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ORIGINAL PAPER

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Influence of Adriblastin and Bleomycin on Wistar rat mothers and fetus development

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ABSTRACT

Introduction. Gestation is a very sensitive time both to mother and child. Any substance, factor, or environmental condition disturbing homeostasis may cause congenital defects, anomalies or even death. Teratology evaluates those potential factors and their influence. Also, medicinal products used during pregnancy may be teratogenic. Adriblastin, also known as Doxorubicin, and Bleomycin are widely used cytostatic drugs in oncology.

Aim. Aim of this study was to evaluate the embryotoxic effects of Doxorubicin and Bleomycin in an animal model.

Materials and methods. Fertilised Wistar rat females were given each drug intraperitoneally between the 8th and 15th gestation day, and compared to control group receiving placebo (distilled water, 0.9% NaCl). Another group received acetyl salicylic acid, as a model, well known teratogen. Changes in mothers' weight from baseline, implantation of embryos, any discrepancies in mothers wombs and health as well as defects in fetuses were evaluated and compared. Fetus skeletons were stained by Dowson's method to visualise bone defects.

Results and conclusion. Both Adriblastin and Bleomycin were teratogenic, producing significantly more embryo absorptions, and fetal defects compared to placebo. The effects of the two cytostatics were similar to the model teratogen acetyl salicylic acid.

Keywords. pregnancy, foetus, congenital defect, teratogen

Introduction

The beginning of teratology as a science dates back to the end of the 18th and beginning of the 19th century.[1,2] Currently, it is defined as the knowledge of inherited defects in body composition developed during gestation and related to fertilisation. Its main task is to investigate causes and effects in structure and function of embryos and fetuses related to factors occurring prior to con-

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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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ception, during gestation and also after birth until early premature age.[3] A fetal defect development is defined as variation in structure and function larger in extent than those observed in standard phenotype variability specific for a given species.[3]

Teratogen, or teratogenic factor is therefore any stimulus (chemical, physical, environmental etc.), which may cause development fetal defects. Teratogenicity of chemical substances occurs when they enter the developing fetus cells or tissues and modify or damage protein synthesis at any stage of DNA translation or RNA transcription. The result of teratogenicity is either a visible malformation of the fetus/new-born, or a latent defect in physiological functioning appearing after birth, or any abnormality during pregnancy (gestation) both in mother and in offspring that leads to miscarriage.[4-6]

Specific names to defects are given based on the development phase acted upon by the teratogen(s) e.g.:

- Genopathy, when a teratogen acted on gametes (or parents) before conception, and mutations in the genes occurred, so we can also talk about chromosomes aberration,
- Blastopathy, when teratogen affected blastogenesis, in humans it is between day 1 to day 14 after conception. Usually blastopathy means total damage of structures and miscarriage.
- Embryopathy, when teratogen works during organogenesis, and congenital defects occur in organs.
- Fetopathy, when teratogen acts in late phase of pregnancy, and defects occur after birth.

In the 20th century, teratogens were preliminarily classified into groups.[1] In 1975 Miller and Yasuda grouped teratogens into:[1-3,5]

a. mechanical and physical, e.g. pressure, injury, irritation, radiation, hyperthermia,
b. biological, e.g. viral or bacterial infections,
c. chemical, e.g. drugs, pesticides, plant or fungal derived toxins, environmental chemical pollutants.

In 1962, drugs were considered as potential teratogens and since then broad, regular testing has begun.[4,6] Many antibiotics, alkaloids, non-steroidal anti-inflammatory, antimetabolics, but also some vitamins happened to be teratogenic.[5] Drugs cause about 5% of congenital defects in new-borns. Causes of the remaining defects have not been precisely identified yet.[5,6,20] Most of known teratogenic drugs easily pass the placenta and these drugs are the most teratogenic. Teratogenicity itself depends on protein binding properties, molecule size, and drug polarisation.[3] If a teratogen passes the placenta, polarised drugs are distributed mainly into intercellular space in fetuses. They are also quickly removed to the amnion and from there do not enter the cells easily. Contrary to this, lipophilic drugs penetrate the placenta and fetal tissues faster and their elimination is poorer. Also, protein binding enhances distribution to tissues and inside cells.[5,9,10]

Currently, many medicines are well established regarding their teratogenicity. Some are absolutely contraindicated during the entire pregnancy period; some are carefully allowed in advanced stages. Adriblastin and Bleomycin belong to the latter.

