty in patient with chronic knee OA. In this study, it was found that majority of subjects that participated were females. It might indicates that knee OA may be commoner among females than male. This is in consistent with the study conducted by Maradit et al, where they reported that OA of knee are more common among females than males.[27]

There was a significant difference in present pain intensity and pain during activity between pre-treatment, 3rd and 6th weeks of treatment in both ultrasound and kneading massage group. However, improvement in pain is more pronounced in patients with pulsed ultrasound which was in agreement with the findings of Yegin et al.[28] The improvement may be due to the process of phonophoresis characterized physiological effect of pulsed US. Pulsed ultrasound has been reported to be used as transdermal delivery of lower molecular weight drug in phonophoresis.[29] The effect of US includes acoustic streaming and cavitation which are more important in treatment of soft tissue lesion to driving topical gel used as medium. A mixture of sound wave, continuous flow and disturbances of cell fluid which is very hard to delineate are usually created by therapeutic US.[30] Also, a term called acoustic is created by US, this is a physical forces of the sound waves which moves ions and small molecules from one place to the other within the tissues.[29] This is the way in which phonophoresis takes place in the cell. Within the cells, there are different size of small organs and molecules, some are on the same sport, while others may be in the interstitial fluid floating which may be forced to move round those at the same sport. The pushing force produced by the wave creates a streamline motion of fluid in the line of the mechanical pressure and around cell membrane this is called continuous flow or streaming.[29] Another effect of US is disturbance of cell fluid called cavitation, this means in the minute surrounding of each cell, there are accumulation of fluid which the sound wave is forcing to vibrate.[30] The movement of the wave of the sound in the fluid around the cells, there is a feature of thinning and size reduction, this enables the minutes thin globe-shaped air filled the film in the tissues to increase in size and to shrink; this process may result into injury to cell structure.[30] The process of cavitation, may be used to explain how US wave can drive methyl salicylate and diclofenac into the deeper tissues of the knee the site of the pain. This probably make US to be more effective than kneading massage.

There was also improvement in pain intensity among patients with kneading massage which supported the work of Atkins et al.[31] Atkins et al., reported that intervention using self-message therapy is highly beneficial to patient with KOA.[31] Literature have reported that massage breaks the cycle of pain, functioning is improved, edema is reduced, it promotes relaxation and healing of many medical conditions is facilitated.[31]

There was also reduction in joint stiffness in both kneading massage and ultrasound group, but improvement is more significant in kneading massage group than ultrasound. This might be due to mechanical effect of kneading massage which was in tandem with the study conducted by Weerapong et al.[32] Their study reported that kneading massage can help to stretch tight muscle and break cycle of pain, stress and depression that accompanied chronic illness. The more reason why kneading massage reduces the joint stiffness of KOA patient. Literature also reported that massage will; make patients to have more sense of relaxation, activate parasympathetic nervous system, reduce heart rate and blood pressure; there is also changes in the activation of the brain cells for which there is reduction in anxiety.[33]

Limitation of the study

Exercise was the base line for subjects in the two groups. This might have contributed to the outcomes of the study. Further study may explore additional group without exercises.

Conclusion

It could be concluded from the study that Ultrasound and KM reduced pain intensity and joint stiffness of KOA effectively; however US reduced pain intensity more than KM while kneading massage reduces joint stiffness better than US.

Recommendations

It is suggested that both pulsed mode ultrasound and kneading massage could be employed as adjunct in the management of chronic knee OA for pain relief and reduction of joint stiffness. These should be incorporated with therapeutic exercises to improve physical functions.

Limitation of the study: There are some limitations with respect to the study. The study was unable to cover the long term effect of the pulse ultrasound and kneading massage. Can the patients sustain the pain reduction after few weeks or months after the intervention? More so, the outcome measures used were self-reported. It is assumed that the patients reported actual feeling of the pain, stiffness and difficulty during the data collection.

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ORIGINAL PAPER

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Low generation polyamidoamine dendrimers (PAMAM) and biotin-PAMAM conjugate – the detailed structural studies by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy

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ABSTRACT

Introduction. The concept of targeted drug delivery is nowadays based on nanoparticle transporters. Such drug delivery systems for cancer cells should follow the requirements like: efficient drug release, selective binding and internalization to cancer cells. The anticancer drug selectivity can be achieved by attachment of cancer cell-recognizing molecules, like biotin. Among nanosized carriers the PAMAM dendrimers are tested intensely, especially they can be modified by covalent attachment of prodrug molecules and biotin as targeting molecule.

Aim. We aimed at construction and characterization of a conjugate formed between PAMAM and biotin (Biot). The nuclear magnetic resonances is powerful tool to determine both the structure and stoichiometry of the conjugate.

Material and methods. PAMAM G0 has been synthesized and functionalized with biotin by reaction with N-hydroxysuccinimide ester of biotin to obtain G0 double-substituted with biotin. All the compound were thoroughly characterized by the NMR spectroscopy.

Results. The conjugate of PAMAM G0 dendrimer with two amide-bonded biotin molecules was obtained and fully characterized by NMR spectroscopy.

Conclusion. N-hydroxysuccinimide ester of biotin spontaneously reacts with PAMAM G0 to obtain the conjugate of 2:1 biotin:G0 stoichiometry. The latter was designed as a targeting molecule in formation of megameric multidrug delivery system.

Keywords. biotin conjugate, nuclear magnetic resonance, polyamidoamine dendrimer

Introduction

Based on WHO data from 2018 cancer was second major cause of death worldwide leading to nearly 9.6 million deaths. Afterwards being leading cause of death cancer is also remarkably harmful to global economy. Direct medical costs for cancer in United States was estimated to $80.2 billion in 2015 which shows range of a problem.[1,2]

Systemic chemotherapy is one of the main approaches in treatment of cancer. In spite of its utility there are some limitations in efficacy of conventional chemotherapy caused by unsatisfying solubility of che-

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motherapeutics, lack of adequate selectivity of therapy, low membrane permeability of drugs and multidrug resistance of cancer cells. To overcome this challenges, conceptions of using nanomaterials as a drug carriers have emerged.[3,4]

Poly(amidoamine) dendrimers are class of hyperbranched nanopolymers tested as potential carriers for drug delivery. PAMAM are characterized by having ethylene diamine core and alkylamido structure of branches terminated by amine groups in case of full generation and carboxyl groups in half generation.[5] Number of terminal groups in full generation PAMAMs doubles with every next generation starting from 8 for generation zero (G0). The main advantages of PAMAM dendrimers are their good water solubility, flexibility, relatively low cytotoxicity and rapid cellular uptake. These properties of PAMAM are used to increase drug bioavailability by conjugation with dendrimers or drug encapsulation. It was shown that conjugation with PAMAM significantly increased water solubility and transepithelial transport of propranolol and naproxen (poorly water-soluble drugs). Moreover, multitude of terminal groups of PAMAM provides an opportunity to conjugate them with multidrug combination or functionalize with other compound such as α-*D*-glucoheptono-1,4-lactone to decrease their toxicity which otherwise is stimulated by free terminal amine groups.[5,6]

There are evidences that some cancer cells overexpress sodium-dependent multivitamin transporter (SMVT) which is responsible for biotin, pantothenic acid and lipoic acid cellular uptake. From certain point of view it also plays a role of biotin receptor. It was demonstrated by *Vadlapudi et al.* that expression of SMVT in T47D breast cancer cell line is significantly higher in comparison to normal mammary epithelial cells (MCF-12A).[7-9]

Overexpression of biotin receptors by specific cancer cells offered the opportunity to increase selectivity of nanoparticle drug delivery systems by biotinylation of nanocarriers. In case of dendrimers such approach seems to be reasonable as enhanced cellular uptake of biotinylated dendrimer conjugates and less cytotoxicity for normal cells in comparison to specific cancer cells lines were shown in several studies.[10-13]

Aim

The aim of our studies was to synthesize biotinylated G0 derivative and then characterize it using NMR spectroscopy. Biotinylated G0 will be used further as substrate in synthesis of megamer of mixed generations G3-G0 PAMAM dendrimers which will be covalently conjugated with anticancer drugs. Such nanocarrier-attached multidrugs will be evaluated *in vitro* studies. We assume that presence of biotinylated G0 in structure of megamer will provide a significant preference in targeting cancer cells overexpressing biotin receptors.

Material and methods

Materials

Solvents, NHS-biotin, ethylenediamine (en), methylacrylate were purchased from Merck (KGaA, Darmstadt, Germany). The polyamidoamine dendrimers of low generation; $G_{-0,5}$ and G_0 were synthesized accordingly to the published procedure.[14]

Synthesis of biotinylated G_0 Dendrimer

Solid biotin N-hydroxysuccinimide ester (NHS-biotin, 690 mg, 2021μmoles) was added in portions to G_0 solution in MeOH (4 ml, 1272 mg, 2460 μmoles) with magnetic stirring. Then 4 ml MeOH and 4 ml of water were added and stirring continued until all reagents dissolved. After 7 hrs gel precipitate was formed which left at 5 °C overnight. The precipitate was filtered off and washed with 30 ml of cold water and dried 12 hours under vacuum. The compound was identified as G_0 substituted with two biotin equivalents (G_0-Biot) by ^{1}H NMR spectrum in dmso-d_6. Yield: 440 mg (45 %). The remaining part of biotin substrate was recovered and identified as G_0 – monosubstituted with biotin contaminated with unreacted G_0.

NMR Spectroscopy

The 1-D ^{1}H and ^{13}C NMR spectra as well as 2-D ^{1}H-^{1}H correlations spectroscopy (COSY), ^{1}H-^{13}C heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation spectra (HMBC) were recorded in deuterated water using Bruker 300 MHz (Rheinstetten, Germany) and worked up with TopSpin 3,5 software at College of Natural Sciences, University of Rzeszów.

Results

The ^{1}H NMR spectra of substrates and G_0-Biot

Polyamidoamine dendrimers were synthesized starting from en core. The methyl acrylate was added to en to obtain generation -0,5 dendrimer ($G_{-0,5}$), which was further converted into zero-generation PAMAM dendrimer, G_0. The dendrimer G_0 was substituted with biotin by reaction with N-hydroxysuccinimide ester of biotin (NHS-biotin) to obtain G_0-Biot conjugate. All the compounds were characterized by the ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectra of $G_{-0,5}$, G_0, and G_0-Biot are presented at Figure 1. The ^{1}H NMR spectral assignments of the compounds were performed according to the atom numbering presented at Figure 2. Additionally the 2-D COSY ^{1}H-^{1}H experiments enabled to join the 3d to 4d triplets into pairs for all compounds by scalar coupling peaks (COSY spectrum for G_0-Biot is shown at Figure 3). Thus, the most upfield shifted triplet

in all derivatives was assigned to 4d methylene protons, at: 2.28 for $G_{-0,5}$, 2.18 for G_0, and 2.18 G_0-Biot. Scalar coupled 3d methylene proton triplets were observed at 2.65 for $G_{-0,5}$, 2.62 for G_0, and 2.62 for G_0-Biot. The 4d methylene proton resonances in all cases showed strong long-range coupling (via two bond) with carbon nucleus (5d) next to 4d (full ^{1}H-^{13}C HSQC and HMBC maps for G_0-Biot are presented at Figure 4).

The addition of en into $G_{-0,5}$ led to amine-terminated G_0 dendrimer. In the ^{1}H NMR spectrum of G_0 additional proton multiplets were observed at 2.53 (t) and 3.01 (q) (Figure 1), which showed scalar coupling in COSY spectrum. Additionally, the scalar coupling cross-peak was observed in COSY spectrum of G_0 between 3.01 quartet and NH (6d) triplet at 7.89 ppm (Figure 1).

Similar pattern of ^{1}H resonances was observed in case of G_0-Biot (Figure 1, upper trace). Based on integral intensity of the biotin resonances and 4d resonances, the latter of intensity corresponding to 8H, we determined the level of G_0 substitution with biotin, which was nearly two. This means that two of four terminal amine groups were converted into amide-linked biotin. In fact we have observed three amide proton resonances in the 7.85-8.00 ppm region, from 6d proton of unsubstituted arm, another one from 6d of substituted arm and 9d' from newly formed amide group (for numbering see figure 2). Simultaneously, the 8d methylene protons of substituted arm shifted from 2.53 ppm into 3.05 ppm as could be determined by integral intensity of overlapped resonances from 7d and 8d'. Such a dramatic chemical shift was due to a change of chemical vicinity of the 8d methylene group from amine ended in G_0 to amide ended 8d', thus similar to 7d which is next to amide.

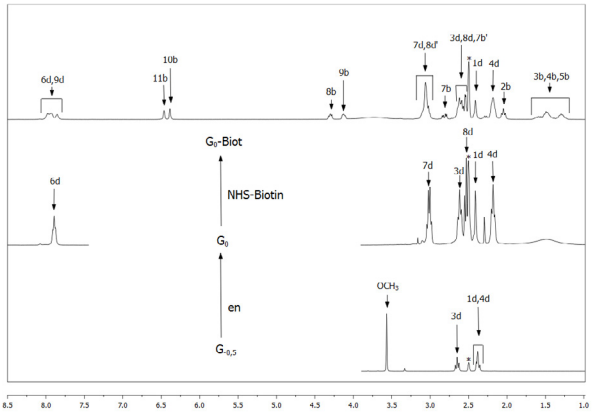

Fig. 1. The relevant fragments of ^{1}H NMR spectra of $G_{-0,5}$ (1), G_0 (2), full spectrum of G_0-Biot (3). The resonances from dendrimer are labeled as d, while resonances of biotin in G_0-Biot are labeled as b and numbered in accordance with figure 2. The residual resonance from dmso-d_6 is labeled with asterisk. The insert illustrates the conversion scheme starting from $G_{-0,5}$ to final G_0-Biot. The structures of compounds are drawn at Figure 2

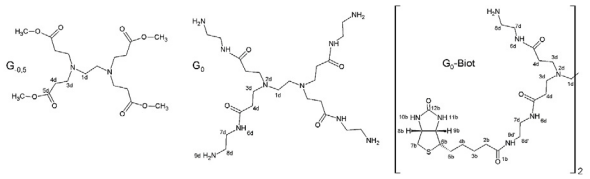

Fig. 2. The formulas of studied compounds with atom numbering.

The homonuclear ^{1}H-^{1}H COSY spectrum is presented at Figure 3. The experiment allowed to assign the resonances of 3d and 4d protons, which are scalar coupled (peak a) and signals of 7d and 8d protons, which are scalar couple (peak b), while 7d and 8d' protons showed scalar coupling with neighboring amide NH protons, namely 6d and 9d (group of cross-peaks labeled as c at Figure 3). The biotin proton cross-peaks are shown in left-upper part of the COSY map at Figure 3. The triplet of 2b methylene proton is coupled (cross-peak e) with 3b methylene protons, while 4d and 5d methylene proton resonances are overlapped within 1.2-1.6 ppm and coupled (groups of d peaks at Figure 3). The resonance of 6b proton is hidden under high-intensity resonance from PAMAM core 7d and 8d' resonances, nevertheless it could be identified through revealed cross-peak f between 6d and 5d protons. Two protons at C-7 are not magnetically equivalent, and the multiplets (dd) of 7d and 7d' are coupled via g cross-peak. The cross-peak j allowed to assign 8b resonance, while coupling between 6b proton and 9b unambiguously assigned the latter by cross-peak h. Consequently the cross-peaks m and n enabled to assign the resonances of 11b and 10b protons coupled with 9b and 8b, respectively. The group of cross-peaks (l) illustrated the three-bond scalar coupling between 7d and 6d of unsubstituted G_0 arm as well as 8d' and newly formed amide proton 9d'.

The ^{13}C NMR spectra of substrates and G_0-Biot

With assigned ^{1}H NMR resonances of G_0-Biot in hand we have performed the 2-D heteronuclear HSQC and HMBC experiments for ^{13}C signal assignments. The combined 2-D map of cross-peaks is presented at Figure 4. The red-yellow contour peaks (obtained in HSQC experiment) correspond to one-bond couplings between hydrogen nuclei and carbon nuclei and are not labeled. They enabled to straightforward assign the ^{13}C resonances of hydrogen-bonded carbon atoms (see Table 1). The magnetization transfer from ^{1}H nuclei to two-bond distant ^{13}C nuclei observed as cross-peaks in the HMBC experiment are shown as black-grey contours at Figure 4. Some of this peaks are crucial for detailed ^{13}C signal assignment. The 12d ^{13}C resonance at 162.5 ppm was then identified by cross-peaks q and s corresponding to magnetization transfer from protons 10b and 11b (q) and from 8b and 9b (s) to 12d carbon nucleus. Another

crucial cross-peaks enabled to assign the amide group carbon resonance 1d (coupled with 2d; cross-peak t), while cross peaks p and r correspond to magnetization transfer from 10b and 11b protons to 8b and 9b carbon nuclei, and from 6d and 9d' protons to 1b carbon nucleus. The entire picture of heteronuclear ^{1}H-H-H

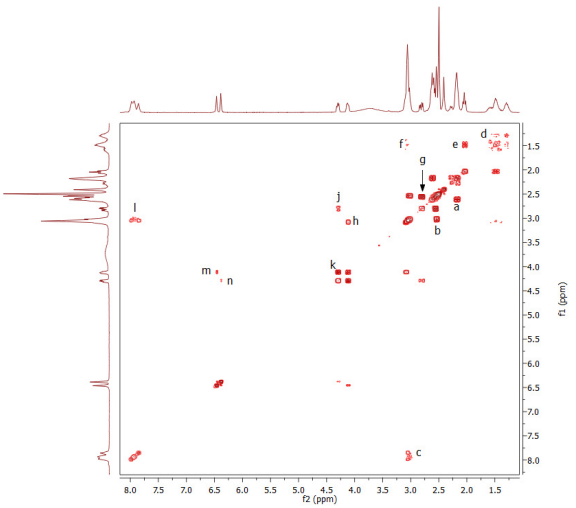

Fig. 3. ^{1}H-^{1}H COSY spectrum of G_0-Biot in dmso-d_6. The relevant off-diagonal peaks are labeled in right-lower part for dendrimer and left-upper part for biotin

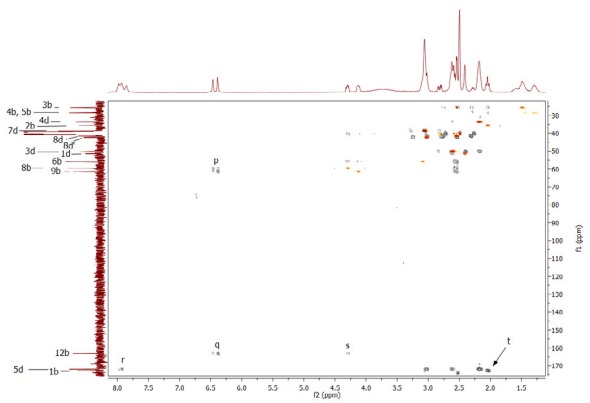

Fig. 4. The combined map of heteronuclear ^{1}H-^{13}C HSQC (red – yellow contours) and HMBC (black – gray)

^{13}C experiments is in precise agreement with ^{1}H spectral assignments described above.

The total assignment of relatively simple molecular system like G_0-Biot is necessary step to construct more composed macromolecules and characterize them structurally by the same set of spectral techniques, which were successfully used here. The ^{1}H and ^{13}C resonance assignments are collected in Table 1.

Discusssion

Despite the starting 1.2:1 G_0:NHS-Biotin molar stoichiometry of the starting mixture, the double-Biotin-substituted dendrimer was the main product isolated from the mixture. We have observed this before for non-steroidal antyinflamatory drug, Nimesulide.[15] It seems that hydrophobic interaction between incoming substituents is a driving force for double substitution instead mono-substitution of G_0. Nonetheless, the isolated conjugate still is equipped with two free amine terminal groups, which can be used for further derivatization.

Table 1. The assignment of ^{1}H and ^{13}C resonances of the conjugate G_0-Biot, G_0 substrate and $G_{-0.5}$ precursor. The upper case letters encounter the cross-peaks observed in HMBC spectrum of G_0-Biot (as specified in Figure 4), which are combining the ^{1}H signals with ^{13}C resonances of two-bond distant carbon-13 nuclei

Compound®	$G_{-0.5}$		G_0		G_0-Biot	
Locant -	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1d	51.8	2.38 (s, [4H])	51.4	2.41 (s, [4H])	51.4	2.42 (s, [4H])
3d	49.7	2.65 (t, [8H])	50.3	2.61 (t, [8H])	50.2	2.61 (t, [8H])
4d	32.5	2.38 (t, [8H])	33.7	2.18 (t, [8H])	33.7	2.18 (t, [8H])
5-OCH$_3$	51.6	3.57(s,[12H])	-	-	-	-
5d	172.9	-	171.8	-	172.0^{r}, 171.9^{r}	-
6d	-	-	-	7.90 (t, [4H)	-	7.93 (bs)^{r}; 7.85 (bs)^{r}
7d			42.7	3.01 (q, [8H])	38.8	3.06
8d			41.8	2.53 (t, [8H])	41.7	2.54
8d′					42.4	3.03
9d′					-	7.98 (bs, [1H])^{r}
1b					172.7^{t}	-
2b					35.8	2.05 (t, [2H])^{t}
3b					25.7	1.44-1.51 (m, [2H])
4b					28.5	1.44-1.57 (m, [2H])
5b					28.7	1.21-1.36 (m, [2H])
6b					55.8	3.06 (m, [2H])
7b, 7b′					40.4	2.81 (dd, [1H]), 2.56 (dd, [1H])
8b					59.7^{p}	4.30 (m, [1H])^{s}
9b					61.6^{p}	4.12 (m, [1H])^{s}
10b					-	6.39 (s, [1H])^{p,q}
11b					-	6.46 (s, [1H])^{p,q}
12b					163.2^{q,s}	-

The detailed NMR spectral characterization of obtained G_0-Biot compound is necessary step to obtain multidrug anticancer conjugates. The conjugates will be obtained as megamers of mixed-generation PAMAM dendrimers. The core dendrimer of third generation G_3^{OH} will be the PAMAM dendrimer totally covered with polyhydroxy substituents. Then terminal hydroxyl groups will be functionalized with NPCF (*para*-nitro-

phenylortochloroformate) and used to bind low-generation G_0 dendrimers containing combination of two anticancer drugs: G_0-nimesulide or G_0-celecoxib and G_0-daunorubicin or G_0-doxorubicine. The third component of the megamer will be G_0-Biot. The latter will play the role of cancer cell targeting molecule. The protocol to obtain such megamer-attached anticancer drug has been already elaborated and published.[15] The megameric conjugates are the macromolecules of an average molecular weight ca 20 kDa which can be characterized by NMR spectroscopy in solution to determine the composition of multicomponent macromolecule. Also the size and zeta potential of the megamers will be determined by Dynamic Light Scattering method in solution.[15] In the solid state Atomic Force Microscopy will be used for imaging the macromolecules deposited on mica, while Differential Scanning Calorimetry will be used to elucidate the flexibility of megameric macromolecules as before.[6,15] Finally, the megameric multidrug associates will be tested *in vitro* on glioblastoma cells and other lines. The PAMAM G3-based multidrug delivery system (MDDS) was already elaborated and applied combination of F-Moc-L-Leu and celecoxib was demonstrated as *in vitro* effective MDDS against glioma.[16] The megameric MDDS is the novel concept which is next to execute. Structurally characterized G0-Biot is part of this MDDS.

Conclusion

Two biotin conjugated with PAMAM dendrimer of generation 0 (G_0-Biot) was characterized by 1-D and 2-D homo- and heteronuclear magnetic resonance spectroscopy. The unambiguous ^{1}H and ^{13}C signal assignments of this molecule will enable to construct high molecular weight associates containing G_0-Biot and combination of anticancer drugs with NMR spectral control of the multicomponent macromolecules composition, which will be used as targeting drug delivery systems.

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ORIGINAL PAPER

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Cervical lymphadenitis as a result of (Hijab) pin prick in north of Jordan

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ABSTRACT

Introduction. Cervical lymph nodes are lymph nodes found in the neck. Hijab is a head cover worn by some Muslim women in the presence of any adult male outside of their immediate family, which usually covers the head, neck and chest. It is strictly forbidden to Muslim woman to unveil any single hair of her head, so they use many pins around the head to fix their Hijab. Often, while using pins they are self-pricked.

Aim. The main aim of our work is to reveal a new cause of lymphadenopathy, which is not known till now.

Material and methods. Retrospective study during the past five years among seventy-five female outpatients, visited our Oral and Maxillofacial clinic in dental department. Our data was collected according to medical history of patients; all female patients with cervical lymphadenopathy were using (A hijab).

Results. Data collected of 75 female patients. Lymphadenopathy causes were various. Most of these causes resulted from nonspecific lymphadenitis (67 patients), 4 tuberculosis, 2 lymphoma, 2 cat scratch disease. Aetiology of 67 nonspecific lymphadenitis was 40 patients of dental cause, 10 of sore throat, 7 of acne vulgaris, 3 of mild facial injuries, and 7 of (Hijab pin pricks).

Conclusion. Hijab pin prick cervical lymphadenitis in Islamic communities is not uncommon and, unexplained cervical lymphadenitis should be considered as potential cause.

Keywords. Hijab, lymphadenitis, tuberculosis

Introduction

Cervical lymph nodes are lymph nodes found in the neck. There is 800 lymph nodes in the human body, 300 of them are in the neck.[1] Cervical lymph nodes are subject to a number of different pathological conditions including tumours, infections and inflammations.[2]

The American Academy of Otolaryngology system (2002) divides the cervical nodes into six levels numbered by Roman numerals I, II, Etc.[3]

Lymphadenitis is enlargement in one or more lymph nodes, usually due to infection. Lymph nodes are filled with white blood cells that help the body fight infec-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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tions. When lymph nodes become infected, it is an indication that an infection started somewhere else in the body. Rarely, lymph nodes can enlarge due to cancer.[4] Lymphadenitis may occur after skin infections or other infections caused by bacteria such as streptococcus or staphylococcus. Sometimes, it results from rare infections such as tuberculosis or cat scratch disease (bacterium *Bartonella henselae*).[5]

Hijab is a veil worn by some Muslim women in the presence of any male outside of their immediate family, which usually covers the head, neck and chest.[6]

It is strictly forbidden to Muslim woman to unveil any single hair of her head, so they use many pins around the head to fix their Hijab. Often, while using pins they are self-pricked. These pins as we know are not sterile or even clean and may cause local infection in the scalp and face, therefore, women start seeking help in our clinic with painful lymphadenitis usually in submental and submandibular region. Most of the patients are cancer phobic and some are as young as 10 years old!

Therapy of cervical lymphadenitis is achieved according to ethology, for nonspecific types we use antibiotic therapy and many times we allow the body to fight infection. For suppurative cases incision and drainage is needed. Surgical excision of the lymph node is often not needed, but only for persistent nodes over one year and for aesthetic reasons, since enlarged inflammatory lymph nodes can persist for many days, weeks, months and even years.[7]

Aim

The main aim of our work is to reveal a new cause of lymphadenopathy, which is not known till now.

Material and methods

Retrospective study, during the past five years from 1.1.2014 to 31.12.2018, seventy-five female out patients visited Oral and Maxillofacial clinic in the dental department of the Princess Basma Teaching Hospital and Al-Yarmuk Teaching Hospital (Irbid city, north of the Hashemite kingdom of Jordan). The annual average number of patients is 1837. Our data was collected according to medical history of the patients. Fine Needle Aspirations (FNA) cytology (Ziehl Neelsen stain), culture and polymerase chain reaction (PCR) were performed to confirm diagnosis. All female patients were using Hijab. Data analysis of the results was processed using the statistical functions of Microsoft Excel.

Results

Data collected of 75 female patients. Lymphadenopathy causes were various. Most of them resulted from nonspecific lymphadenitis 67 patients, 4 patients had tuberculosis diagnosed by histopathology test by detecting chronic granulomatous cells and to be followed by tuberculin skin test, 2 patients had lymphoma, 2 patients had cat scratch disease (Table 1).

Table 1. Aetiology of all cases of lymphadenopathy

	Diagnosis	Number	Percentage
1	Nonspecific lymphadenitis	67	89.33%
2	Tuberculosis	4	5.33%
3	Lymphoma	2	2.66%
4	Cat scratch disease	2	2.66%
	Total	75	100%

The mean was 24.13, standard error 16.09, median 18.75, standard deviation 32.18, sample variance 1035.58, range 65, minimum 2, maximum 67, sum 75, count 75, kurtosis 17.48 and skewness 17.99. 67 nonspecific lymphadenitis patients have age categories as in table 2.

Table 2. Age categories of lymphadenitis

Age (years)	Number of patients	Percentage
10-20	35	52.23%
20-30	15	22.38%
30-40	10	14.92%
40-50	4	5.97%
50-60	2	2.98%
over 60	1	1.49%
Total	67	100%

Aetiology of 67 patients was nonspecific lymphadenitis, 40 patients of dental cause, 10 of sore throat, 7 of acne vulgaris, 3 of mild facial injuries, and 7 of (Hijab pin pricks) - Figure 1.

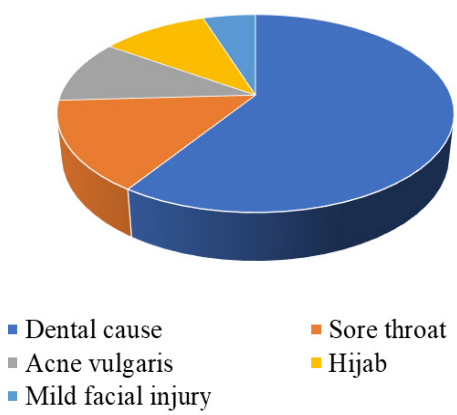

Fig. 1. Ethology of nonspecific lymphadenitis

The mean was 11.17, standard error 5.24, median 7, standard deviation 12.82, sample variance 164.57, range 34, minimum 1, maximum 35, count 67, kurtosis 8.87 and skewness 1.62

Hijab pin-prick patients' total number was 7, most of them were among young age (Table 3).

Table 3. Age categories of hijab patients

Age group by years	Number	Percentage
10y – 20y	4	57.14%
20y – 30y	2	28.57%
over 30y	1	14.29%
total	7	100.00%

It has been noticed low incidence in older ages as shown in table 3.

Therapy of lymphadenopathy was according to aetiology. We treated Hijab pin prick infections with cervical lymphadenitis by cephalosporins of first generation orally, as we treat skin infections, which is most common caused by *Staphylococcus aureus*. All cases cured and lymph nodes enlargement disappeared after few days, however, they can last up to several weeks.

Discussion

This study is the first of its kind, in which lymphadenitis has been associated with hijab pin pricks. Therefore, it is encouraged to continue this research to further obtain insight into this field.

Lymphadenitis has high prevalence in children.[8] The immune system is strong in the first three decades of life. When a juvenile female uses Hijab, it means that she is not well experienced to fix it, that leads to self-pricking more than older women, further juvenile is more sensitive to head and face infections.[8]

Pins used for Hijab purpose are not sterile or even clean. So self-pricking leads often to infection of the inner layers of skin (cellulitis), the bacteria most commonly involved are *Streptococcus ssp.* and *S. aureus*. Physicians must treat it as serious skin infection by antibiotics and to use mild analgesics to relief tenderness of the reactive lymph node in the neck. In children sometimes reactive lymph nodes become suppurative and need incision and drainage.[9]

We used first-generation cephalosporin, because is currently recommended for cellulitis without abscess.[10]

It is highly recommended for physicians working in Islamic countries or Islamic communities in non-Islamic countries to consider the medical challenge. When physician faces unexplained cervical lymphadenitis in female head veiled patient, he should think about self-pricking of Hijab pins.

The biggest problem was to persuade the patients that this painful enlargement of the mass is not a cancer and, when they were told that we must take a biopsy they became more anxious and cancer phobic. Phobia was dismissed when we read the pathological report for FNA (Fine Needle Aspiration), which took two to three weeks to be finalised. It was at this point that we were able to convince the patient that the biopsy was not cancerous.[11]

Conclusion

Hijab pin prick cervical lymphadenitis in Islamic communities is not uncommon and, unexplained cervical lymphadenitis should be considered as potential cause. Treatment of choice is first generation of cephalosporins and if lymph node becomes suppurative, incision and drainage should be performed but it is rare in such conditions.

In the media we recommend awareness campaigns to avoid this phenomenon and cancer phobia of affected females.

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REVIEW PAPER

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Pathophysiological roles of ERα in the ER signaling mediated oncogenesis of breast cancer

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ABSTRACT

Introduction. Estrogen receptors (ER) are members of nuclear receptors that act in the ER signaling pathway regulating the pathophysiology of hormone-responsive target cells including breast tissue.

Aim. This detailled review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Material and methods. This review was performed according to systematic literature search of three major bibliographic databases (Scopus, PubMed, and Cochran).

Analysis of the literature. In this pathway, estrogen receptor alpha (ERα) is a key estradiol-17β (E2) induced transcription factor that has been implicated in the initiation and development of the major fraction of breast cancers. Hence understanding the ERα-mediated ER signaling that results in alterations from normal phenotypic features of breast tissue to the oncogenic features of breast cancer is important. The oncogenic effect of ERα in ER signaling is driven by combinations of molecular assets within the cancer cells. Normally, the transcriptional activity of ERα is controlled by tight regulation of its protein level inside the cells. Altered stability and activity of ERα due to its phosphorylation, ubiquitination, glycosylation, sumoylation, and acetylation events can trigger oncogenic ER signaling.

Conclusion. The function and activity of ERα is also modulated by its interaction with coregulators as well as crosstalk with oncogenic factors from other oncogenic pathways. These all events increase the complexity of the progression of ER+ breast cancer and its response to endocrine therapy.

Keywords. breast, cancer, estrogen receptor alpha, oncogenesis

The list of abbreviations:

AF-1 - activation function 1, **AF-2** - activation function 2, **AIB1** - amplified in breast cancer 1, AIs - aromatase inhibitors, **AP-1** - activator protein 1, **CYP19** - cytochrome p450 family 19, **CYP2D6** - cytochrome p450 family 2 subfamily D member 6, **DBD** - DNA binding domain, **DCIS** - ductal carcinoma in situ, **DNA** - deoxyribose Nucleic Acid, **DR** - drug resistance, **E2** - estradiol-17β, **EGFR** - epidermal growth factor receptor, **ER+** - estrogen receptor positive, **ERE** - Estrogen response element, **ERα** - estrogen receptor alpha, **FISH** - fluorescence in situ hybridization, **GREB1** - growth regulation by es-

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trogen in breast cancer 1, **GSK3** – glycogen synthase kinase 3, **HER2** – human epidermal growth factor receptor 2, **IDC** – invasive ductal carcinoma, **IHC** – immunohistochemistry, **ILC** – invasive lobular carcinoma, **LBD** – ligand binding domain, **LCIS** – lobular carcinoma in situ, **MRI** – magnetic resonance imaging, **NRF-1** – nuclear respiratory factor 1, **NRF2** – Notch, nuclear factor erythroid-derived 2, **PAM50** – prosigna of A 50-gene qPCR assay, **PCR** – polymerase chain reaction, **PEST** – proline (P), glutamic acid (E), serine (S), and threonine (T) region, **PI3K** – phosphatidylinositol 3-kinase, **PIN1** – peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, **PR** – progesterone receptor, **RE** – response element, **RNA** – Ribonucleic acid, **SP1** – Specificity protein 1, **Src** – tyrosine-protein kinase, **TF** – transcription factor

Introduction

Cell signaling is a complex network of the communication process that cells normally use to respond to their microenvironment. Oncogenic signalling that drives oncogenesis happens when cellular signaling interactions and information processing is altered.[1,2] Cytogenetic aberrations in signaling pathways that control cell growth, apoptosis and cell-cycle progression are common hallmarks of cancer. The cancer genome atlas (TCGA) study on the oncogenic signaling pathways in 33 cancer types found alteration of cell cycle, Hippo, Myc, Notch signalling, nuclear factor erythroid-derived 2 (Nrf2), phosphoinositide 3-kinase (PI3K/Akt), receptor tyrosine kinase (RTK-RAS) signalling, tumor growth factor betta (TGFβ) signaling, p53 and Wnt/β-catenin signalling pathways.[3] On top of deranged expression of such potentially oncogenic factors in a given pathway, oncogenic signalling pathway cross-talk is also a common phenomenon as most solid tumors often undergo clonal evolution on top of primary cancer initiating oncogenic mutation.[4] The extent, mechanisms, and patters of co-occurrence of alterations in these pathways differ between individual tumors and tumor subtypes.

ER signalling pathway is important in tissue expressing ER for the normal development of breast tissue. However when this pathway is deranged, it has been implicated to trigger the oncogenesis of breast cancer.[5,6] Due to the central importance of ERα in the pathophysiology of E2 target tissues, understanding of E2-ER signaling events that results in alterations from normal phenotypic features to the oncogenic features of breast cancer is important.

So far there have been many studies on the mechanisms of E2/ERα-mediated breast cancer development where ERα plays critical roles among which epigenetic regulation of ERα expression, altered ERα stability and identification of many of ERα coregulators and their association with breast cancer.[7-21] Moreover, the oncogenic event of ER+ breast cancer has been found to more complex especially when pathway crosstalk happens with other oncogenic signals.[22,23] This implicates that on top of the current ERα targeted endocrine therapy, there is a need of systematic analysis of oncogenic events and the resistance mechanisms of endocrine therapy for such major subtype of breast cancer. Hence, this detailled review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Aim

This detailled review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Breast cancer: Overview

Breast cancer is the most common malignancy as well as the leading cause of cancer death in women with increasing incidence rate all across the world.[24,25] According to global cancer incidence, mortality and prevalence (GLOBOCAN), breast cancer accounts for 25.1% of all cancers with a higher incidence rate in developed countries and relative greater mortality in less developed countries.[26]

Risk factors for breast cancer include being female, menarche at early ages and menopause in old ages, the use of preventive pregnancy hormones, opting not to have children, obesity after menopause, use hormones to prevent pregnancy, physical inactivity and alcohol consumption. In contrast, having children and breastfeeding are preventive factors.[27]

Formation of a lump in the breast tissue is the most common symptom of breast cancer, but symptoms vary in many cases. The other common symptoms of breast cancer include irregular enlargement of the breast, abnormal or bloody discharge from the nipple, dimpling and rash on the nipple. It has been reported that only one in 10 lumps is diagnosed as malignant.[28]

Primary screening for diagnosis of breast cancer commonly includes clinical examination followed by mammography which is specialized medical imaging system that uses a low-dose x-ray to detect breast cancer. Adjunctive screening for breast cancer uses breast ultrasonography and magnetic resonance imaging (MRI) which uses radio waves & magnetic fields to produce detailed images of breast tissue.[29] A biopsy of a small sample of breast tissue or fluid taken from the suspicious area is also analyzed to know the type of breast cancer cells, the grade of cancer as well as the hormone receptor status that can influence the patient treatment options.

Subtypes of breast cancer

The choice and improvement of best diagnosis, prognosis and treatment of breast cancer rely on the knowl-

edge of the clinical heterogeneity, genetic and intrinsic heterogeneity of breast cancer. In this regard breast cancer is classified into multiple subtypes based on the following three major subtyping features. These subtyping features are defined by histological analysis, molecular characterization and functional subtyping of the breast cancers.[30]

Histological subtyping classifies breast cancers based on their histological features and growth patterns. Breast cancer can be broadly categorized into pre-invasive (25%) or invasive carcinoma (75%).[28,29] Pre-invasive carcinoma can then be sub-divided into ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). The invasive carcinomas are more heterogeneous in that they can be categorized as invasive ductal carcinoma (IDC) which is the most common one, invasive lobular carcinoma (ILC), tubular carcinoma, infiltrating ductal carcinoma, mucinous, and medullary carcinoma.[29-31] The traditionally used histological staining like fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) allow us to classify the clinical specimen of breast cancer into ER+, progesterone receptor (PR+), and/or containing an amplification of the human epidermal growth factor receptor 2 (HER2).[32] But it does not segregate the luminal A and B subtype of ER+ cancer that may have a distinct clinical response to the given therapy.[33,34] The emerging needs for personalized therapy and prognostics also require more advanced breast tumor classification with greater diagnostic precision.

Molecular subtyping is used as a compliment to primary screening and histological subtyping, as a prognostic indicator and to inform the choice of therapy. Molecular subtyping of breast tumors means categorizing tumors according to microarray analysis of their gene expression patterns. The two genomic tests are bluePrint/mammaPrint and the prosigna breast cancer prognostic gene signature assay (PAM50 assay). Based on the expression analysis of ER, PR, HER2, Claudin, epidermal growth factor receptor (EGFR), Keratin 5/14, E-cadherin, Vimentin and its major combinations, breast cancers are grouped into four major molecular subtypes. These are Luminal A (ER/RP+ HER2- and low Ki-67 expression), Luminal B (ER/RP+ and HER2+ or HER2- but high Ki-67 expression), HER2-enriched and Basal subtype (triple negative). In addition to these four subtypes, a normal-like, Basal-like and Claudin-low subtype have also been identified.[33-35]

The functional subtyping of breast cancer is an extension of molecular subtyping. It is based on the newly emerging hypothetical concept that the functional outcome of the tumor may depend on the perigenetic alterations present in the tumor-initiating mammary stem cell or progenitor cell being transformed by various oncogenes.[36,37] Molecular features associated with a biological function or clinical outcome of particular subtypes within a given tumor may lead to the heterogeneity of breast carcinoma.[36,38] Hence functional genetic screening is needed to identify genetic signatures that play a critical role for growth and drug response of specific subtype of breast cancer. For example, the patterns of sensitivities to the targetable oncogenes such as PTEN mutation or functional genetic screening of a given kinase inhibitors helps to define the functional viability profiles of breast cancer.[39]

Treatment strategies and endocrine resistance

The treatment plan for breast cancer depends on the biology and behavior of cancer and the status of the patient. As a result of this, treatment recommendations and options are very personalized and many factors are often taken in to account including, the tumor's subtype, stage of the tumor, the presence of an inherited mutation and genomic markers, the patient's general health status, age, menopausal status and patient preferences.