Aim of the study

The aim of this investigation was to evaluate the effects of Adriblastin and bleomycin administered to gravid Wistar rat females during organogenesis. Both mother and foetus drug effects were observed and evaluated. Comparison between drugs was performed with regards to prespecified parameters. Also, both drugs were evaluated as potential model teratogenic factors for future animal studies and comparators to other compounds, e.g. new candidates for drugs in pharmacotherapy.

Material and methods

The study was approved by the Bioethical Committee in Lublin, Poland. We used white Wistar rats females, at an age of 4-5 months, and weigh of 200 to 250 grams, derived from a certified breeding laboratory. The total number of females in the experiment was 125 of whom 595 fetuses were delivered. Animals were kept in natural day-night light exposure, at temperatures between 18-22 °C and 60% humidity. 5-6 females were placed in one standard plastic cage of 0.5 m^2, in accordance to conditions recommended in the literature.[11] Water supply and granulated feed "LSM" were available for animals ad libitum. The feed was made as per Polish Academy of Science recipe (Zakład Hodowli Zwierząt Laboratoryjnych). Regular sawdust was used as litter and stress avoidant conditions were assured. To reduce seasonal variations, the experiment was conducted in 2 three-months-long restricted cycles: March-June and September-November. Also, daily procedures were performed at regular time-schedules to avoid stress.[4] Virgin females were quarantined for 10-14 days after transferring from the breeding lab to our experiment premises to adapt to new settings.[12] Animals of doubtful health conditions were excluded during that accommodation phase. The oestrus in females was verified by vaginal smear, and matching 5 females with 2 males took place thereafter for one night. Vaginal smears followed next morning. If spermatozoa were found in the smear or sperm-slime head in the vagina the 1st gestation day was assumed since then.

Inseminated females were divided into experimental (active) and control groups, each consisting of 10-15 animals, and allocated to cages. The cages of every group were properly marked.

A. The first control group did not receive any chemical substances.
B. The second control group received 0.9% NaCl (saline) intraperitoneally, at 1 ml/kg b.w., once daily

C. The third control group received distilled water via the gastric sound, at 5 ml/kg b.w., once daily.

In the experimental (active treatment) groups the females received intraperitoneally:

A. Bleomycin at 20 mg/kg b.w. once daily (manufacturer Nippon Kayaku, Tokyo, Japan)
B. Adriblastin at 16 mg/kg b.w., once daily (mnufacturer Farmitalia Carlo Erba, Italy).

An additional control group was created to receive acetyl salicylic acid as a benchmark (model) teratogen.

Bleomycin and Adriblastin were diluted in 0.9% NaCl prior administration. Acetyl salicylic acid was diluted in water with Twin 80 prior to administration via gastric sound.

All drugs were administered between gestation day 8 to 15 which outlines organogenesis in rats.

Methods of examinations of mothers and fetuses

Daily life activities of females were noted during the experiment. Their weight was measured at day 1, 8, 15, and 21. Weight gain was calculated between the measurement days. At day 21, females were decapitated. Linear incision of the abdominal wall was cut to reach wombs with ovaries and upper part of vagina. Wombs were cut along its antimesomatrial margin. The followings were checked and counted: luteal corps, implantations, early and late resorptions, dead and alive fetuses. Alive foetuses were ranked in 3 level original scale:

1. R-1, foetus vivid, spontaneously active, pink skin
2. R-2, minimal spontaneous movements
3. R-3, alive, not active, limp, but reactive to touching.

Macroscopic evaluation of fetuses

Skin evaluation for any signs of bruises (hematomas), oedemas, excessive folds, hernias was carried out. Head size and shape, ear shapes, tongue size, limbs size and shapes, finger counts, and adhesions were noted. Any abnormalities found macroscopically resulted in placement of the foetus in formalin for microscopic evaluation.

Skeleton assessment was done according to Dowson's method.