The common breast cancer treatment includes surgery, hormonal therapy, radiation therapy, chemotherapy and targeted therapy. ER and PR are standard biomarkers used in clinical practice to characterize breast cancers. ERα+ breast cancers can be effectively targeted endocrine therapy which includes selective ER modulators such as Tamoxifen, selective ER downregulators such as Fulvestrant and Aromatase inhibitors (AIs). About 30% of cases of ERα+ breast cancers treated with Tamoxifen develop resistance.[40] In some patients, this *de novo* tamoxifen resistance could be culminated by treatment with Fulvestrant and AIs indicating that hormonal-based therapy reduces the recurrence risks and confers survival benefit for ER+ breast cancer.[41-43] However, the risk of disease recurrence albeit 5 years after adjuvant-based tamoxifen treatment is still substantial.[44,45] Activation of an alternative signalling pathway during endocrine therapy remains a challenge as it results in the growth of treatment resistance clones, recurrence of cancer and treatment failure.

So far several acquired endocrine resistance mechanisms have been proposed. Upregulation of the ERα co-regulator Amplified in breast cancer 1 (AIB1) potentiates tamoxifen agonistic effects, especially in the presence of HER2 expression.[46] Phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinases (MAPK) pathways activation by aberrant growth factor signaling have also been implicated in resistance to tamoxifen, as well as AIs.[47-50] Aberrant expression of genes such as c-Myc, BCL2 associated agonist of cell death (BAD) and apoptosis regulator B-cell lymphoma 2 (BCL2) and breast cancer anti-estrogen resistance 3 (BCAR3) have been reported to allow cancer survival and proliferation under endocrine therapy.[51,52] Efficacy of tamoxifen and AIs is also influenced by functional

polymorphisms, with cytochrome p450 family 2 subfamily D member 6 (CYP2D6) and cytochrome p450 family 19 (CYP19), being the most widely studied.[53,54]

Taking the central role of ERα in the tumorigenesis and drug response of breast cancer, understanding the regulation of cellular status of ERα is by far important. Altered ERα expression or mutations which give rise to an active ligand-independent form or epigenetic silencing also contribute to endocrine resistance.[8-12] One of the identified modifications which have been reported to induce resistance to tamoxifen is serine-305 phosphorylation at the hinge region of ERα.[55] ER+ tumours generally respond less well to chemotherapy.[56,57] It was observed that if ERα is reconstituted back to ER-negative cells it became less responsive to chemotherapeutic agents, raising the possibility that ERα modulates chemo-response.[58,59] Since the underlying mechanisms are barely known and likely complex, tamoxifen co-administration with chemotherapy has not proven effective choice to treat cases of primary breast cancer.

Estrogen Receptor Signaling in Normal Human Physiology

Estrogen receptors are members of a large superfamily of nuclear receptors that act as transcription factors. The activity of estrogen receptors is modulated by steroid hormones; hydrophobic hormones generally synthesized from cholesterol in the gonads and adrenal glands. The best-characterized estrogen receptors are those responsible for membrane-initiated estradiol signaling. These include the classical ERα and Estrogen Receptor beta (ERβ) isoforms. ERα and ERβ are encoded by separate genes and each has different and specific roles in mammals.[60-63]

Estrogens are one class of steroid hormones that include estriol, estradiol and estrone. E2, the most potent circulating hormone involved in the detailed array of important physiology. E2 regulates development of reproductive organs, regulation of musculoskeletal, cardiovascular as well as immune system, and homeostasis of the central nervous system.[5,6] ER signaling in normal breast tissue is activated by E2 and it modulates the normal development of the mammary gland. The activity and expression of ERα are tightly controlled at transcriptional and post-translational levels.[64]

E2 can enter cells and interacts with the ER in two ways. Being lipophilic, E2 passes through the plasma membrane of any cell freely and then interact with cytoplasmic ER. E2 can also enter the cell through ER-mediated membrane signaling. Starting from such slightly distinct intracellular localization paths, E2 triggers three major ER signaling events that can follow five interconnected signalling pathways (Figure 1). The three signaling events are ER-mediated membrane signaling event, ER-mediated mitochondrial events and ER-mediated nuclear signaling events.

The plasma membrane-initiated signaling is a rapid and transcriptional-independent E2 signaling event. Following binding of E2 to ER, the membrane-integrated and palmitoylated ER monomer dimerizes as shown on the second (II) signalling pathway in Figure 1 below. Once dimerized, it detaches from the membrane and localizes to the nucleus where it induces nuclear ER signaling. The E2 induced plasma membrane-associated ER dimerization may also allow the recruitment of G protein that can collaterally activate kinases allowing the activation of the tyrosine-protein kinase (Src) and RTK oncogenic signaling pathway.[65-68] ERα palmitoylation was found to be important in this signaling cascade, as mutation of Cystine-451 residue to Alanine within the ligand binding domain (LBD) prevents the trafficking of the receptor to the membrane thereby abrogating nuclear ER-mediated transcriptional events. Introducing this mutation into mice resulted in infertility, abnormal regulation of the pituitary hormone, abnormal ovaries, arrested the development of mammary gland and altered vasculatures.[69,70]

The mitochondria are an important target of ER-mediated E2 action in which both ERα and ERβ can localize to inhibit early stage of apoptosis induced by several stimuli.[71-74] The E2-ER complex alters mitochondrial function by directly acting on mitochondrial deoxyribose nucleic acid (DNA) to induce mitochondrial gene expression; including expression of mitochondrial adenosine triphosphate (ATP) synthase subunits and manganese superoxide dismutase.[75,76] Here the superoxide generated by E2 induced mitochondrial biogenesis is counterbalanced by manganese superoxide dismutase encoded from mitochondrial DNA to prevent apoptosis (Figure 1: signaling pathway V). The E2-induced nuclear receptor signalling can also indirectly act on mitochondria by activating the expression of Nuclear respiratory factor 1 (NRF-1) that can enter into the mitochondrial scaffold to regulate mitochondrial function and maintain cellular integrity.[71,77,78]

Nuclear ER-mediated signaling is the major effector arm of the ER signaling pathway regulating the pathophysiology of hormone-responsive target cells. ER has a nuclear localization signal that interacts with importin, a nuclear membrane component, thus maintaining ER's nuclear localization.[79] Once E2-ER complex moves to the nucleus, it follows two well-known separate modes of action. These are the E2 response element (ERE)-dependent (sometimes called the genomic) signaling pathway and ERE-independent or non-genomic signaling pathway.

In the ERE-dependent pathway, the E2-ER complex directly binds to the consensus motif sequence (5'-GGTCAnnnTGACC-3') often found on the promoter of ER target genes to regulate its expression (Figure 1: signaling pathway I and II). The E2 liganded ERα

binds to ERE through the sequence specificity P-box residue (Glutamine-203, Glycine-204 and Alanine-207) and conserved Arginine-211 residue recruiting co-regulators, chromatin modifiers as well as the transcription initiation complex.[80-83] Briefly, the E2-ER complex first recruits the p160 co-activator family members (Sirtuin 1-3, AIB1, Nuclear receptor coactivator 2) which have histone acetyltransferase activity to open-up the chromatin. This phenomenon allows the recruitment of the p300 co-integrator that further relaxes the chromatin. p300 activates the subsequent binding of RNA polymerase II on to the transcription initiation sites to start transcription of the target genes.[84-86]

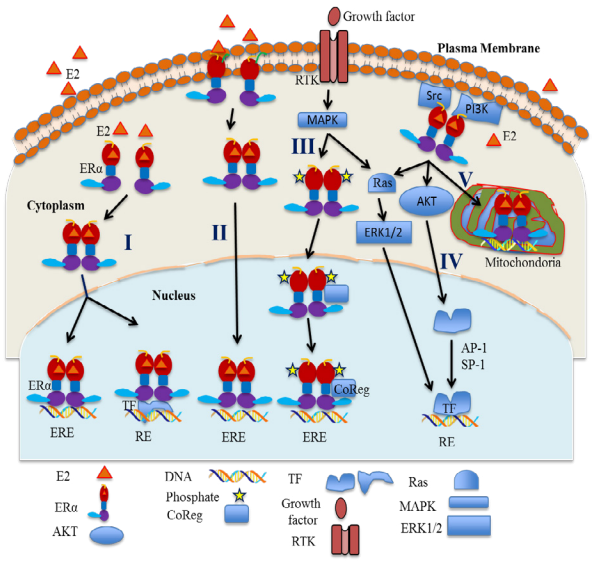

Fig. 1. Estrogen Receptor Signalling Pathways: I) In ER-dependent nuclear signalling, E2 can freely inter the cell, bind and dimerize ERα. Once ERα dimer localizes to nucleus it may bind directly to an ERE or binds to another response element (RE) through transcription factors (TF). II) ER-mediated membrane signaling pathway. Here receptor associated ER monomer binds to E2 and dimerizes. III) E3-independent signalling pathway/growth factor-mediated ER signalling. Here MAPK/Ras-mediates the cascade or MAPK phosphorylates ERα. Phosphorylated ERα dimerize and localizes to the nucleus where it recruits co-activators and bind to ERE. IV) ER-mediated Src, PI3K/AKT signalling crosstalk which involves other TF (AP-1 and SP-1). V) ER-mediated mitochondrial signalling events also happen when ER localizes and binds to mitochondrial DNA (modified)[87,88]

The ERE-independent pathway involves the mechanism whereby E2 liganded and/or antagonist/agonist liganded ER dimer interacts with co-regulatory proteins or transcription factors such as specificity protein 1(SP-1) and activator protein 1 (AP-1) that have their own cognate response elements to modulate the expression of genes involved in cell proliferation, differentiation or cell death.[89-96] The critical involvement of co-regulators accordingly makes a good platform to diversify the E2 responsive genes (Figure 1: signalling pathway IV). The involvement of co-regulators in this pathway diversifies the chromatin binding coverage for the genes responsive to ER signalling pathway.

To further understand the role of the ERE-independent pathway, the P-box residues (Glycine-204, Glutamine-203 and Alanine-207) found on the DNA binding domain of ERα were mutated in human cell lines and in mice thereby ERE independent E2 mediated signalling pathway model were developed [97]. Later-on to reduce the effect of such mutation on the co-regulator binding, Arginine-211 was mutated to Glutamine-211 along with ER203/204 thereby created ERE-binding null ER. These mutations were sufficient to abrogate the cardinal ERE response while still allowing activation of subsets of ERE-independent E2 responsive genes. The *in vivo* mouse knock-in model of these mutants showed the phenotype of hypoplastic uteri, hemorrhagic ovary, reduced mammary gland development which are phenotypic features of ERα-knockout mouse.[98,99] Furthermore, ERE oligonucleotide used as decoy DNA transfected into ER+ breast cancer cells were shown to halt E2 induced growth.[100] These examples indicate that the ERE-dependent signalling pathway accounts for the majority of physiologically relevant E2-ER signalling.

Structure and function of ER

Estrogen receptors (ERα and ERβ) have some basic structural features that underlie the similarity of their function. Linking the ER structural topology to its function, there are five segments of ER functional domains encoded by 8 exons. ERα is encoded on chromosome 6 and is 595 amino acid residues in length or 66-kD when translated. In comparison, ERβ is smaller in size, encoded from chromosome 14 and it is 530 amino acid in length, or 60-kD when translated. The homology of the two ER domains varies across the domain with the highest similarity (97% homology) in C segment of DNA binding domain (DBD) (Figure 2).

The amino-terminus A/B domain of ER is highly disordered AF-1 region but co-operatively assemble to keep the structural integrity of activation function 2 (AF-2) region of LBD in accordance with the signaling milieu.[103-107] The A/B domain of ER can display a variety of dynamic conformations that change with modifications such as phosphorylation or upon interaction with another protein partner.[108] Compared to ERα, ER-β has a truncated AF-1 region and thus it's interaction with other protein partner is impaired.[109-112]

In ERE-dependent ER signaling, ER bind to chromatin through the C domain called DBD. This domain is highly conserved between the two estrogen receptors and it dimerizes and assembles itself on the DNA

double helix forming a zinc finger module, the P-box and D-box determining half-site spacing and DNA-sequence binding specificity.[80-82] It has been also reported that this domain is linked to the hinge region (D domain) containing nuclear localization signal and allow the binding of associated transcription factor during modulation of ERE-independent signaling pathway activation [113]. The D segment of ER also contains multiple ubiquitination residues that undergo posttranslational modification to determine the half-life of ERα.[114]

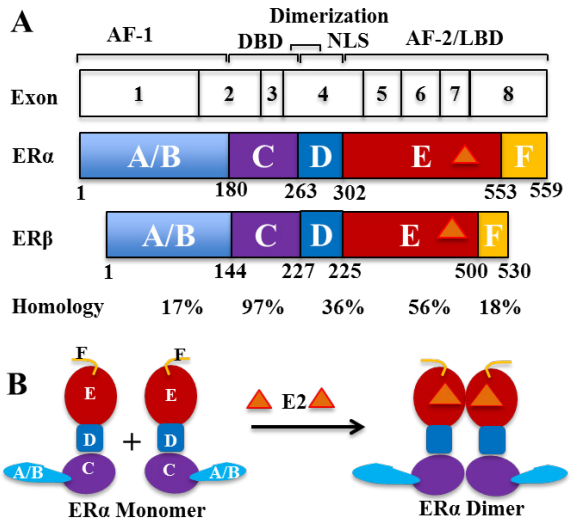

Fig. 2. Human ERα and ERβ Domain Structure. A) Schematic presentation of ERα and ERβ domains with corresponding percent homology. Both ERα and ERβ genes are expressed from 8 exons and have five interconnected segments of functional domains. The percent homology between the two ER for each segment is indicated. B) ERα domain arrangement and conformation change during E2 induced dimerization (adapted)[101,102]

The LBD of ERα is predominantly involved in the dimerization of ERα following the formation of ligand binding scaffold; a very important segment for ligand-dependent activation of ER signalling.[115] E2 binds to this AF-2 domain thereby recruit co-activators.[116-118] The conformational status of the dynamically mobile H12 helicase motif in the LBD determines the choice of agonist or antagonists as well as the co-activator binding events. Thus LBD has been chosen as the best pocket-docking site for drugs.[117,119]

Roles of ERα in ER Signaling in Breast Cancer

The oncogenic effects of ER signalling are driven by combinations of molecular assets within the cancer cells. E2 induced ER signaling has been also implicated in the initiation and development of breast cancer.[5,6] Functional study in this pathway also showed that impairing estrogen signalling by removing the ERα causes the defects on the reproductive system and brain in both female and male mice, whereas prolonged exposure of exogenous E2 by using contraceptives or other hormone therapy has been shown to promote the incidence and progression of many hormone-dependent; breast, ovary, and prostate cancers.[120-124]

Normally, the transcriptional activity of ERα is controlled by tightly regulating its protein level inside the cells. In the oncogenesis of breast cancer, E2 influences uncontrolled cell proliferation or promote ER independent signalling to sidestep the physiologically controlled ER signalling.[125,126] Few reports also showed that breast cancers express a protein that stabilizes ERα to promote proliferation.[127,128] Thus understanding the cellular and molecular events in breast cancer that regulate ERα stability and function is very important.

Altered ERα stability triggers oncogenic ER signaling

Given that the majority of breast cancers are ERα+ and the cellular level ERα is a critical determinant both as a diagnostic marker as well as a targetable molecule, understanding the mechanisms that underlie the tight regulation of ERα will greatly impact breast cancer therapy. It has been reports that ERα undergoes an intricate interplay of post-translational modifications such as phosphorylation, ubiquitination, glycosylation, sumoylation, and acetylation events that can modulate un-liganded or liganded ERα stability as well as function thereby triggers oncogenic ER signaling in breast cancer.[13-19]

The post-translational modification of ER that regulate ER stability in cancer is often mediated by several proteins that interact and protect ER from degradation by the ubiquitin-proteasome system.[129] Through diverse mechanisms, these proteins prevent polyubiquitination and degradation of ER, leading to an increase in ER protein levels; consequently, estrogen signaling and its physiologic effects are enhanced in breast cancer cells. Thus, increased protein stability seems to be one of the main reasons that ER is upregulated in breast cancer. For instance there are coactivators that stabilize the level ER protein, the kinases like GSK3, LMTK3, and ABL interact with and stabilize ER, non-nuclear mechanisms for maintaining ER stability and involvement of other proteins like MUC1, PIN1, GSK3, LMTK3, RNF31, RB, and ABL have been reported to be involved in ER stability in breast cancer.[127-138] More recently TRIM56 has been identified to be a novel regulatory factor prolongs ERα protein stability in breast cancer, through targeting ER alpha K63-linked ubiquitination.[139] Some of the depicted mechanism how such post-translatioal modification affect ER stability is diacussed below.

ERα Ubiquitination

The cellular level of ERα is primarily regulated by ubiquitination, especially when ERα is transcriptionally ac-

tive. Reports have shown some proteins dynamically interact with and prevent degradation of ERα by the ubiquitin-proteasome system.[114,127,128,134] The oncogenesis of breast cancer could emanate evolving the molecular mechanisms whereby cancer cell bypasses this polyubiquitination and degradation of ERα, thus increases its stability.

An oncogenic glycoprotein Mucin-1(MUC1) has been reported to binds directly to the ERα DBD and stabilize ERα by blocking its ubiquitination and degradation.[137] Another protein called Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) interacts with ERα to prevent the binding of E3 ligase, E6AP, to ERα thereby prevents the ubiquitination and degradation of ERα.[138] Recently, a Ring-between-Ring E3 ligase family protein; Ring Finger Protein 31 has been found to associate with ERα and catalyzes ERα monoubiquitination but blocking its polyubiquitination thereby increasing ER protein stability.[127] Retinoblastoma transcriptional corepressor (RB) has also been reported to interact with and stabilize ERα, protecting it from degradation by the ubiquitin-proteasome system (UPS) in breast cancer.[139,140]

ERα Phosphorylation

Phosphorylation of ERα also regulates its stability and function. ERα is phosphorylated following the activation of various kinases. It has been reported that MAPK, AKT, PKA, RSK and Src kinase-associated pathway phosphorylates various residues of ERα.[141-143] The kinases like glycogen synthase kinase 3 (GSK3), lemur tyrosine kinase 3, abelson tyrosine-protein kinase and casein kinase 2 also phosphorylate and stabilize ERα.[130-135] Phosphorylation impacts ERα in various ways including altering its ubiquitination, chromatin interactions, recruitment of coregulators and the expression target genes that trigger the growth of breast tumor and patient response to endocrine therapy.[40,144-147] Concomitant to phosphorylation, there are also other mechanisms and proteins associated with ERα stability. For example, phosphatidylethanolamine binding protein 4 that competes with ERα for components of the UPS and other posttranslational modifications like acetylation, palmitoylation, can also affect ERα stability by affecting its phosphorylation.[148-150] On top of this, c-Abl regulates ERα transcription activity through its stabilization by phosphorylation.[132]

ERα Glycosylation

So far, there is no experimentally validated glycosylation of human ERα. However, mouse ERβ has been reported to undergo an alternative O-glycosylation/O-phosphorylation. This posttranslational modification occurs on Serine-16 near to the transactivation domain and Threonine-575 as part of a PEST region (a peptide sequence that is rich in proline, glutamic acid, serine, and threonine which acts as a signal peptide for prompt protein degradation) on mouse ERβ suggesting that glycosylation may regulate transactivation and turnover of ERβ indicating that such saccharide modification may also play a role in modulating the dimerization, stability, or transactivation functions of Estrogen receptors.[151-153] More recently ERα Glycosylation by N-Acetylgalactosaminyltransferase 6 (GALNT6) found to affect its nuclear localization in breast cancer cells.[154] Moer recently GREB1 a well known top E2 responsive ER target that regulate ER signaling in breast cancer was computationaly identified as a putative glycosyltransferase enzyme.[155,156] Another study also showed that the amino-terminal of GREB1 interracts with ERα thereby trigger progression of breast cancer.[157] Nevertheless whether the conserved c-terminal GREB1 domain predicted to have glycosyltransferase activity affects the transcriptional co-activator function of ERα or albeit affect ERα stability remains to be biochemically investigated.

Mutation of ERα

ERα gene (*ESR1*) somatic mutations have been linked to the acquired resistance to endocrine therapies in breast cancer.[12,158-161] The most prevalent point mutations of ERα are Y537S and D538G.[158] These mutations which are found on the LBD of ERα have been reported to affect the ERα co-activator binding conformation as well as chaperone-mediated regulation of ER stability.[158] In breast tumour cells, ERα mutations at the sites linked to ERα degradation were also reported to regulate its stability.[114,134]

Altered ERα expression

The deregulation of E2–ER signaling plays a critical role in the initiation and progression of target tissue malignancies as well as in ER-driven neoplastic processes and also the development of endocrine resistance in the treatment of estrogen target tissue malignancies, exemplified by breast cancers.[19,162-164]

The regulation of ERα signaling could be altered due to alteration of ER expression via epigenetic events that leads to the initiation and/or progression of numerous types of cancer including breast cancer. Epigenetic dysregulation of the GC-rich promoter of ER due to methylation-mediated ERα gene silencing during tumor progression happens in one-third of breast cancers that initially express ERα.[165]

Altered reregulation of ERα function and activity

The E2 induced ER signaling could underlay the carcinogenesis of breast cancer if the ERα function and activity is altered. For instance the loss of control over cell cycle progression following overexpression of Cyclin D1 could be due to heightened E_2 induced ER sig-

naling that recruits various transcription factors that involve ATF-2 and c-Jun without ERE requirement on the cyclin D1 promoter.[166,167] The extranuclear actions of ER also affect breast cancer cell proliferation, migration, drug resistance, and apoptotic inhibition by stimulating various pathway cross-talks.[168,169] Rapid E_2 action leads to the activation of IGF-1R and EGFR as well as stimulation of the Src kinase, MAPK, PI3K, and protein kinase C (PKC) pathways in the cytosol for breast cancer cells. [22,23]

On top of this, there several studies that show ERα coregulators in ER signaling also play role in the development breast cancer. Many well-characterized coregulators with the potential to influence the ERα-mediated breast cancers by interacting with ERα thereby regulate chromatin remodeling and directly or indirectly regulate target gene expression.[20,21] For instance, the AIB1 coactivator activates ERα-dependent transcription by recruiting HAT such as p300 and P/CAF to ERα target gene chromatin.[170] AIB1 interacts with ERα in a ligand-dependent fashion and it leads to ERα stabilization in the presence of E2, thereby regulating ERα activity, as well as ERα protein degradation mediated by the ubiquitin proteasome pathway.[171]

Recently, GREB1 was reported to function as a transcription co-activator of ERα. Loss or dysregulation of GREB1 substantially decreased ERα-mediated gene transcription and reduced tumor growth.[155,172-174] One study found GREB1-ERα interactions in 50% of ER+ cancers and showed GREB1 expression was correlated with a good clinical outcome.[151] Nevertheless, little is known about the exact role of GREB1 in the cascade of hormone action, though it appears to be a key E2-induced gene having a role in ER signaling. MUC1 is also a potent coactivator of ERα. It regulates ERα activity by directly binding to the DNA binding domain of ERα and stabilizes ERα by blocking its ubiquitination and degradation in breast cancer cells.[175]

In contrast to coactivators, corepressors recruit histone deacetylases (HDACs) to ERα target gene chromatin, which leads to the chromatin condensation and the inhibition of ERα target gene expression in breast cancer cells.[7] The corepressors counterbalance the actions of coactivators to orchestrate the magnitude of E2 responses, which leads to the inhibition of ERα target gene expression. Therefore, the loss of ERα corepressors promotes breast cancer.[176] For instance, MTA1 containing nucleosome remodeling and histone deacetylation complex (NuRD) suppresses ERα-mediated gene expression, resulting in invasive breast cancer phenotype.[177] While Nuclear receptor corepressor 1 (NCOR1) is another well-defined corepressor of ERα that inhibits ERα transcriptional activity by binding to the ligand-binding domain. Low expression of NCOR1 is associated with shorter relapse-free survival in breast cancer patients, which shows that loss of NCOR1 enhances breast cancer development.[178] As ERα-mediated physiological response results from the coordination between ERα, coactivators, and corepressors, targeting expression-profiles for all coregulators may help patient diagnosis and treatment of the breast cancer subtypes.

Conclusions

ER+ breast cancer constitutes a major fraction of breast cancers. Thus, tremendous efforts have been made to explore ERα function and its relevance to breast cancer. As a result, many novel mechanisms of E2/ERα-mediated breast cancer development were discovered including epigenetic regulation of ERα expression, altered stability and identification of hundreds of ERα coregulators and their association with breast cancer development. The extensive posttranslational modification of estrogen receptor regulating ERα stability shows the complexity of ER signals, especially when pathway crosstalk happens with other oncogenic signals. Hence, a better understanding of oncogenic events that drives expression, activity, stability of ERα may play a critical role in the development of diagnostic and prognostic biomarker as well as to overcome endocrine therapy resistance.

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REVIEW PAPER

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Journey so far with COVID 19 – a comprehensive review

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ABSTRACT

Introduction. The occurrence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has emerged as a global pandemic with huge death tolls. Coronavirus disease 2019 (COVID-19) may progress from minimal infection to serious respiratory failure mandating treatment for a continuum of developed disease condition.

Aim. The purpose of this review is to summarize the findings related to epidemiology, clinical manifestations, modes of transmission, diagnosis and the treatment modalities (both experimental and repurposed) for COVID-19.

Material and methods. Literature were searched using various search engines like PubMed, SCOPUS, EMBASE, J-Gate, Google Scholar to look for review articles, randomized controlled trial results, prospective studies and, retrospective studies done on COVID-19 for the purpose of this comprehensive review.

Analysis of the literature. The transmission seems to be occurring through droplet, fomite and aerosols (rarely). Currently there is no specific/targeted vaccine available. Priority is highly placed to identify possible treatment approaches to circumvention this disease.

Conclusion. Till we find a vaccine, we have to strategize to optimally use the existing evidence of the indirect effects of these various available drugs for therapy and maintain a strict protocol for prevention and we must use triage system to admit only those critically ill or having severe disease.

Keywords. clinical trials, respiratory infection, treatment strategies

Introduction

The trigger of coronavirus disease 2019 (COVID-19) by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has expanded all over the globe culminating in a pandemic. Coronaviruses are morphologically spherical or pleomorphic in nature, with a mean diameter of approximately 80–120 nm having distinct large (20 nm) "club-like" surface extensions, which are the glycosylated spike proteins of the virus.[1] The revelation of a novel human coronavirus, SARS-CoV-2, has now become a global health issue that causes serious human respiratory tract infections. Incubation times between 2-10 days have been identified for human-to-human transmissions, facilitating their spread through droplets, contaminated hands or surfaces.[2] On inanimate surfaces, coronavirus continue to exist for up to 9 days at room temperature while the related survival of the viruses declines at a temperature of 30°C or higher

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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and disinfectants like ethanol (62-71%), hydrogen peroxide (0.5%) or sodium hypochlorite (0.1%) can inactivate the virus within 1 minute.[2]

It is pertinent to admonish readers that new data specific for COVID-19 emerge around every hour concerning clinical features, treatment choices and therapeutic outcomes. Optimized support treatment regimen remains the bedrock of intervention, and the clinical effectiveness of subsequent treatments is still being researched upon. So many of the preclinical and clinical data on antiviral therapy are derived from the research studies on other viruses such as severe acute respiratory syndrome-1 coronavirus-1 (SARS-CoV-1), Middle East coronavirus respiratory syndrome (MERS-CoV) and non-coronavirus (Ebola). In addition, the clinical prominence of *in vitro* anti-viral activity remains uncertain given the lack of pharmacokinetic/pharmacodynamic or clinical evidence equating realistic doses to a treatment impact compared to such values. Hence, it needs to be noted that in vitro details should be appropriately evaluated across findings given the possible variation in test methodologies that could affect purported behavior.

Aim

This narrative review summarizes the epidemiology, clinical manifestations, modes of transmission, diagnosis and the treatment modalities (both experimental and repurposed) for COVID-19. It is an attempt that could provide needful references for further research endeavours.

Material and methods

Literature were searched using various search engines like PubMed, SCOPUS, EMBASE, J-Gate, Google Scholar to look for review articles, randomized controlled trial results, prospective studies and, retrospective studies done on COVID-19 for the purpose of this comprehensive review.

Viral structure in nutshell

The virus responsible for COVID-19, SARS-CoV-2, is 125 nm in size which is slightly larger than the influenza, SARS and MERS viruses.[3] It shares the identical morphology of "spike-protein" with other coronaviruses and this spike latch onto human cells, then fuses with the cell membrane through structural change and, this gives entry of the viral genes into the host cell to be copied, producing more viruses.[4] It is known to be a descendant of a bat corona virus, the closest being originated from the Rhinolophus bat which is > 96% homologous with stated one.[3]

The viral membrane consists of four proteins: 1) the spike (S) glycoprotein, 2) a small glycoprotein making up the envelope (E), 3) a glycoprotein forming the membrane (M), and 4) the nucleocapsid (N) protein.[5] The membrane (M) glycoprotein, spanning the membrane bilayer three times, is the most abundant one.[6] A study performed next-generation sequencing from samples of bronchoalveolar lavage fluid and found that the ten 2019-nCoV genome sequences from these nine patients were quite similar, almost in excess of 99.98% for sequence similarity.[7] There was homology (88%) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 but found to be more distinct from SARS-CoV and MERS-CoV by about 79% and 50%, respectively.[7] Homology modeling prominently demonstrated that 2019-nCoV had a receptor-binding domain architecture close to that of SARS-CoV, given the differences in amino acids at some key residues.[7] The spike (S) glycoproteins consist of two subunits, namely S1 and, S2.[5] Out of these two parts, Part S1 determines host virus range and cellular tropism along with the formation of receptor binding domain while S2 mediates virus fusion and transmitting to host cells.[8] Homotrimers of S proteins make up the spikes on the viral surface which direct the connection with the host receptors.[9] The most important inducer of neutralizing antibody is the spike protein. The dynamics of CoV pathophysiology and virulence, including that of SARS-CoV-2, have ties to the role of non-structural proteins (*nsps)* and structural proteins. There are 16 *nsps* identified so far and they play various roles like IFN signaling inhibition and host innate immune response blockage by promotion of cellular degradation and blockage of translation of host's RNA, protein binding prohibition, cytokine protein expression and viral polyprotein cleavage, contributing to formation of transmembrane scaffold protein and many others.[5] Researches have shown that *nsp* can suppress the innate immune response of the host.[10]

Epidemiology

According to the situation report-194 released by the WHO, the entire world appears to have been infected by the virus, but the influx of new outbreak trends has migrated from China to the European countries drastically and finally to encompass the Asian subcontinent also.[11]

According to the WHO situation report-194, a summary of the outbreak in the different zones of the world are presented in Table 1.[11]

Some countries have adopted to test only the seriously infected patients. This has eventually led to a false statistics related to high death rate but it is a fact that the actual burden of the disease can be measured only by mass testing. South Korea took up massive testing from the beginning of the outbreak and has a very low fatality rate and could bend the pandemic curve very early while UK, Italy and France started late and the fatality rates are disastrous now.[12]

Table 1. Summary of total confirmed cases and deaths as per Situation Report-194

Area	Confirmed COVID 19 cases	Deaths due to COVID 19
Global	17396943	675060
Western Pacific region	312771	8388
European region	3357465	212978
South-East Asia region	2072194	44900
Eastern Mediterranean Region	1544994	40019
Region of the Americas	9320330	355217*
African Region	788448	13545

* highest contributing zone towards deaths from COVID 19; WHO, World Health Organisation

The proportion of the elderly population, health care facilities and known co-morbidities also play a key role in assessing the fatality rate as a result of this infection, as the majority of older people with co-morbid conditions suffer from serious disease.[13]

Clinical features

Table 2. Summary of the characteristics of clinical symptoms

Type of clinical symptoms	Characteristics
Mild	Clinical symptoms present but there is no evidence in chest imaging
Moderate	Respiratory problems with fever and positive chest imaging findings
Severe	If any of the criteria is satisfied then that patient is designated to be suffering from severe disease: 1) Oxygen saturation ≤ 93% during resting phase, 2) Shortness of breath (RR ≥ 30 times per minute), 3) Higher than 50% progression of the lesions in a time period of 24-48 hours or, 4) Inspiration oxygen fraction ≤300 mm Hg but this should be adjusted in the plateau region according to the different atmospheric pressure to get the partial pressure arterial oxygen and fraction of inspired oxygen (PaO_2/FiO_2 ratio)[18]
Critically severe	If the patient satisfies any of these criteria: 1) Failure of organ needing ICU admission followed by treatment, 2) Shock or, 3) Respiratory failure with a need for mechanical ventilation

The clinical manifestations of COVID 19 are multifaceted which varies from being asymptomatic to the precipitation of acute respiratory distress syndrome to dysfunction of multiple organs. In general, mild or asymptomatic variety comprises almost 80% of infections, 15% are severe requiring oxygen, and 5% are critical who might require ventilation.[14] The disease might progress to pneumonia, respiratory failure and even death for a sub-group of patients by the end of the first week. This phenomenon has to do with a significant rise in inflammatory cytokines namely IL2, IL7, IL10, TNFα, GCSF.[15] Patients with COVID-19 usually experience the associated symptoms such as mild respiratory problems and fever in a time period of 5 to 6 days on an average after infection.[16]

Table 3. Extra-pulmonary manifestations of COVID-19

System	Manifestations
Hematologic	Low total white blood cell count (Lymphopenia) or even leukocytosis, increased neutrophil count or low platelet count. Elevated CRP, ferritin, Interleukin-6, LDH, D-dimer, fibrinogen, P Time and partial thromboplastin time
Thrombotic	Arterial thrombotic complications: type 1 and 2 MI, ischemic variety of stroke, acute ischemia of limb, and mesenteric ischemia; venous thrombotic complications: deep vein thrombosis and PE; thrombosis related to catheter: thrombosis found in venous and arterial catheters, as well as in extracorporeal circuits
Immune system-related	Cytokine-release syndrome comprising of high-grade fevers, hypotension requiring inotrope support and, multi-organ dysfunction
Cardiovascular	Myocardial ischemia and type 1 and 2 MI; myocarditis; new onset Arrhythmias - sinus tachycardia as well as bradycardia, atrial fibrillation and flutter, QTc prolongation (often associated with use of drugs) resulting in torsades de pointes and even sudden cardiac death, PEA; new onset cardiomyopathy resulting in biventricular or isolated right or left ventricular dysfunction and, even cardiogenic shock
Renal	Acute kidney injury; electrolyte abnormalities such as hyperkalemia and, hypo or hypernatremia; urinary protein and blood loss; metabolic acidosis
Gastrointestinal	Diarrhea, nausea and/or vomiting, abdominal pain; rare cases of mesenteric ischemia and gastrointestinal bleeding and, elevated liver enzymes and low albumin
Endocrinal	Hyperglycemia in a patient of diabetes that might lead to ketoacidosis, stress hyperglycemia (non-diabetic patient); ketosis with no or minimal elevation in blood glucose level
Neurologic and ophthalmologic	Headache, dizziness; anosmia, loss of taste sensation (ageusia), anorexia and feeling of extreme fatigue; ischemic stroke; acute hemorrhagic necrotizing encephalopathy, encephalitis, GBS, and, conjunctivitis

MI, myocardial infarction; ESR, ESR erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactate dehydrogenase; P Time, prothrombin time; PE, pulmonary embolism; PEA, pulseless electrical activity; GBS, Guillain-Barré syndrome

Clinically SARS-CoV-2 infection can be categorized under the following sub-headings based on the symptoms shown by the patients (Table 2).[17]

COVID-19 has shown to affect most of the systems of the body and many extra-pulmonary manifestations are also seen which are given in Table 3 below.[19]

Radiological features

The prevailing CT outcomes included opacification, consolidation, bilateral involvement and peripheral and diffuse distribution.[20] A radiological reterospective study involving patients with RT-PCR confirmed COVID-19 pneumonia admitted to one of two hospitals in Wuhan were subjected to sequential chest CT scans and were further divided into four treatment groups based on the mean of the total involved lung segments: group 1 having 10·5 ± 6·4, 2·8 ± 3·3% involvment, group 2 having 11·1 ± 5·4% involvement, group 3 having 13·0 ±5·7% involvement, and group 4 having 12·1 ±5·9% involvement. Out of the total 849 affected segments, the principal types of abnormalities noted were bilateral (79%) involvement, peripheral (54%) involvement, ill-defined (81%) lesions and ground-glass opacification (65%), which majorly involved the right lower lobes (27%).[21] The study interpreted that COVID-19 pneumonia demonstrates abnormalities in chest CT imaging, also in asymptomatic patients, with rapid change from focal unilateral to diffused bilateral ground-glass opacities which progressed to or co-existed within 1–3 weeks. The pictorial representations of COVID-19 pneumonia were observed to be highly unspecific and were most frequently bilateral with subpleural and peripheral diffusion and varied from ground-glass opacities in milder forms to more extreme consolidations.[22]

Predictors of severity

The results of a retrospective study conducted in children in China stated that lower count of lymphocytes, raised body temperature, and high concentrations of procalcitonin, D-dimer and creatine kinase MB were significantly associated with the presentation of COVID-19 intensity.[23] All the enrolled subjects (children) received interferon-α twice daily via aerosolisation, 14 (39%) received lopinavir – ritonavir syrup twice daily and six (17%) required oxygen inhalation. In the present study, it was observed that age was the predominant factor for the incidence of increased risk of severe illness and related death.[24] In a study by Xiaobo Yang and colleagues done on 32 non-survivors from a group of 52 patients in intensive care unit due to COVID-19, it was found that Cerebrovascular diseases (22%) and diabetes (22%) were the dominant comorbidities.[25]

Another research study involving 1099 COVID-19 patients reported that 23.7% had serious disease with hypertension comorbidities, 16.2% with diabetes mellitus, 5.8% had coronary heart disease while 2.3% suffered from cerebrovascular disease.[26] Similar reports was also published from another study conducted in Wuhan, China showing 91 (48%) patients had some type of comorbidity.[27] This study found that hypertension was the most common (30%) comorbidity and, was followed by diabetes (19%) and coronary heart disease (8%). Immunosuppression was likely to be effective in hyperinflammatory conditions. Re-analysis of results from a phase 3 randomized controlled trial of IL-1 blockade (anakinra) in subjects with sepsis, demonstrated major survival benefits in patients with hyperinflammation without elevated incidence of adverse effects.[28] A research indicated to evaluate all patients with extreme COVID-19 to be screened for hyperinflammation employing laboratory indicators such as elevated levels of ferritin or declining platelet countsor erythrocyte sedimentation rate as well as H-Score (which creates a propensity of secondary haemophagocytic histiocytosis) to classify the sub-group of patients for whom immunosuppression would increase the mortality rate.[29]

Pathogenesis and inflammatory cytokine storm

The process of pathogenesis and initiation of inflammatory cytokine storm are detailed as follow:

Entry and cellular replication

The virus enters into the host cell by a specific protein known as corona virus S protein.[30] In SARS-CoV-2, the envelope spike glycoprotein binds to the cellular receptor, ACE2.[31] The viral RNA genome is transferred into the cytoplasm as the virus enters the cell which is further translated into two polyproteins and structural proteins furthering to replication of the viral genome.[32] The newly shaped envelope of glycoproteins is introduced into the endoplasmic reticulum or Golgi membrane, and the nucleocapsid is developed by the fusion of genomic RNA and nucleocapsid protein. The viral particles then germinate into the intermediate reticulum-Golgi endoplasmic compartment (ERGIC) leading to the fusion of the vesicles containing the virus particles with the plasma membrane leading to the virus release.

Antigen presentation

When the virus reaches the cells, it will be presented to the antigen presentation cells (APC), which is the fundamental anti-viral mechanism of immune system in the body. The viral antigens are presented by the major histocompatibility complex (MHC; or human leukocyte antigen (HLA) in humans) followed by recognition by virus-specific cytotoxic T lymphocytes (CTLs).[33] Till date there are no reported evidence supporting the same and some information can be retrieved from the previous research work involving SARS-CoV so MERS-CoV.[33] In SARS-CoV both MHC I and II helps in antigen presentation.[34] HLA polymorphisms correlating to the susceptibility of SARS-CoV are HLA-B∗4601, HLA-B∗0703, HLA-DR B1∗1202.[35]

Role of immunity at humoral and cellular levels

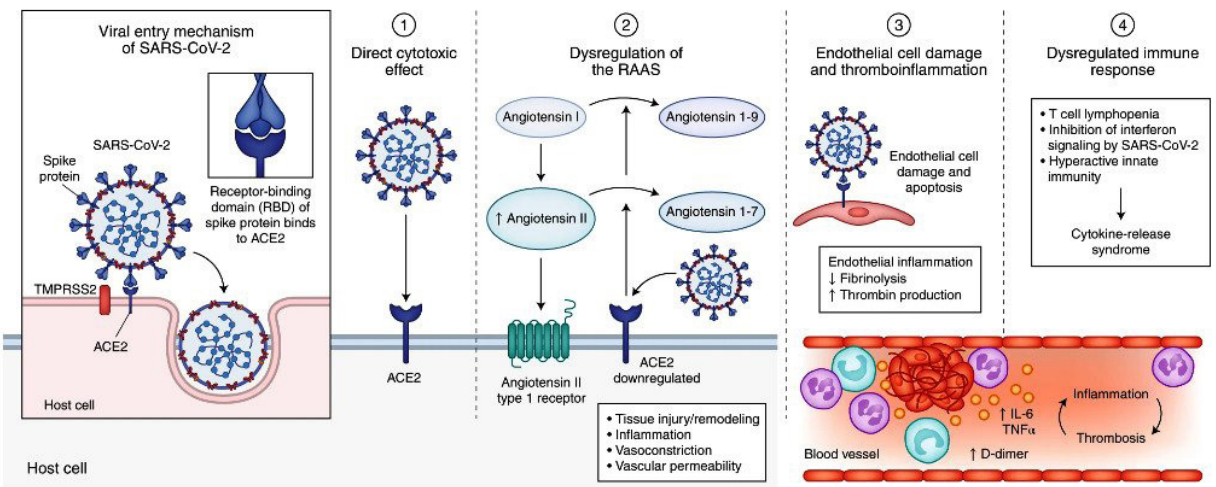
Consequentially, antigen exposure activates the humoral and cellular immunity of the body, which is regulated by the B and T cells unique to viruses. The antibody profiling against SARS-CoV virus has a characteristic trend of IgM and IgG levels, relative to common acute viral infections.[36] At the completion of week 12, the SARS-specific IgM antibodies vanish while the IgG antibody last for a longer duration of time period, suggesting that IgG antibodies would serve a defensive role.[37] Approximately, 1.48% of antibody-secreting cells (ASCs) emerged in the blood at the time of viral clearance on day 7 while on day 8 it peaked to 6.91% in a patient with mild COVID 19 symptoms prompting hospitalisation. At day 7 (1.98 percent), the development of cTFH cells occurred simultaneously in blood, rising on day 8 (3.25 percent) and day 9 (4.46 percent). The above analysis revealed a prominent presence of both ASCs (4.54%) and cTFH cells (7.14%) during convalescence (day 20).[38]

Cytokine storm in COVID 19

Reports suggest acute respiratory distress syndrome (ARDS) to be the most common immunopathological incidence for SARS-CoV-2, SARS-CoV and MERS-CoV infections and high concentrations of cytokines in severely ill patients are a common finding indicating the role of cytokine storm as a core driving factor for deterioration.[39] ARDS leads to damage of lung microvasculature, interstitium, and alveolar space by accumulation of neutrophils and the release of inflammatory cytokines.[40] A hyperinflammatory syndrome called Secondary haemophagocytic lymphohistiocytosis (sHLH) has the hallmark features of fulminant and hypercytokinaemia subsequently leading to fatal multiorgan failure.[41] A cytokine profile similar to sHLH is often identified with prevalence of COVID-19. It is characterized by enhanced interleukin (IL)-2, IL-7, stimulating factor granulocytecolony, inducible protein 10, protein monocyte chemoattractant 1, inflammatory protein 1-α macrophage, and factor-α tumor necrosis. One of the key mechanisms for ARDS is release of significant levels of pro-inflammatory cytokines like interferon-alpha (IFN-a), IL-12, etc. and chemokines like CCL2, CCL3, etc. by immune effector cells.[42,43] The cytokine storm may also lead to ARDS and multiple organ failure, and ultimately lead to death in serious cases of SARS-CoV-2 infection, as evidenced in SARS-CoV and MERS-CoV infection.