Fetuses were first eviscerated, then dehydrated in 96% ethanol, and overexposed to 1% KOH. Staining with alizarin red dye followed and visual assessment took place. Any deficiencies, malformations, or anomalies were noted and compared to skeletons of control groups.

Numbers of intrauterine resorptions of fetuses were statistically compared between groups. Post implantation fetus mortality was counted, and rates of alive fetuses to all implantation nests in uterine were calculated. Those figures reflected eventual embryotoxic effects of studied drugs.

The teratogenic effect was estimated by comparing numbers and scale of defects in live-born fetuses in experimental groups versus control groups in which eventual defects were considered as spontaneous.

Statistical analysis

Arithmetical averages and standard deviations for obtained parameters were used to analyze and compare groups. Comparative tables were prepared for qualitative features e.g. skeleton defects. Analysis of variance (ANOVA) was used for quantitative parameters including doses and administration times of drugs. Kruskal-Wallis non-parametric test or interval estimation (Takey confidence interval) method were used in abnormal variable distribution. Chi-square statistic (Yates modification for small cardinality) was used for obtained variables and Fisher's exact test for multipartite tables. A P value < 0.05 was considered as statistically significant. Statistical analysis of the data was performed using the STATISTICA software package (version 12. StatSoft Inc. 2014, Tulsa, OK, USA, www.statsoft.com).

Results

In the Bleomycin group, mean weight gain in females from day 1 to 21 was 11.13 ± 4.1 g, which was only 10.5% of weight gains in the control group receiving distilled water. In the Adriblastin group, weight gain was 23.67 ± 5.1 g which was only 21.3% of control group receiving distilled water. In acetyl salicylic model teratogen group, weight gain was 11.00 ± 6.2 g, which was only 9.9% of control group receiving distilled water (Table 1 and 2).

Adriblastin and Bleomycin significantly decreased weight gains of pregnant females after day 15 and 21 as compared to control groups. The same effect was observed in acetyl salicylic acid model teratogen group (Table 3).

Post-implantation resorptions and fetus mortality rate was 57.9% in Bleomycin, and 37.2% in Adriblastin. Both were significantly higher than in control group. In both Adriblastin and Bleomycin receiving groups in part of fetuses the following defects were observed: spina bifida, meningocele, macroglossia, syndactyly of fingers IV and V in lower limbs, and hematomas (Table 3).

Skull bone evaluation in the Bleomycin group revealed lessening in the parietal bone (50% of fetuses), interparietal (28%), frontal (25%), hyoid (12.5%), and lack of 1st, 2nd, 3rd ossification spot in sternum in 25%, 18.7% and 9.4% respectively. In 6.2% of foetuses, lessening in lumbar or sacral parts of vertebral column was found.

Skull bone evaluation in the Adriblastin group revealed lessening in the parietal bone (25% of fetuses), frontal (7.5%), and a lack ossification spots in sternum in 7.5% of fetuses.

Both tested drugs became significantly teratogenic compared to control groups (Fig. 1-3).

Table 1. Weight of pregnant females in control and active treatment groups. Baseline – 1st day. *- p<0.05, ** - p<0.001 compared to Control H_2O, ^ - p<0.001 – compared to baseline.

Measurement	Administered substance at dose: mg/kg b.w.					
	Control 0	Control 0.9% NaCl	Control H_2O	Acetyl Salicylic Acid [250.0]	Bleomycin [20.0]	Adriblastin [16.0]
I day 1	233.47 ± 3.58	230.73 ± 3.69	208.3 ± 4.71	209.3 ± 6.91	186.87 ± 5.79*	215.22 ± 9.2
II day 8	257.32 ± 3.51^	252.6 ± 3.42^	244.6 ± 3.95^	223.8 ± 7.96	201.25 ± 5.29**	227.89 ± 9.1
III day 15	289.31 ± 3.57^	285.22 ± 4.36^	273.8 ± 4.0^	213.6 ± 7.7**	195.13 ± 7.34**	245.78 ± 9.33*
IV day 21	339.03 ± 3.94^	336.89 ± 3.9^	319.3 ± 6.71^	220.3 ± 10.64**	198.0 ± 13.92**	238.89 ± 11.91**

Table 2. Weight gain of females in control and active treatment groups between day 1 and 21. ANOVA was used for comparison ^ p<0.001 day 21 to baseline (day 1) * p<0.001 vs Control H_2O.