Evading the immune response

Due to lack of research data related to SARS-CoV-2, the emergence to extrapolate the available data on



SARS-CoV-2 enters host cells through interaction of its spike protein with the entry receptor ACE2 in the presence of TMPRSS2 (far left). Proposed mechanisms for COVID-19 caused by infection with SARS-CoV-2 include (1) direct virus-mediated cell damage; (2) dysregulation of the RAAS as a consequence of downregulation of ACE2 related to viral entry, which leads to decreased cleavage of angiotensin I and angiotensin II; (3) endothelial cell damage and thromboinflammation; and (4) dysregulation of the immune response and hyperinflammation caused by inhibition of interferon signaling by the virus, T cell lymphodepletion, and the production of proinflammatory cytokines, particularly IL-6 and TNFα.

Fig. 1. COVID-19 pathogenesis in a nutshell

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SARS-CoV and MERS-CoV is highly needful. The pattern recognition receptors (PRRs) can identify the evolutionarily preserved microbial structures called pathogen-associated molecular patterns (PAMPs). Nonetheless, SARS-CoV and MERS-CoV may induce the development of double-membrane vesicles that lack PRRs and further replicate in these vesicles, thus preventing the detection of their dsRNA by host.[44] The antigen presentation could be suitably affected by the coronavirus as exemplified by gene expression in relation to down regulation of antigen presentation following MERS-CoV infection.[45] The process of virus attachment and proposed mechanisms for COVID-19 caused by infection with SARS-CoV-2 are beautifully depicted in Figure 1.[25]

Transmission

The fact that this virus attaches to the human cell surface receptors called angiotensin-converting enzyme 2 (ACE2) 10 to 20 times more than the previous SARS virus in 2002, might have increased the spreading potential of SARS-CoV-2 from person to person than the earlier virus.[4] The cause of increased transmissibility of this virus has been reported by Korber et al. to be highly linked to the mutation seen in the amino acid sequence of the spike protein D614G.[46] The key framework for the transmission of the disease was perceived to be transmission from animal to human, given the case of the initial straight forward contact with the Huanan Seafood Wholesale Market, Wuhan. Still, there was no connection between this responsiveness phase and subsequent events. Mode of transmission was also suspected to be from human to human, and that the most prevalent cause of COVID-19 transmission are the symptomatic individuals.[47,48] The probability of transmission seems infrequent before symptoms occur but it cannot be excluded.[49] Data shows that the best way to control this outbreak is "self-isolation".[50] Transmission through coughing and sneezing is presumed to occur by respiratory droplets. Infections of the respiratory tract can be transmitted by various sized droplets. When the particles of the droplets exceeds5-10 μm in diameter are termed as droplet particles when they are < 5 μm in diameter, they are called droplet nuclei.[51]

Airborne transmission is distinct from droplet transmission as it corresponds to the inclusion of microbes within droplet nuclei, which has the ability to stay in the air for long periods of time, and can be transmitted over distances greater than 1 metre.[52] Reports suggest that infection with COVID-19 can cause intestinal infection and the virus can be found in the feces as shown by one Chinese study which cultured COVID-19 virus from a specimen of stool.[53]

Diagnosis

Diagnosis of SARS-COV-2 infection is generally done by a combination of molecular, serological and radiological findings in a patient having typical symptoms described above or by a combination of molecular testing with radiological help in asymptomatic individuals.[33] Amongst the nucleic acid detection technologies, Real-time quantitative polymerase chain reaction (RT-qPCR) and high-throughput sequencing are the most widely used for COVID-19.[33, 54] As the latter approach is expensive and burdensome, hence RT-qPCR is primarily used for viral RNA detection.[55] The validity of use of real time RT-PCR test has been questioned by a study from Wuhan, China, as they found high false negative results with this method only and suggested to include clinical features and radiological parameters to be taken into consideration for diagnosis.[56] The samples used for SARS-CoV were nasopharyngeal aspirate, throat swab, urine and stool and almost similar specimens were used for SARS-CoV-2 but bronchoalveolar lavage, endotracheal aspirate and tissue from biopsy or autopsy including from lung have also been approved by WHO for SARS-CoV-2.[57, 58] Studies suggest that the viral RNA yield varies from patient to patient depending on the day of collection after symptom onset, site of collection, technical errors associated with sample collections and the methods applied for detection.[59] A nasopharyngeal swab gives a better yield than an oropharyngeal swab collection but the yield is always highest with the bronchoalveolar lavage collection.[60, 61] While considering serological assays like IgM and IgG for diagnosing SARS-COV-2 infection, we must remember that IgM is notoriously non-specific and both takes almost 2 week time to reach highest levels.[60, 62] Neither RT-PCR,

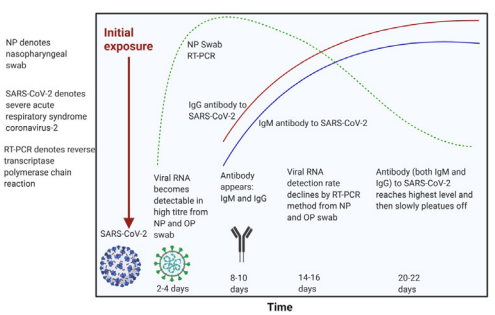

Fig. 2. Timing of applicability of various diagnostic tests for SARS-CoV-2
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nor computed tomography (CT) thorax should be used solely for COVID-19 diagnosis purpose since both have their own limitations and imaging facilities can be used to aid diagnosis of early and missed, suspicious, symptomatic cases when RT-PCR might come as false nega-

Table 4. Summary of treatments under trial and approved on emergency basis

Drug generic name	Class of drug	Possible mechanism of action	Trial type with number of participants (n)	Trial No.	Reference
Hydroxychloroquine; Chloroquine	Anti-malarial	Viral enzyme pathway inhibition including viral DNA and RNA polymerase, glycosylation of viral proteins and its assembly, transport of new virus particles followed by their release. Other mechanisms can also include cellular receptor inhibition of ACE2, cell membrane acidification inhibiting virus fusion, and immunomodulation of cytokine release.	Adaptive, randomised open clinical trial Phase 2, International Multi-site, Bayesian Adaptive, RCT (double-Blind, Placebo Controlled) (n=55000)	NCT04315948 NCT04333732	58,67
Azithromycin	Antibacterial	Down regulation of inflammatory responses with reduction in the elevated production of cytokines correlated with viral infections in the respiratory tract; furthermore, their direct impact on viral clearance is unknown. Mechanisms might also involve immune-modulation comprising of reduction of development of reactive oxygen species and neutrophil chemotaxis, acceleration of apoptosis of neutrophil and disruption of nuclear transcription factor activation as well as hypersecretion of mucus.	Randomized, placebo control, parallel arm trial (phase 4). N=40. Randomized, placebo controlled in mild to moderate COVID-19 patients. N=2271.	NCT04359316 NCT04332107	68,69,70
Azithromycin + chloroquine/ hydroxychloroquine		As stated above in the respective sections.	Randomized, open label, parallel assignment trial. N=750. Randomized, quadruple masked, parallel assignment trial. N=140	NCT04370782 NCT04374552	
Anakinra	Interleukin-1 receptor antagonist	Inhibition of IL-1β and IL-1α from binding which thereby blocks signal transduction pathway.	Randomized, triple masked, parallel assignment trial. N=30. (Phase = 3) Non-randomized, sequential assignment open label, Phase 2 trial. N=90.	NCT04362111 NCT04148430	71
Remdesivir (GS5734)	Inhibitor of RNA-dependent RNA polymerases	The compound undergoes a metabolic pathway, which stimulates the nucleoside triphosphate metabolite to inhibit viral RNA polymerases.	Phase 3 RCT (double-Blind, Placebo Controlled), Multicenter Study (n=308). Phase 3 RCT (double-Blind, Placebo Controlled), Multicenter Study (n= 453) Phase 2 Multicenter, Adaptive, RCT (double--Blind, Placebo Controlled) (n=440)	NCT04252664 NCT04257656 NCT04280705	64,72

Glucocorticoids	Steroids	Patients with refractory shock or an acute respiratory distress syndrome could be usually prescribed; however corticosteroid treatment for viral pneumonia is not suggested.	Randomized, open label parallel group trial (phase 2). N=478. Observational, retrospective, case-control study. N=50. Randomized, open label trial (phase 2, 3) with Lopinavir-Ritonavir combination, Hydroxychloroquine, Corticosteroids, Azithromycin, Convalescent plasma, Synthetic neutralizing antibodies or Tocilizumab. N=15000.	NCT04416399 NCT04445506 NCT04381936	[73]
Tocilizumab	Human, monoclonal antibody inhibiting the interleukin-6 (IL-6) pathway	Enhanced IL-6 is a characteristic inflammatory hallmark noted in the serum of patients with acute respiratory distress related to incidence of COVID-19. Tocilizumab acts by inhibiting IL-6-mediated signaling pathway (competitive binding to IL-6 receptors).	Non-randomized, single group assignment, open label phase 2 trial. N=32. Randomized, double blinded, placebo controlled phase 3 trial. N=300.	NCT04331795 NCT04412772	[74]
Baricitinib	Janus kinase (JAK) inhibition	Inhibition of intracellular enzymes,JAKs, which are responsible for transmitting cytokine signals or receptor growth factor interactions involving hematopoiesis and immune cell function.	Randomized, double blinded, placebo controlled, phase 3 trial. N=600. Single arm, open label, single site, adaptive phase 2/3 trial. N=80.	NCT04421027 NCT04340232	[75,76]
Lopinavir/ ritonavir combination	HIV Protease Inhibitor	Inhibition of Mpro, a key enzyme for coronavirus replication.	Adaptive, randomised open clinical trial (n=3200)	NCT04315948	[77,78]
Favipiravir	Anti-viral	Favipinavir acts by selectively inhibiting the RdRP of influenza virus. It is reported to be approved in China for COVID 19.	Randomized, phase 2, double blinded, placebo controlled, trial. N=120. Proof-of-Concept, randomized, phase 2 open label study. N=50.	NCT04346628 NCT04358549	[65,79,80]
Inhaled GMCSF	Recombinant humanized granulocyte-macrophage colony stimulating factor	Acts by targeting both leukopenia and innate immune cell sensitivity that can play a factor in the treating patients at high risk.	Randomized, phase 2, open label study. N=60. Randomized, prospective, open label, phase 4 trial. N=80.	NCT04411680 NCT04326920	[81]

Mesenchymal stem cell (MSC); Derived Mesenchymal stem cell (DMSC)	Stem cells	Preventing the stormy release of cytokines by the immune system and encouraging endogenous repair through stem cell reparations.	Randomized, prospective, open label, phase 2 trial. N=20. Randomized, parallel assignment, single masked, phase 1 study. N=20.	NCT04444271 NCT04346368	[82]
Convalescent Plasma therapy	Plasma	Plasma obtained from patients recovering from COVID-19 may harbor SARS-CoV-2 antibodies. Clinical studies are underway to assess the use of COVID-19 convalescent plasma in treating patients with severe or infections due to COVID-19; however it is not anticipated for infection prevention.	Randomized, open label, phase 2, multi-centre trial. N=278. Randomized, prospective, single-institution, single-arm, phase 1/2 study. N=67.	NCT04345523 NCT04438694	[83]
Sarilumab	Human, monoclonal antibody	Inhibition of the interleukin-6 (IL-6) pathway	Randomized, open label, phase 2, pilot study. N=30.	NCT04357808	[84]
Nitric oxide Inhalation	Inhalation	Acts as a targeted pulmonary vasodilator that is employed in refractory hypoxemia as a rescue treatment because of the precipitation of acute respiratory distress syndrome (ARDS). Even, in-vitro and clinical results shows that nitric oxide gas (iNO) inhaled has antiviral activity against other coronaviral strains.	Randomized, parallel assignment, triple masked, phase 2 study. N=260.	NCT04338828	[85]
Losartan potassium	Angiotensin receptor blocker	It was shown that losartan-treated mice after acid-induced acute lung injury (with the addition of SARS-CoV spike protein) had substantially reduced lung injury and pulmonary oedema relative to placebo-treated mice. Reports suggests an improvement in lung injury in patients with SARS-CoV infection	Open label, phase 1 trial. N=50. RCT, placebo controlled, phase 2 trial. N=200.	NCT04335123 NCT04312009	[86]
Aviptadil	Synthetic version of Vasoactive Intestinal Polypeptide (VIP)	Aviptadil is a vasodilator which reduces blood pressure, when intravenously administered. This can significantly reduce inflammatory mechanisms by functioning on the white blood cells implicated in granulomal formation.	RCT, placebo controlled, phase 1 trial. N=80. Randomized, placebo-controlled, phase 2 trial. N=144.	NCT04536350 NCT04311697	[87]
CD24Fc	Non-antiviral Immunomodulator	It is a biological which acts by preventing TLR activation and also involved in activating Siglec signalling.	Randomized, placebo-controlled, double-blinded, multicentre phase 3 study. N=241.	NCT04317040	[88]

RCT, randomized controlled trial

tive but CT aids in diagnosis by generating the typical findings seen in COVID-19 pneumonia like peripheral, subpleural ground-glass opacities, often in the lower lobes.[59] The cycle threshold (Ct) value of RT-PCR can help in detecting patients who are infectious and who are not, since one study found that a Ct value of more than or equal to 34 seems to be non-infective.[62] The stages of appearance and waning of viral RNA material and antibodies are depicted in figure 2.[63]

Treatment

There is currently no clinically approved, appropriate vaccine available for the treatment of COVID-19. Few drugs have been approved recently depending on the disease severity like remdesivir, favipiravir, and, dexamethasone.[64,65,66] The most effective management technique remains supportive care, including oxygen therapy, fluid control and use of wide spectrum antibiotics to mitigate secondary bacterial infections. Several therapeutic interventions are still under evaluation, and further research is anticipated. The details of available treatment modalities which are mostly on basis of compassionate use in emergency condition and trial purposes are illustrated in Table 4.

Potential vaccines

There is comprehensive work in the advancement of vaccine development for COVID 19 as it is believed to be the only viable effective prevention technique like all other viral epidemics to improve the community's herd immunity and thereby limit the spread. According to the WHO draft (dated 20 March 2020), there were 2 vaccines in the clinical evaluation phase (Phase 1-ChiCTR2000030906; Phase 1-NCT04283461) with 42 more candidate vaccines in the pipeline of pre-clinical research evaluation.[89] The various constituents that can be used for vaccine development are depicted in figure 3.

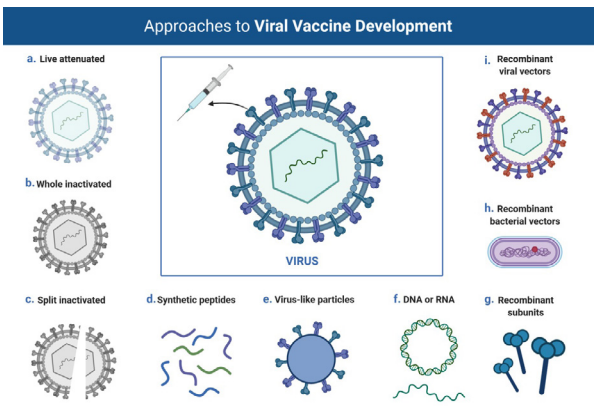

Fig. 3. Concept of potential SARS-CoV-2 vaccine development
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Table 5. Vaccine candidates with sponsor names and trial phase

Candidate	Sponsor	Trial phase
AZD1222	The University of Oxford; AstraZeneca; IQVIA	Phase 2/3
BBV152 Covid vaccine (Covaxin)*	Bharat Biotech International Limited (BBIL); National Institute of Virology	Phase 2/3
Inactivated vaccine	Institute of Biological Products (Wuhan); National Pharmaceutical Group (Sinopharm)	Phase 3
CoronaVac	Sinovac	Phase 3
mRNA-1273	Moderna	Phase 3
BNT162	Pfizer, BioNTech	Phase 2/3
Gam-COVID-Vac	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Phase 1/2, 3
Ad26COVS1	Janssen Pharmaceutical Companies	Phase 1/2, 3
Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M.	Novavax	Phase 1/2, 2b, 3
Ad5-nCoV	CanSino Biologics	Phase 2
Adjuvant recombinant vaccine candidate	Anhui Zhifei Longcom Biopharmaceutical, IMCAS	Phase 2
BBIBP-CorV	Beijing Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	Phase 1/2
GX-19	Genexine	Phase 1/2
ZyCoV-D	Zydus Cadila	Phase 1/2
Self-amplifying RNA vaccine	Imperial College London	Phase 1/2

IMCAS, Institute of Microbiology of the Chinese Academy of Sciences

For now, a nasal vaccine for COVID 19 has been developed by Bharat Biotech® which has presented to be safe and effective in phase I and phase II of the clinical trials and will be further rendered by introducing gene sequences from SARS-CoV-2 into M2SR (influenza virus self-limiting version) so that the new developed vaccine can also trigger immunity against coronavirus.[90] In

addition, on April 2nd2020, the University of Pittsburgh unveiled a possible SARS-CoV-2 vaccine that can be administered through a fingertip patch which generates SARS-CoV-2-specific antibodies in amounts considered adequate to neutralize the virus.[91] A recent epidemiological research has shed some light on the usage of BCG vaccination to lower down COVID 19 related wellness and risk of death.[92] Few of the vaccines which are in phase 1/2 or phase 3 with their sponsor are listed in Table 5 below which are being adopted from the WHO vaccine landscape.[89]

Existing treatment protocol in adults with COVID-19 as per severity

The treatment protocol varies marginally from one country's guideline to the other, but the essential components of addressing the underlying pathology and critically ill intervention remain unchanged. The summary of existing treatment protocol has been summarized in figure 4.[93, 94]

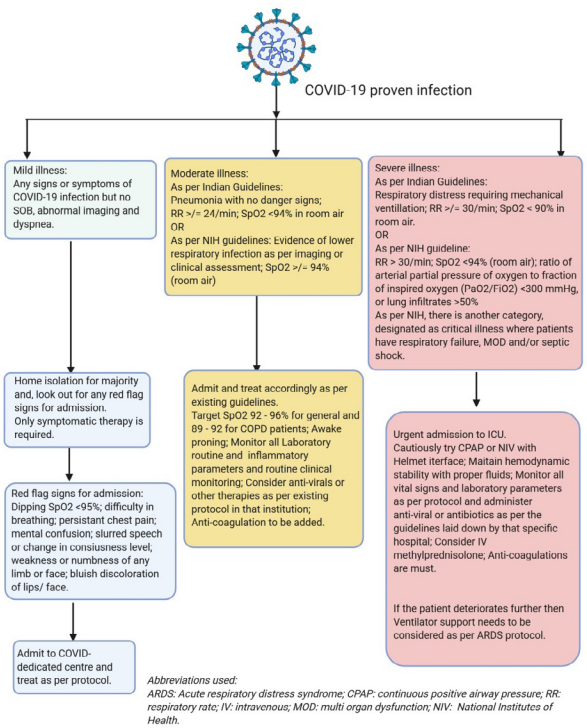

Fig. 4. Summary of treatment protocol as per disease severity
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New learning points from this review

COVID-19 related publications have reached a magnitude of 23000, 5898, and 5393 publications in PubMed, Elsevier, and RG (research gate), respectively, over a few months.[95] Review articles have highlighted the importance of hand-hygiene, travel restrictions, mask use, and physical distancing continuously. Rapid diagnostic assays, effective therapeutic strategies, and prevention through the rapid development of vaccines are the most important step in our battle against this virus.[96, 97] Travels, both international and national, should be restricted only for medical emergency purposes with screening at all levels and self-reporting of the passengers' symptoms. Quarantining of suspected people and their close contacts, increasing public awareness of the non-specific symptoms of the disease, and maintaining proper personal hand hygiene and mitigating social gatherings all have been suggested to play crucial roles in preventing this virus from spreading.[98] This review attempts to summarize the epidemiology, pathogenesis, clinical features, mode of transmission, diagnostic tests with their correct interpretation, available treatment modalities, and on-going vaccine research for COVID-19.

Conclusion

COVID-19 is a pandemic similar to the H1N1 outbreak in 2009 and appropriate action should be taken to curtail the spread of the virus, especially when considering the high-risk population comprising of children, elderly population and the healthcare professionals. The outbreak began in mainland China, with a regional concentration at Wuhan City, Hubei, thereby spreading its arm in rest of the world at an alarming rate with an increased incidence when compared to the epicenter. Clinical research suggest that patients routinely exhibit symptoms consistent with viral pneumonia with the commencement of COVID-19, sore throat, cough, myalgia, fatigue and fever being the common ones. Estimated 80 percent of documented hospitalized patients had mild to moderate form of the stated disease, which happened to involve cases of non-pneumonia and pneumonia, and 13.8 percent of the population presented with serious illness. In such a complex scenario, compelling multifarious intervention against COVID-19 should be imposed to initiate the deceleration phase of the disease. Promoting social isolation, avoiding crowds, wearing mask and gloves along with washing hands with soap and water needs to be advocated to limit the exponential spread. Because asymptomatic patients may spread the disease, studies about its transmission needs to be explored. The current treatment initiatives are geared towards symptomatic diagnosis and oxygen therapy. However, prophylactic vaccination is the need of the hour, which would help to avoid COV-related epidemics or pandemics in the future.

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CASUISTIC PAPER

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Idiopathic transient osteoporosis a rare and underdiagnosed entity a case report with a review of the literature

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ABSTRACT

Introduction. Idiopathic transient osteoporosis of the hip is a rare but underdiagnosed condition. It is common in middle-aged men and pregnant women. The exact etiology is unknown.

Aim. We present a 52-year-old man presented with progressively increasing pain left hip for two months.

Description of the case. The radiograph showed osteoporosis localized to the proximal femur. Magnetic resonance imaging showed bone marrow edema. He was diagnosed as a case of idiopathic transient osteoporosis of the left hip (ITOH) after ruling out other causes. He was treated nonoperatively with analgesics and rest. He was given daily calcium and monthly ibandronate 150mg. His symptoms subsided after 3 months. There was no recurrence of symptoms.

Conclusion. We present this case to describe the clinical, radiological features, diagnosis, and treatment of ITOH. Idiopathic transient osteoporosis is a rare condition. It is often not diagnosed because of a lack of awareness and also being a self-limiting condition. The radiogram may be normal. So a high index of suspicion is needed for its diagnosis.

Keywords. bisphosphonate, idiopathic transient osteoporosis of the hip, regional migratory osteoporosis

Introduction

Idiopathic transient osteoporosis of the hip is a rare but underdiagnosed condition. It is common in middle-aged men and pregnant women. The exact etiology is unknown. The possibilities, like reflex sympathetic dystrophy, small vessel ischemia, obturator nerve compression, intramedullary hypertension, fatty marrow conversion of the proximal femur, and hormonal and chemical change during pregnancy are some of the postulations. It is a self-limiting condition with radiological evidence of localized osteoporosis around the hip. The diagnosis is usually made by excluding other cases.[1,2]

ITOH is an uncommon condition. Most cases are misdiagnosed due to a lack of awareness among surgeons.

Aim

Our objective is to describe the clinical features and diagnosis of a 52-year-old man with ITOH along with a literature review. We think this case will be a re-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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minder for us to consider transient osteoporosis as a differential diagnosis in adult patients with sudden onset hip pain.

Description of the case

A 52-year-old man presented with gradual onset of progressively increasing pain left hip for two months. There was no history of trauma or constitutional symptoms. No hip ailment in childhood, a non-smoker and non-alcoholic. He had an antalgic gait, with no limb length discrepancy. There was tenderness in the left hip. Range movements were normal. No abnormalities were detected in his routine blood and urine examinations. Radiogram showed osteoporosis in the left proximal femur extending from the head to the trochanteric region, without loss of shape of the femoral head, or subchondral fracture (Fig 1).

An ultrasound scan showed minimal effusion in the left hip. MRI scan revealed diffuse bone marrow edema in the proximal femur with minimal effusion. No evidence of subchondral fracture or collapse of the head. The lesion was hypointense on the t T1W image and hyperintense on the T2W image (Fig 2, 3).

An evaluation to rule out a bone metastasis was negative. After excluding other possibilities a diagnosis of ITOH was made. He was treated symptomatically with analgesics and rest. The graduated exercises were done as tolerated by him. He was given daily calcium supplementation with ibandronate 150mg orally once in a month. His symptoms subsided after 3 months and no recurrence of symptoms or other joint involvement during follow-up was noted.

Discussion

ITOH is a non-destructive and self-limiting condition of the hip. It is a poorly understood and forgotten clinical entity.[2] It is an uncommon disorder characterized by transient pain and disability with osteopenia in the hip area.[3] Curtiss and Kincaid first reported ITOH as 3 cases of hip pain in women in the third trimester of pregnancy in 1959. There was periarticular osteoporosis and no cause was found. The symptoms subsided spontaneously. Later similar cases were described by De Marchi, Santacroce, and Solarino in men also. ITOH is also known as transient bone marrow edema syndrome (TBOS). It may be associated with migrating arthralgia of weight-bearing joints of the lower limbs called regional migratory osteoporosis (RMO).[4] It is more common in males. Common in the 5th and 6th decades. The patients usually present with a dull aching pain in the groin progressively increasing in intensity over two to three months. There will be difficulty in bearing weight. Wasting of muscles may be present in some cases. Minimal signs will be present during the examination of joints. The range of movements will be full though terminally painful. This discrepancy between disability and lack of signs during examination is an important clue to the diagnosis of ITOH. The symptoms may last for 7 to 9 months thereafter it completely resolve. The natural history of ITOH has 3 phases. The initial phase lasts for

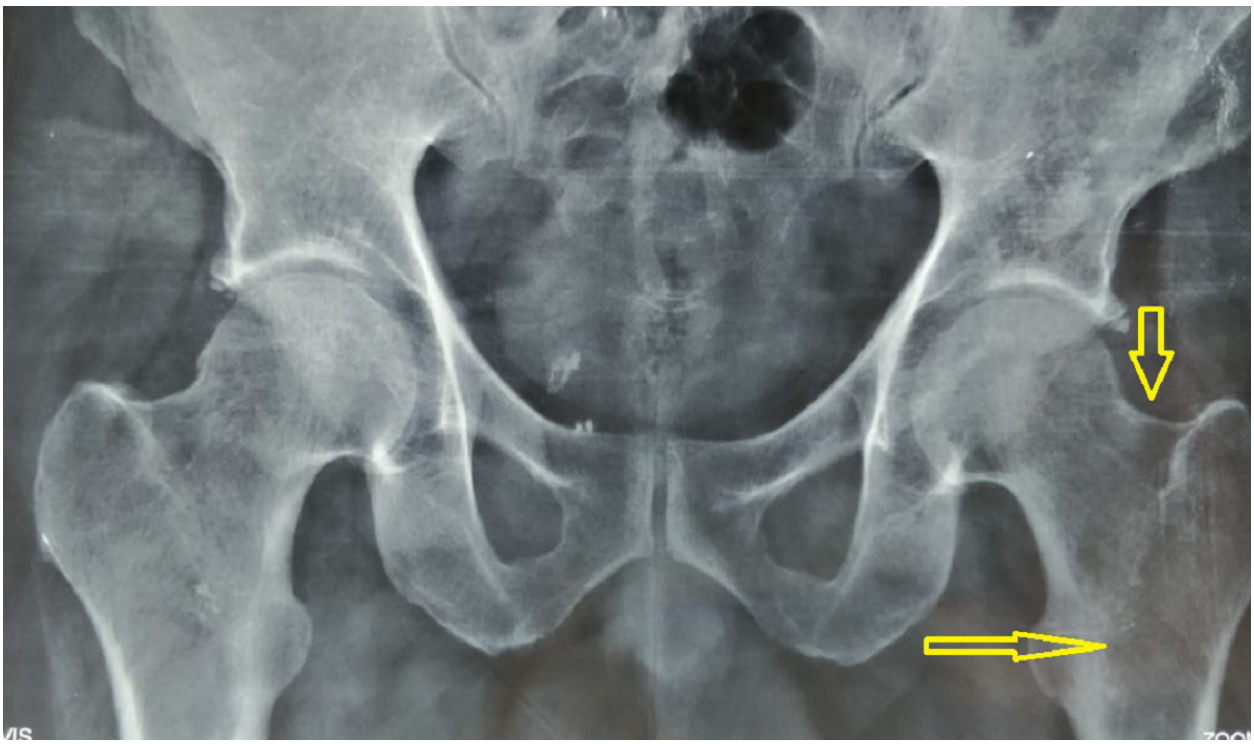

Fig. 1. Radiograph of the pelvis anteroposterior view showing the diffuse osteoporosis in the left proximal femur including head, neck, trochanteric region (arrows)

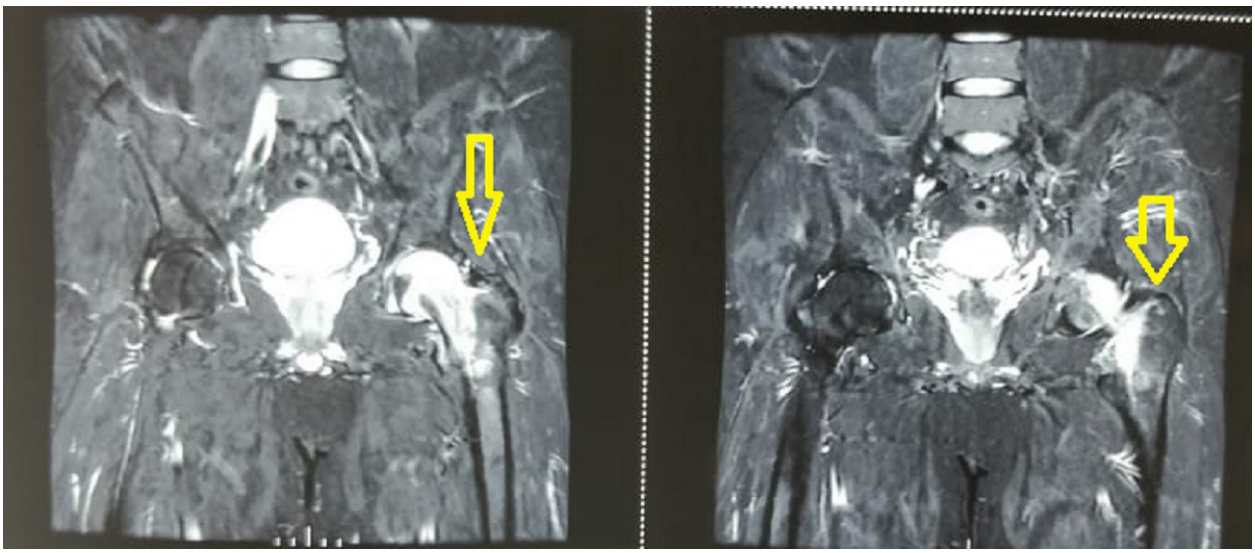

Fig. 2. MRI Scan of the pelvis showing a hyperintense lesion in the head, neck, and trochanteric region of the left hip. There is no evidence of a subchondral fracture of the collapse of the femoral head

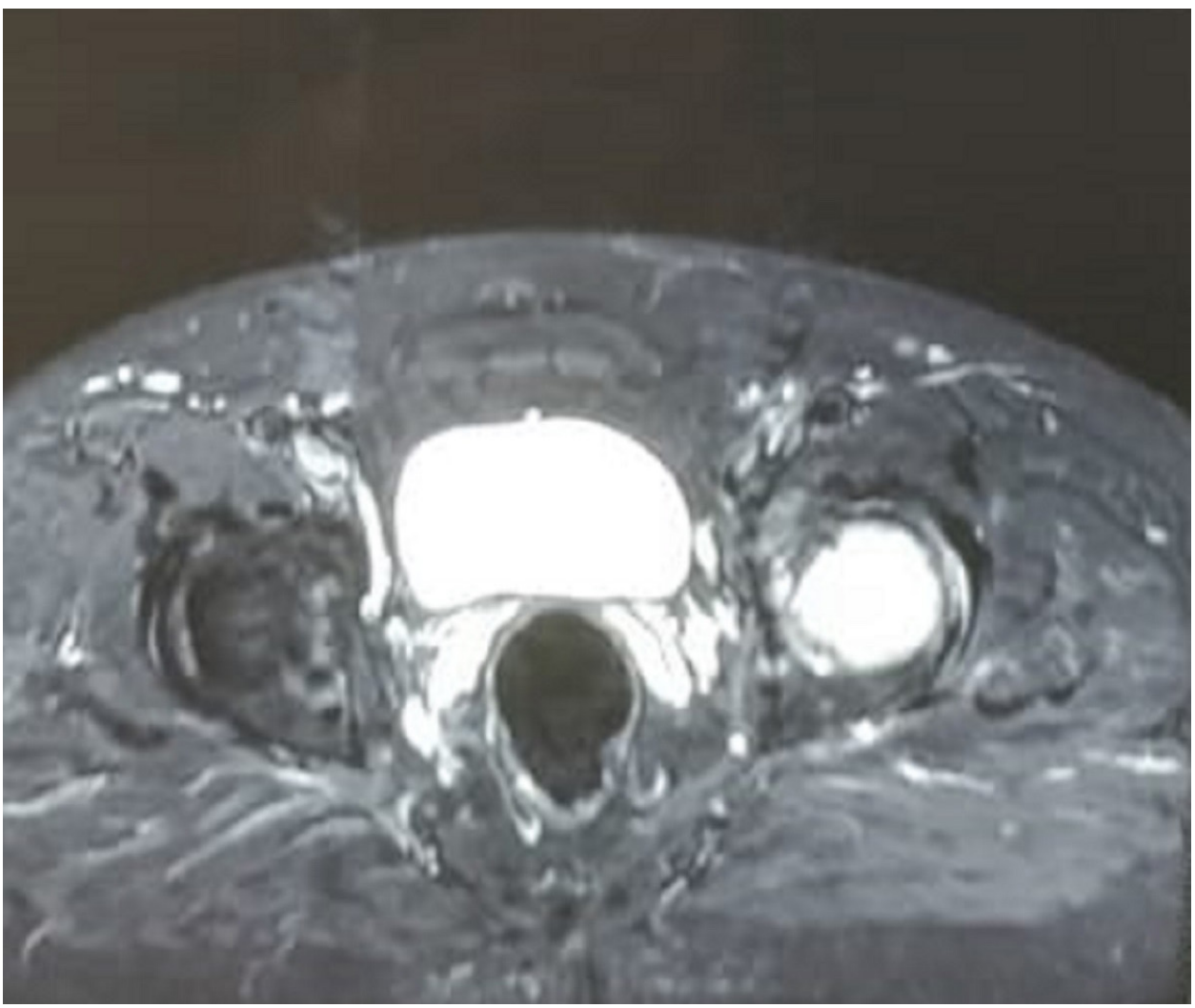

Fig. 3. Transverse MRI image showing a hyperintense lesion in the head, the neck of the left hip

about a month. In this phase, there is a sudden onset of pain and functional limitations. This is followed by plateauing of symptoms for about 2 to 3 months this is the second phase. The third phase is characterized by a gradual decline in symptoms followed by spontaneous regression. It usually takes four months. There is osteopenia during the second phase and bone density returns to normal in the final stage.[1] During pregnancy, it usually starts during the 3rd trimester and subsides by the 3rd month postpartum. It is usually diagnosed as symphysiolysis without tenderness over the pubic symphysis. There can be involvement of other joints in ITOH. It can precede or follow the involvement of the hip. Then it is called RMO. Usually, the migration occurs from proximal to distal joints. Intraarticular migration is described between different compartments of the knee, and between different joints of the foot. Most of the time migration to other joints occurs within a year. Axial skeletal involvement with compression fractures is described in RMO. No involvement of upper limb joints is described in RMO. The diagnosis of ITOH is usually by exclusion.[4] Rarely fracture of the neck of the femur can occur in ITOH. This complication is commonly seen in transient osteoporosis in pregnancy.[5]

The radiogram shows uniform diffuse radiolucency from the femoral head to the intertrochanteric region. There is no cavitation or subchondral collapse. In some cases, the X-ray may be normal. Rarely acetabulum may show osteoporosis. The joint space is preserved. The loss of subchondral cortical shadow is the pathognomonic sign of transient osteoporosis of the hip in the radiograph.[6] There is an increased uptake bone scan. Increased uptake is in areas from head to the intertrochanteric region.[7] Radiological findings can be confused with infection, avascular necrosis, or malignancy. The MRI scan shows bone marrow edema in the proximal femur. There is decreased signal intensity of the bone marrow on T1W images and increased signal intensity relative to normal marrow in T2W images. These are seen both in the epiphysis and metaphysis predominantly along the primary trabeculae. These findings distinguish it from senile osteoporosis and atrophic osteoporosis where there are no signal intensity changes of bone marrow in MRI. Neoplasms and infection can mimic ITOH but they predominantly affect metaphysis. The absence of a well-defined demarcation zone and the area of decreased signal intensity on long SE sequences helps to differentiate it from avascular necrosis in an MRI scan. Bone necrosis with increased turnover and inflammatory changes are seen in bone biopsy.[8-10]

Treatment of ITOH aims at the reduction of pain and disability. Judicious use of NSAIDs protected weight-bearing and graduated physiotherapy is the mainstay of nonoperative treatment. Intermittent traction is used to reduce pain and spasm, but not in pregnancy. Oral, intravenous, intramuscular bisphosphonates are reported to be helpful. Antiresorptive agents like calcitonin have shown to reduce the duration of illness. Bone sparing steroids like deflazacort were also tried. A prostaglandin analog Ilaprost has shown some promising results.[11] Hyperbaric oxygen is also tried in the treatment of ITOH.[3] But lack of randomized control trials, small sample size, and the self-limiting nature of the disease question the usefulness of these drugs.[12-15] Core decompression has shown early relief of pain in ITOH. But it should be used only in prolonged and incapacitating cases.[16] The core decompression can give faster recovery. But many authors suggest it as an unnecessary surgery in ITOH.[17]

We presented this case to make us all aware of this condition. Most of the time we miss the diagnosis and the patient gets relief from his symptoms due to self-regression. A strong index of suspicion is required for its diagnosis.

Conclusion

In conclusion, transient osteoporosis of the hip is an underreported entity. Pain and disability out of proportion to clinical signs give us a clue to its diagnosis. More researches in the future may help us to better understand the idiopathic transient osteoporosis of the hip.

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CASUISTIC PAPER

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Gastrointestinal symptoms as antecedent signs of severe acute respiratory syndrome coronavirus 2 infection

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ABSTRACT

Introduction. The coronavirus disease 2019 (COVID-19) is an acute infectious disease of the respiratory system caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2).

Most patients present with typical, respiratory symptoms. Common signs include cough, fever, dyspnea and shortness of breath. In this case we provide atypical indications of COVID-19, which may occur earlier than respiratory symptoms.

Aim. This case is an example of an unusual course of SARS-CoV-2 infection.

Description of the case. This article describes a case of a 63-year-old man and his wife, a 60-year old woman who were admitted to the emergency department with a few days' history of gastrointestinal symptoms. Both patients presented with the digestive symptoms of nausea, diarrhea and loss of appetite. They denied abdominal pain and the loss of smell or taste. Due to suspicion of SARS-CoV-2 infection a nasopharyngeal swabs of both patients was taken.

The results of real- time reverse transcriptase-polymerase chain reaction were positive. When the final diagnosis of COVID-19 was established they were transported to another hospital.

Conclusion. COVID-19 may manifest with atypical indications such a nausea and diarrhea. An atypical indications of COVID-19 may occur earlier than respiratory symptoms. It is important for clinicians to remain alert.

Keywords. COVID-19, diarrhea, gastrointestinal, SARS-CoV-2

Introduction

The coronavirus disease 2019 (COVID-19) is an acute infectious disease of the respiratory system caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2). The first cases were reported in Wuhan City, Hubei Province, China in December 2019. The most common, typical symptoms include: cough, fever or dyspnea. However unusual manifestations of the disease are also observed.[1,2]

COVID-19 may be initially masked by gastrointestinal symptoms. Human intestinal epithelial cells are susceptible to SARS-CoV-2 infection. SARS-CoV-2

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tropism to the gastrointestinal tract involves angiotensin-converting enzyme (ACE)-2 receptors, which are also present in the intestines.[3,4]

Aim

This case is an example of an unusual course of SARS-CoV-2 infection.

Description of the case

A 63-year-old Caucasian man and his wife, a 60-year old Caucasian woman were admitted to the emergency department on July 5, 2020 with a few days' history of weakness, nausea, and diarrhea. The first symptoms in both patients about one week ago appeared. They denied contact with individuals exhibiting respiratory symptoms and any travels. The man's past medical history included arterial hypertension and benign prostatic hyperplasia. He was undergoing treatment with angiotensin-converting enzyme inhibitor (ramipryl) and alpha-1-adrenergic receptor antagonist (tamsulosin). He mainly reported nausea, a loss of appetite, weakness, and diarrhea for 3 days, and to a lesser extent, mild coughing. He denied vomiting, abdominal pain, loss of smell and taste. His body temperature was 36.4°C and blood pressure was 140/90 mmHg; his heart and respiratory rates were normal at 80 bpm and 16 breaths/min, respectively. The saturation on pulse oximetry was 92%. Auscultation revealed cracking in the lower right lung field.

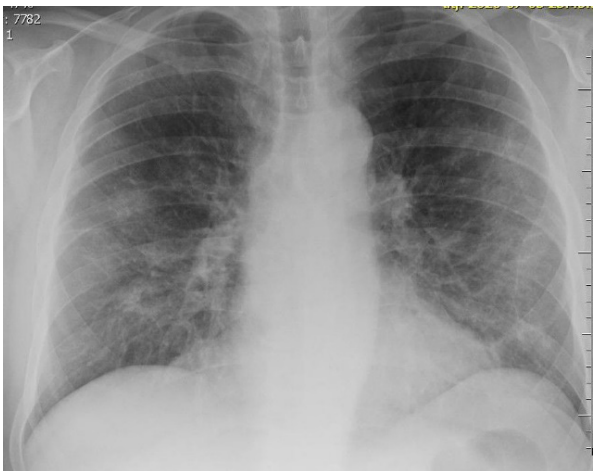

Fig. 1A. Imaging findings of a 63-year-old man with coronavirus disease 2019. Chest X-ray showing bilateral pneumonia affecting the lungs

The woman's past medical history included arterial hypertension. She was undergoing treatment with angiotensin-converting enzyme inhibitor (ramipryl) and beta-adrenergic receptor antagonist (metoprolol). She mainly reported nausea, a loss of appetite, diarrhea for 4 days and weakness. She denied abdominal pain, loss of smell and taste. A few days ago she had one-off an episode of vomiting. Her skin was sweaty, body temperature was 36.8°C, and blood pressure was 150/90 mmHg; her heart and respiratory rates were normal at 90 bpm and 20 breaths/min, respectively. Her saturation on pulse oximetry was 93%. The lungs appeared normal on auscultation. Both patients underwent chest radiography of the lungs, revealing bilateral inflammatory changes in the man (Fig. 1A) and basal, bilateral inflammatory changes in the woman (Fig. 1B).

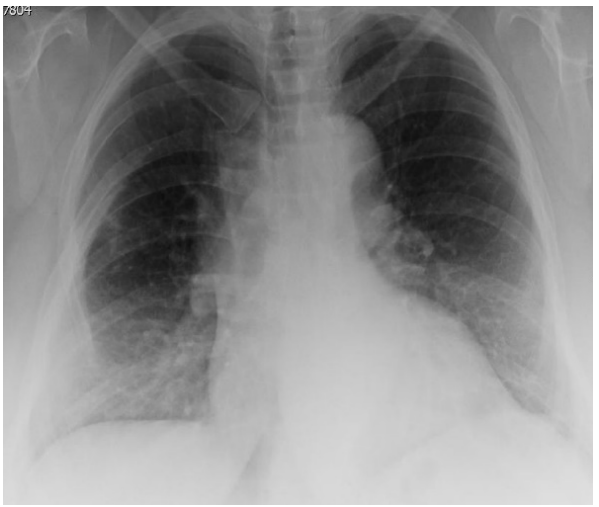

Fig. 1B. Imaging findings of a 60-year-old woman with coronavirus disease 2019. Chest X-ray showing basal, bilateral inflammatory changes in the lungs

The man's laboratory values on admission were as follows: leukocytosis with a predominance of neutrophils (neutrophil count, 10820 cells/μL; normal, 1900–7500), elevated C-reactive protein (46.9 mg/L; normal, <10), elevated D-dimer (1126 ng/mL; normal, <500), normal electrolyte levels, increased aspartate transaminase (47 U/L; normal, <34), and low procalcitonin (0.06 ng/mL; normal, <0.5).

The woman's laboratory test showed a normal white blood cell count, elevated C-reactive protein (52.1 mg/L; normal, <10), normal electrolyte levels, increased aspartate transaminase (110 U/L; normal, <34), low procalcitonin (0.04 ng/mL; normal, <0.5), increased gamma-glutamyltransferase (367 U/L; normal, <38), and increased alkaline phosphatase (233 U/L; normal, 46–116). With COVID-19 suspicion, nasopharyngeal swabs were obtained from the patients. While awaiting the results, the man one dose of an intravenous infusion of ceftriaxone (2 g) and fluids received. The women anti-emetics and fluids received.

Realtime reverse-transcriptase polymerase chain reactions of the nasopharyngeal swabs of both patients revealed positivity for the SARSCoV2 nucleic acid. Due to desaturation, both patients underwent oxygen therapy with a face mask (3 L/min O_2) and were admitted to a hospital specializing in infectious diseases, which was further than 20 km from our emergency department.

Unfortunately, we do not know the outcome of the disease in both patients.

Discussion

Gastrointestinal symptoms are reported in the literature as less common indications of COVID-19, which may occur earlier than respiratory symptoms and fever.[4-8]

The analysis of the available data presented by D'Amico et al. revealed an overall diarrhea rate of about 10% in COVID-19 patients.[3] The incidence of diarrhea in COVID-19 is certainly underestimated. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) which is expressed not only in lung but also in the esophagus, liver, small intestinal and colon epithelia.[4]

In a study from hospitals in China presented by Guan et al, it reported nausea or vomiting in 55 (5%) and diarrhea in 42 (3,8%) patients.[5]

Nausea and diarrhea may be the only manifestations in the early stage.[6-8]

Furthermore, some studies have demonstrated the presence of viral RNA in stool or rectal swabs of COVID-19 patients.[9,10] This suggested that fecal source can also lead to viral transmission.

Conclusion

Digestive symptoms are becoming increasingly more common in patients with COVID-19 and may be an antecedents signs of SARS-CoV-2 infection. In this case study, we find that COVID-19 may manifest with gastrointestinal indications such a nausea and diarrhea. An atypical indications of COVID-19 may occur earlier than respiratory symptoms. It is important for clinicians to remain alert.

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CASUISTIC PAPER

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Sciatic vessels – a case report

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ABSTRACT

Introduction. Sciatic vessels most often accompany the sciatic nerve. Sciatic vessels are very rare.

Aim. In this paper we determine the procedure in sciatic vessels surgical treatment.

Description of the case. We present the case of a 75-year-old patient with symptoms of acute right lower limb ischemia. The patient was discharged home in good condition, and remains in outpatient control to this day.

Conclusion. The popliteal artery proved to be available, but much deeper than usual.

Keywords. artery, sciatic vessels, surgical treatment

Introduction

Sciatic vessels most often accompany the sciatic nerve (nervus ischaicus).[1-3] Sciatic arteries (arterial ischiadica) extend into the popliteal artery and then 2/3 of the distal lower limb draws blood from the internal iliac artery. In young mammalian embryos, the sciatic artery is still the main vessel of the free part of the lower limb and only in later periods of its development does it replace the femoral artery (arteria femoralis).[4,5]

Aim

The aim of this study was to present a case of the acute vascular disease.

Description of the case

A 75-year-old office worker was admitted to the Department of Vascular Surgery with symptoms of acute right lower limb ischemia. In the history of tobacco smoker in the past diagnosed COPD/bronchial asthma, hyperthyroidism during thyrostatic treatment. On the day of illness, the following ailments appeared: sudden, severe right lower leg pain, cooling down, disturbed sensation of the lower leg and right foot. In addition, the patient gave limited mobility and hindered the ability to walk and load the right lower limb, which so far did not occur. In the physical examination: the skin appendages were symmetrical, veins of

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the right foot collapsed, venous return in more than 2 seconds. Shortly after the first symptoms appeared, the patient was taken to the Emergency Department of the Poviat Hospital, where basic laboratory tests with the coagulation system were taken and Duplex Doppler ultrasound was performed. Then the patient was transferred to the Admissions Room of the Vascular Surgery Ward, after analyzing the above information, acute ischemia was diagnosed - right lower limb arterial embolism. However, the etiology of its creation was unknown. Due to long-term nicotinism, arterial thrombosis seemed highly likely. An ultrasound examination was performed again confirming the lack of flow in the femoral vessels of the right lower limb. The patient was qualified for urgent surgery. Shortly after being admitted to the Department of Vascular Surgery, the patient was taken to the operating theater, where, after proper preparation, he was spinal anesthetized. Typical vessels were not seen in the properly dissected groin. Therefore, using the operating room with constant access to angiography through a deep vessel found in the groin not reminiscent of a typical femoral artery, arteriography was performed, which visualized the contrasting of vessels above the inguinal ligament in an unusual arrangement, the branches of the external iliac artery were contrasted with a small diameter, which had their end sections on the medial side of the thigh, while the internal iliac artery had a clearly enlarged trunk that suddenly ended.

There was a difficult question in surgery, what's next??

The next floor, where the cut appeared, was access to the popliteal artery above the right knee, because there was an outline of contrasting vessels, of a fairly large diameter.

Reasoning and treatment proved effective. The pulse on the left leg was well felt under the buttock. An interview was completed in which the patient reported that while sitting felt the pulse, he could not sit in the chair/armchair for a long time.

Discussion

The study found right-sided occlusion of the external iliac artery, femoral joint, deep femoral, superficial femoral artery, while the flow in the arteries below the knee was seen - like 'behind obstruction'. In the basic studies, no significant deviations were found, in the ECG examination without arrhythmias, the history of vascular malformations, including negative aneurysms. The popliteal artery proved to be available, but much deeper than usual. However, having access to a vessel with a diameter of about 10 mm, it was possible to carry out further diagnostics that gave a specific answer.[7-9]

Conclusions

The initial diagnosis was confirmed - the acute vascular disease was caused by an embolism that arose in the aneurysmal altered "persistent sciatic artery", which is extremely rare as anatomical variability. After the saphenous vein was dissected, a bridge was made, while the sciatic artery was ligated above the anastomosis (cutting off the pathway to subsequent embolisms). We report herein the unique case. In most cases, the sciatic artery is the main dominant inflow vessel to the lower extremity and persistent sciatic artery is strongly associated with aneurysmal disease, with a high potential for thromboembolic events.

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CASUISTIC PAPER

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Numerous gastrointestinal tumors

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ABSTRACT

Introduction. Gastrointestinal stromal tumor (GIST) is most often locate in the region of the stomach and the proximal part of the small intestine.

Aim. The multiple histopathological examination is described.

Description of the case. Multiple GISTs are rare neoplasms that originate from the interstitial cells are described.

Conclusion. GIST can occur in any part of the gut, they are most common in the stomach and small intestine, and less frequent in the colorectum and esophagus. Although their pathogenesis and clinical manifestations are different, these tumor syndromes confer a high risk for developing multiple neoplasms.

Keywords. gastrointestinal stromal tumor, histopathological examination, multiple neoplasms, small intestine

Introduction

Gastrointestinal stromal tumor (GIST) arises from CD34 positive Cajal cells.[1] Although they may occur along the entire length of the digestive tract, they most often locate in the region of the stomach and the proximal part of the small intestine.[2] Occurs at a frequency of 0.68 per 100,000.[3] The main method of treatment is surgery, however, after describing the KIT or PDGFRA mutation, imatinib-directed therapy has become possible.[4] In addition, many types of mesenchymal tumors may arise in the gastrointestinal tract. Their names refer to the cells they resemble or the tissues from which they originate. Neoplasms of nerve casings are schwannomas or schwannomas. Their location in the small intestine is extremely rare.[5] Pre-operative diagnosis of this cancer is difficult. In Japan, none of the reported cases were correctly diagnosed before surgery.[6] Benign neoplasms of smooth muscle are myomas. They

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constitute about 30-35% of benign small intestine tumors.[7] These tumors are made of spindle cells and require differentiation between themselves.[8]

Aim

The study presents a female patient with the presence of a small intestine tumor and a stomach tumor. Histological analysis assist in establishing a correct diagnosis.

Description of the case

A 75-year-old female patient without a history of chronic disease was admitted to the Gastroenterology Clinic due to changes in the abdominal CT scan suggesting the presence of a small intestine tumor and a stomach tumor. In the gastroscopy performed, the impression of the posterior gastric wall is described. In balloon-derived enteroscopy, the oral jejunum was visualized in the jejunum, approximately 40-50 cm after the pylorus (below the Treitz ligament), a prominent fragment of the intestinal wall with pronation. 20 x 30 mm. Specimens from the lesion and from the macroscopically normal part of the jejunum were collected. In the results obtained from the Department of Pathology, the correct intestinal membrane was described, with edema, congestion and dilated vessels. After the surgical consultation, the patient was qualified for surgical treatment in planned mode. A month later, the patient was admitted to the Surgery Clinic. After the preparation, it was operated. Peripherally, the tumor was found in the distal part of the stomach, jejunum and ileum. Three preparations were sent for histopathological examination:

a. Fragment of the small intestine (3.5 cm) with frill for 2.5 cm. In the muscle membrane of the intestine, the gray cohesive node is 0,3 cm without communication with the mucous membrane or mesentery.
b. Fragment of the small intestine (3.5 cm) with a tumor 4.5 x 4.5x2 cm. Macroscopically, a gray tumor, cohesive bound to the muscle membrane.
c. A fragment of the stomach measured along the curvature of greater 10 cm, along a 9 cm curvature with a 2.5 x 2.5 x 2.5 cm tumor located on the posterior wall and the greater curvature. At a distance of 3cm from the cut border and 4.5cm from the proximal border, a gray solid tumor limited to the muscle membrane. Intra-and postoperative course uncomplicated. The patient was discharged home.

Results

The majority of GISTs occur as sporadic solitary neoplasms resulting from somatic mutations. Currently, there are no criteria for the diagnosis of primary versus metastatic tumors in patients with multiple tumors. The prognosis of patients with multiple GISTs is similar to that of patients with solitary tumors.

The results (Figures 1-6) in histopathological examination was as follow:

a. Neuroma from Schwann cells of the small intestine: IHC: S100 (+), Desima (-), SMA (-), CD34 (-), CD117 (-), Ki67 (+) about 1%
b. Leiomyoma of the small intestine: IHC: SMA (+), CD (-), CD117 (-)
c. Gastrointestinal stromal tumor (GIST) pT2NoMX, Mitosis index 2/50 dpw IHC: CD117 (+), CD34 (+), SMA (-)

Discussion

GISTs belong to a group of cancers called soft-tissue sarcomas. Soft-tissue sarcomas develop in the tissues that support and connect the body. Widely used diagnostic endoscopy allows to reduce the extent of the procedure.[9] However, it should be remembered that there is still no consensus of experts on important issues regarding gastrointestinal tumors. An example is the presence of necrosis in GISTs, and its translation into prognosis in patients.[10] **CT or CAT scan are** uses x-rays to make detailed pictures of the inside of soft-tissue sarcomas your body. CT scans can often show the size, shape, and place

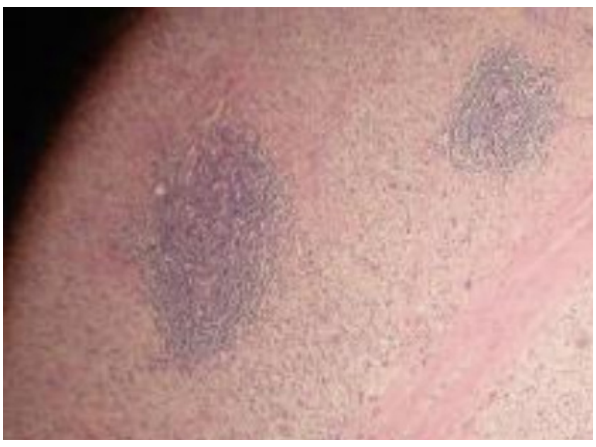

Fig. 1. Schwannoma HE 40x (Schwan4)

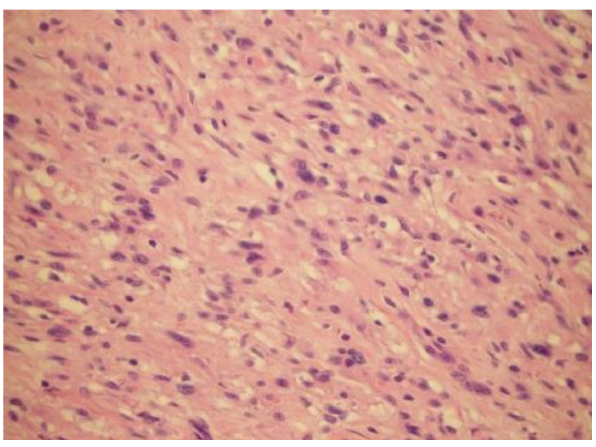

Fig. 2. Schwannoma HE 400x (Schwan40)

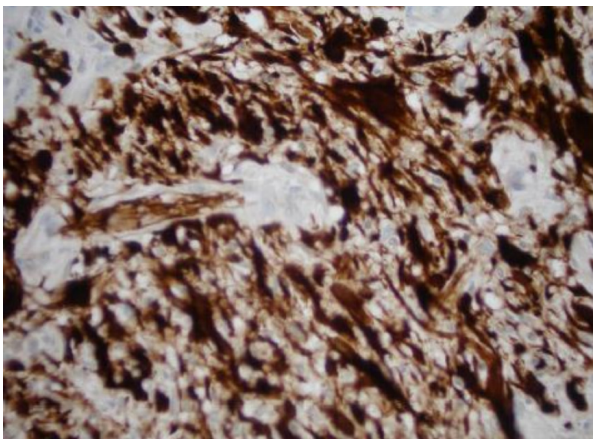

Fig. 3. Schwannoma S100 400x (SchwanS100)

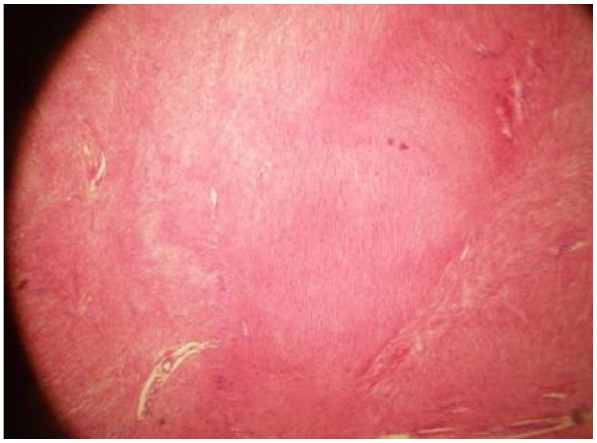

Fig. 4. Leiomyoma leiomyoma 40x (Myo4)

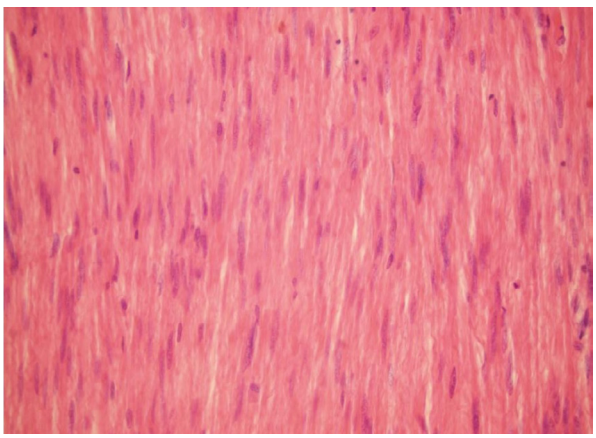

Fig. 5. Leiomyoma leiomyoma HE 400x (Myo40)

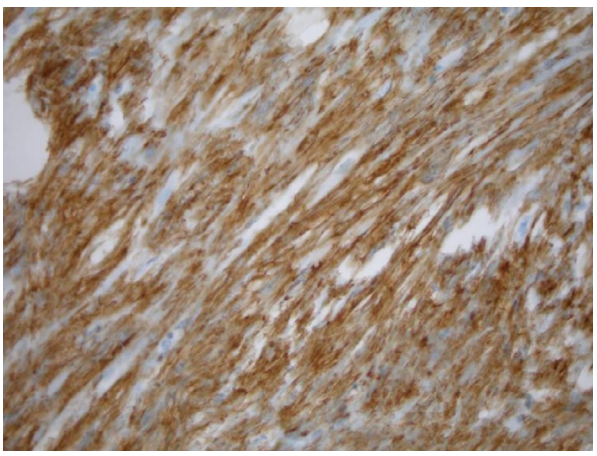

Fig. 6. Leiomyoma leiomyoma SMA 400x (MyoSMA)

of tumors in the GI tract. This test may also be done to see if cancer has spread.

Conclusion

In clinical practice, if there are multiple tumors, we first think of a diffuse or inflammatory process. The described case reminds that one patient may have multiple, different tumors in one time.

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CASUISTIC PAPER

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Primary breast angiosarcoma – a case report

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ABSTRACT

Introduction. Angiosarcoma is a rare breast cancer that can be primary or secondary after surgery or after breast cancer radiotherapy. It is important that breast angiosarcoma belongs to tumors with a non-specific clinical and radiological picture.
Aim. The study of the biopsies contained aggressive vasomotor hyperplasia.
Description of the case. The presented case concerns the primary angiosarcoma of the right breast in a 56-year-old woman who had never had a surgical procedure before, nor radiotherapy in the area of the breast.
Conclusion. Histopathological examination supported by immunohistochemistry is a reliable and indispensable diagnostic element in the diagnosis of vascular sarcoma.
Keywords. breast biopsy, histopathological examination, immunohistochemistry

Introduction

Breast angiosarcoma occurs in two variants as secondary cancer, associated with lymphatic edema after breast cancer surgery (Stewart-Treves syndrome) or after radiotherapy for breast cancer, usually about 3-12 years after irradiation. The dose of radiation does not correlate with the development of cancer. This type of tumor usually occurs with inflammatory changes in the skin of the breast. The second type, primary, occurs without previous diseases of the breast gland as a painless fast-growing tumor, without accompanying changes on the breast skin.[1-3]

Aim

Therapy of a 56-year-old woman with the presence of an irregular 50 mm size change in the right breast.

Description of the case

In a 56-year-old woman who had no breast surgery or radiation therapy in the past, during the periodic mam-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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mographic examination, asymmetric compaction in the right breast was revealed (BIRADS 4). The patient was referred for further diagnosis to the oncological clinic in the future. The completed ultrasound examination confirmed the presence of an irregular 50 mm size change. A thick-foot biocidal biosynthesis was performed to collect tissue from the change to histopathological examination. The obtained material was fixed in buffered 10% formalin, and tissue and H + E staining was performed. At the same time, in order to accurately assess the extent and size of the pathological change, a breast magnetic resonance (MRI) was performed, confirming the presence of a tumor size 55x50x46cm (BIRADS 6).

Results

In the microscopic examination, the biopsies contained aggressive vasomotor hyperplasia (Figure 1), which was confirmed by immunohistochemistry (positive reactions of endothelial markers CD31 and CD34) (Figure 2). The whole picture was complemented by a high proliferative marker Ki67 (Figure 3). Vascular sarcoma of intermediate grade (G2) was diagnosed. The patient was transferred for further treatment to the reference center.

Discussion

Angiosarcoma - a malignant tumor of vascular origin 1. Angiosarcoma of the breast is a rare malignant tumor and constitutes about 0.04% of the primary malignant tumors of this organ.[1-5]

Angiosarcoma in the breast may be secondary to surgery or radiotherapy usually after 3-12 years 1. This type occurs in older women (average age 67). The primary angiosarcoma occurs in younger women with an average age of around 40 years. A major role in the development of primary angiosarcoma is attributed to exposure to vinyl chloride, arsenic, thorium oxide, local injuries and inflammatory changes induced by foreign bodies.[2-7] Cancer is clinically present as a non-painful tumor. Sometimes with symptoms of tissue pulsation and redness of the skin. It usually does not cause the nipple to contract. Very rarely gives metastases to the lymph nodes. Usually metastasis is present in the lungs, second breast, or in the bones.[1-8] The tumor may be visualized in mammography as a homogeneous mass. In an ultrasound scan, the picture may be heterogeneous: hypoechogenic or hyperechoic. The Doppler ultrasound examination reveals a rich tumor vascularization. In the MRI examination, the extent of the tumor can be clearly visualized, especially in the secondary type after radiotherapy.[1-4] The tumor usually located deeply in the breast parenchyma. The size of the tumor usually exceeds 2 cm.[1-7] In the macroscopic image, the tumor has the appearance of a spongy congested structure or solid fibrous mass in the case of a low-differential tumor.[4] A certain diagnosis of angiosarcoma can be determined

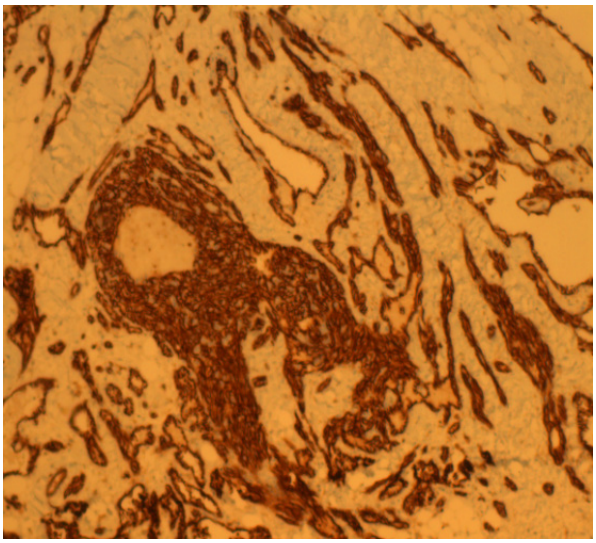

Fig. 1. Breast Angiosarcoma (H&E, 10x)

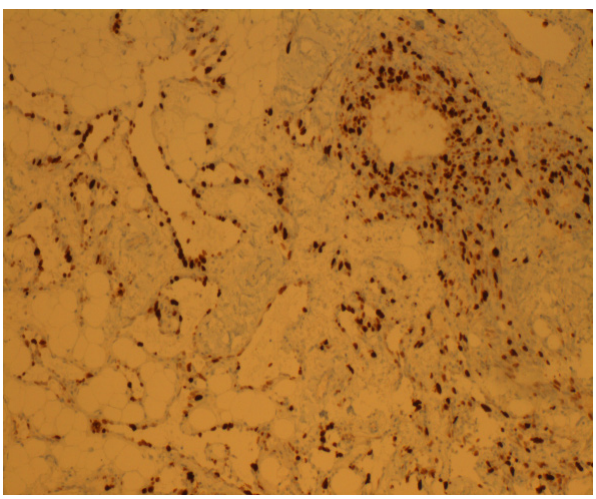

Fig. 2. Breast Angiosarcoma (CD31, 10x)

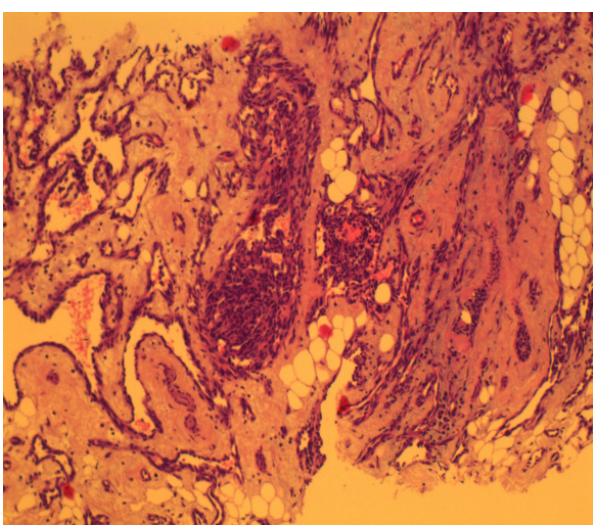

Fig. 3. Breast Angiosarcoma (Ki67, 10x)

based on histopathological and immunohistochemical examination, which exposes the vascular character of proliferation using markers to endothelial cells.

Mainly CD31 and CD34 and factor VIII. A proliferative marker Ki 67 showing a high proliferative index is also helpful. Based on the histopathological picture, three angiocarcinoma groups can be defined depending on the degree of cancer maturity. A well-differentiated cancer (G1), an intermediate-grade tumor (G2), a poorly differentiated tumor (G3). The prognosis of cancer and the form of therapy depend on the degree of differentiation.[1-8] In the treatment of cancer, the main role is played by surgical complete removal of the tumor as a result of quadrantectomy or mastectomy. Adjuvant treatment with radiotherapy and chemotherapy after surgery is usually not effective. There are, however, cases where such combined treatment gives good results and prolongs the time free from tumor recurrence.[2-8] Supplementary treatment may bring benefits in the G3 and large tumors, over 5 cm. Recently, research on vascular endothelial growth factor (VEGF) and the VEGF-R1 and VEGF-C receptor, give hope for targeted anti-angiogenic treatment.[9,10] In histopathological diagnosis it is very important to differentiate this tumor with other malignant, benign and non-neoplastic tumors. Angiosarcoma in G1 and G2 must be differentiated with angiomatosis, pseudoangimatous stromal hyperplasia (PASH) and angiomas. This is especially a challenge when we have a thick-needle biopsy material. Helpful then is the overall picture including imaging tests (benign tumors are less than 2.0 cm), histopathological and immunohistochemical.[11-30]

Conclusion

In histopathological diagnosis it is very important to differentiate this tumor with other malignant, benign and non-neoplastic tumors. Angiosarcoma in G1 and G2 must be differentiated with angiomatosis, pseudoangimatous stromal hyperplasia (PASH) and angiomas. This is especially a challenge when we have a thick-needle biopsy material. Helpful then is the overall picture including imaging tests (benign tumors are less than 2.0 cm), histopathological and immunohistochemical.

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CASUISTIC PAPER

Joanna Gustalik [1(ABCGF)], David Aebisher [2(DFG)], Ewa Kaznowska [1(DFG)], Dorota Bartusik-Aebisher [3(DFG)]

Mixed adenocarcinoma-neuroendocrine cancer – a case report

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ABSTRACT

Introduction. Mixed adenocarcinoma-neuroendocrine cancer (MANEC) is a rare cancer that is characterized by aggressive course and poor prognosis.

Aim. A case report and literature review.

Description of the case. This article presents the case of a 63-year-old patient who was hospitalized due to the occurrence of neurological symptoms such as nausea, dizziness and headache as well as double vision and numbness of the hands. Suspected ischemic stroke, meningitis with bacterial etiology or brainstem pathology**.**

Conclusion. MANEC composed of large neuroendocrine cells have better survival and clinical behavior than patients with small, intermediate or mixed large and intermediate cells.

Keywords. central nervous system, MANEC**,** mixed adenocarcinoma-neuroendocrine cancer

Introduction

Mixed adenocarcinoma-neuroendocrine (MANEC) is a rare change, which is primarily located in the gastrointestinal tract and bile ducts.[1] It is characterized by two ways: epithelial and neuroendocrine, each of which must constitute a minimum of 30% of weaving of change.[1] This unit was introduced to the classification of neuroendocrine tumors by WHO (World Health Organization) in 2010.[2-4] The majority of cancers in this group have an aggressive course and thus a poor prognosis.[2-4] In addition, MANEC also has a large potential to provide distant metastases. This feature is primarily attributed to the neuroendocrine component.[5] Clinical symptoms are non-specific and mainly result from tumor mass or the ability of the neuroendocrine component to produce endogenous active substances.[4] Standard medical treatment includes total resection of the lesion and complementary chemotherapy.[1-8]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Aim

In this paper, we present one unusual case of MANEC. The pathophysiological specimen confirmed the presence of malignant cells.

Description of the case

A 63-year-old patient came to the Admission Room because of severe dizziness, headache, nausea, double vision and numbness in his hands. He was admitted to the Department of Neurology. From the interview it was known that these ailments appeared the day before. In addition, the patient was previously hospitalized in the Department of Laryngology in connection with deafness of the right ear and deep hearing of the left ear. Extensive diagnostic imaging of the central nervous system was performed – MRI of the brain, angio-CT of the head and UDP of the intracerebral arteries. He was never treated chronically and did not report allergies or drug intolerances.

At the time of admission, significantly increased blood pressure (170/110 mmHg) and tachycardia (110/min) were observed. In the neurological examination, among other things, it was found that the patient was conscious, but restless, he had a dia-tellic speech, palatal reflexes were present, and presented bilateral features of nerve injury VII. In the CT scan of the head ordered at admission the hypodense focus in the left cerebellar hemisphere and small painting cavities at the level of subcortical nuclei were described. In the cerebrospinal fluid found increased cellularity, reduced glucose levels and elevated protein levels. lactic acid and chlorides. Ceftazidime, metronidazole, acyclovir, dexamethasone, mannitol, enoxaparin, furosemidum, atorvastatin, perindopril, diazepam and acetylsalicylic acid were used in the treatment. On the second day after admission, the patient's condition deteriorated rapidly, circulatory arrest was stopped, cardiopulmonary resuscitation was successfully performed, but soon the cardiac arrest was resumed and, despite a prolonged resuscitation action, the circulation was not restored.

Results

The whole picture of the autopsies was the reason for the diagnosis of MANEC type of cancer with spreading mainly to meninges and lungs. Figures 1, 2 and 3 show infiltrates of signaling cells in spinal cord cerebellum plains. Figure 4 presents infiltration of brain cells in the brain's cerebrospinal meninges, additional staining with mucicarmine for the presence of mucus. Figures 5 and 6 presents gastric tumor.

Discussion

To date, according to literature, MANECs has been identified in various organs, such as the stomach.[1] Diagnosis is mainly based on tumor cytology and architecture and is completed by immunostaining with specific neuroendocrine markers. In the macroscopic part of the autopsy examination, congestion and edema of the brain, few ecchymoses in the white matter of both hemispheres of the brain, bilateral foci of soft-

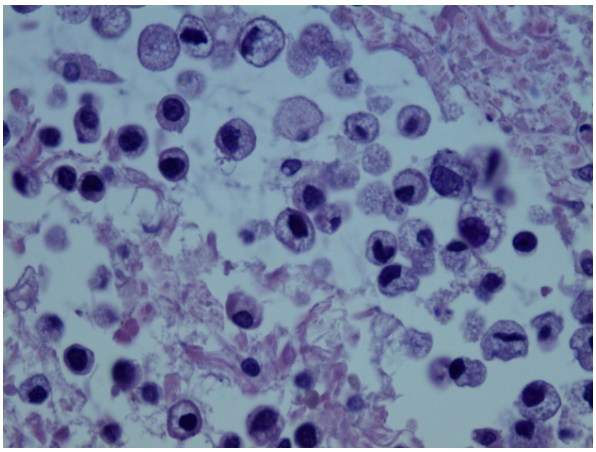

Fig. 1. Infiltrate of signaling cells in spinal cord cerebellum plains (H&E, 630x)

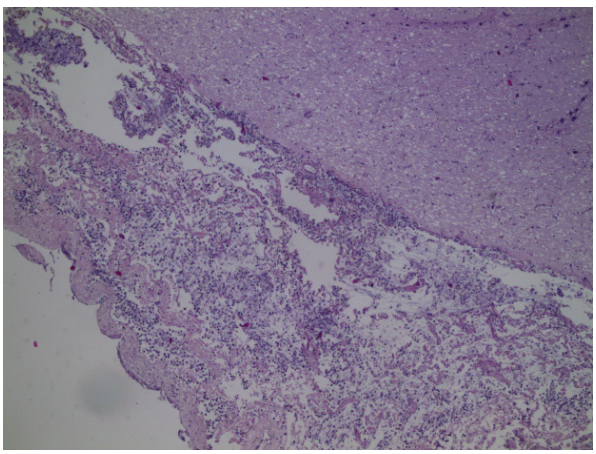

Fig. 2. Cerebrospinal meningin infiltrate by atypical plaintain cells (H&E, 40x)

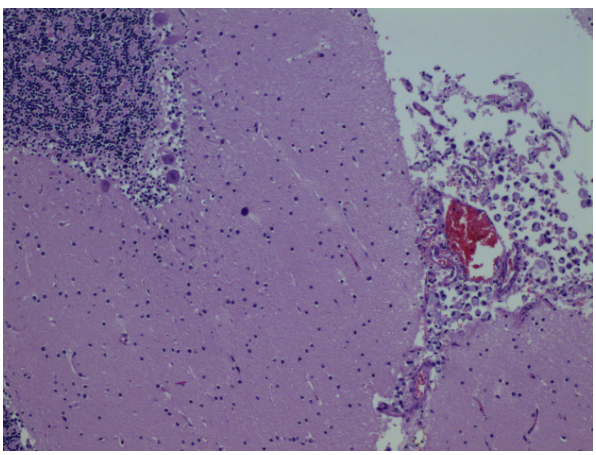

Fig. 3. Infiltrate of signaling cells in spinal cord cerebellum plains (H&E, 100x)

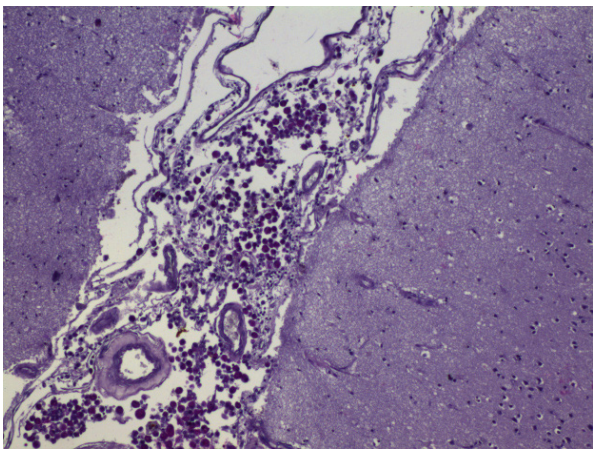

Fig. 4. Infiltration of brain cells in the brain's cerebrospinal meninges, additional staining with mucicarmine for the presence of mucus (H&E, 100x)

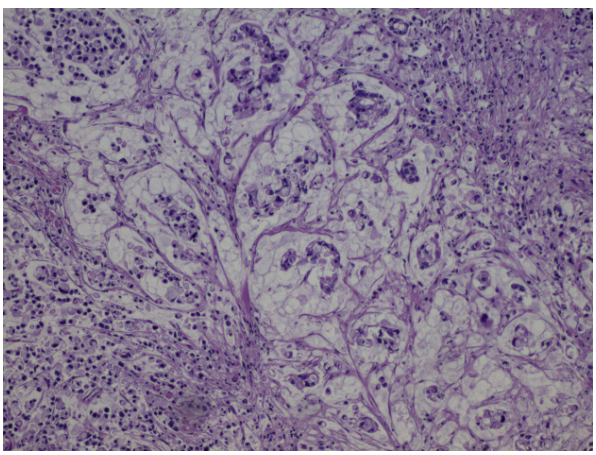

Fig. 5. Gastric tumor (H&E, 200x)

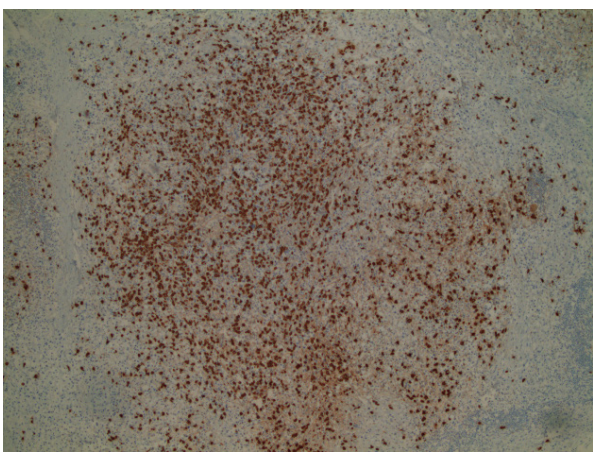

Fig. 6. Gastric tumor – positive chromogranin reaction (immunohistochemical staining, 100x)

ening in the cerebellar body, around the toothed nuclei, congestion and pulmonary edema and a single white-yellow nodule in the back wall of the stomach diameter 1 cm. In addition, there are no other significant deviations from the norm.

In the microscopic part of the autopsy examination in the cerebrospinal meninges a very abundant infiltration of atypical signet cells was found. In the brainstem there was an outbreak of softening with the presence of the same atypical signet cells.[2-5] Their presence was also demonstrated in the cerebellum and in the form of vascular occlusion in the lungs. The tumor in the stomach, however, after immunohistochemistry, turned out to be a mixed glandular-neuroendocrine tumor.

Conclusion

From a clinical point of view, this case is very instructive, because it shows that the results of imaging and laboratory tests are not always able to explain symptoms. Radiological studies suggested cerebral ischemic stroke, cerebrospinal fluid - bacterial meningitis, and clinical symptoms - brain stem pathology. In the end, all doubts were resolved only by the sectional examination, which showed that the basis for these changes was the dissemination of the MANEC-type stomach tumor to the central nervous system.

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— References

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References should be prepared according to the AMA style. The list of references should be placed at the end of an article and prepared according to the order of citation in the text.
Citations in the article should be placed after a sentence ending with a full stop and edited as the so called 'superscript'. In-text citations should only be placed at the end of a sentence or a paragraph, not in the middle.
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. Wnen 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 number of sources cited for an opinion article/ a review article should be between 40 and 50, and from 20 to 40 for other articles. A minimum of 50 % of literature should come from the last 5 years.

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	Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. Comput Methods Programs Biomed. 2016;134:79-88. de Kam D, Kamphuis JF, Weerdesteyn V, Geurts AC. The effect of weight-bering asymmetry on dynamic postural stability in people with chronic stroke. Gait Posture. 2016;53:5-10.
Article from a journal, number of authors more than 6	Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. Cell Rep. 2017;18:1215-28. 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;34:1678-88.
Article from an online journal	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;25:43-9. 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	Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiti-cholera/. 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	Naish J, Syndercombe Court D. Medical Sciences. 2nd ed. London, Elsevier;2015. Modlin J, Jenkins P. Decision Analysis in Planning for a Polio Outbreak in the United States. San Francisco, CA:Pediatric Academic Societies;2004.
Chapter in a book	Pignone M, Salazar R. Disease Prevention & Health Promotion. In: Papadakis MA, McPhee S, ed. Current Medical Diagnosis & Treatment. 54th ed. New York, NY: McGraw-Hill Education; 2015:1-19. Solensky R. Drugallergy: desensitization and Treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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.

TABLES AND FIGURES

All tables and figures should be inserted in the text. They must have captions.

Tables should have the Arabic Numerals and a caption inserted above a table, in the sequence of appearance of the first reference in the text. One should ensure whether every table is mentioned in the text. When constructing tables, avoid vertical separators.

Figures should have the Arabic Numerals and a caption placed under it. They should be numbered in a sequence of appearance of the first reference in the text. One should ensure whether every figure is mentioned in the text.

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