Administered substance	Dose (mg/kg b.w.)	Route of administration	Body mass (g)		Mean weight gain (g)
			1st day of gestation	21st day of gestation	
			X ± SE	X ± SE	
Control 0	-	-	233.47 ± 3.58	339.03 ± 3.94^	105.5 ± 3.14
Control H_2O	5 ml/kg	p.o	208.3 ± 4.7	319.3 ± 7.7^	111.0 ± 4.1
Control 0.9% NaCl	1 ml/kg	p.o.	230.7 ± 3.7	336.9 ± 3.9^	106 ± 3.1
Acetyl Salicylic Acid	250 mg/kg	p.o	209.3 ± 6.9	220.3 ± 10.2*	11.00 ± 6.18*
Bleomycin	20 mg/kg	i.p.	186.9 ± 5.8	198.0 ± 13.9*	11.13 ± 4.13*
Adriblastin	16 mg/kg	i.p,	215.2 ± 9.2	238.9 ± 11.9*	23.67 ± 5.14*

Table 3. Visible defects in fetus skeletons staining. Chi-square test *- p<0.01.

Finding	Administered substance											
	Control 0		Control H_2O		Control 0.9% NaCl		Acetyl Salicylic Acid		Bleomycin		Adriblastin	
	n	%	n	%	n	%	n	%	n	%	n	%
Total foetuses	121		92		115		58		48	100	64	
Stained skeletons	80	100	58	100	60	100	30	100	32		40	100
Skull												
Frontal bone loss									8	25.0*	3	7.5*
Parietal bone loss					2	3.3	10	33.33*	16	50.0*	10	25.0*
Interparietal bone loss	1	1.2					11	36.67*	9	28.13*		
Hyoid bone loss									4	12.5*		
Bone palate defect												
Sternum												
Lack of ossification points												
I												
II												
III			1	1.72					8	25.0*		
IV												
Ribs									6	18.75*	3	7.5
Shortening of ribs			1	1.72			5	16.67*	3	9.38*	2	5.0
Fusion of ribs												
Vertebral column									2	6.25*		
Vertebrae loss												
Cervical												
Thoracic							4	13.33*	1	3.12		
Lumbo-Sacral							7	23.33*	2	6.25*		

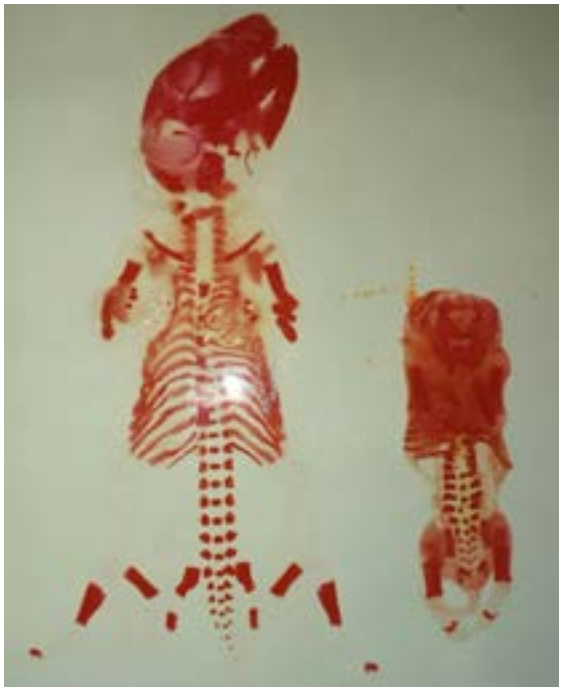

Fig. 1. *LEFT*: Normal skeleton of a foetus delivered from a mother in Control Group. *RIGHT*: Skeleton of a foetus delivered from a mother treated with Bleomycin at dose of 20 mg/kg b.w. Apparent are: ribs malformations and shortening, lack of interparietal bone, decrement of thoracic vertebral, different lengths of bodies

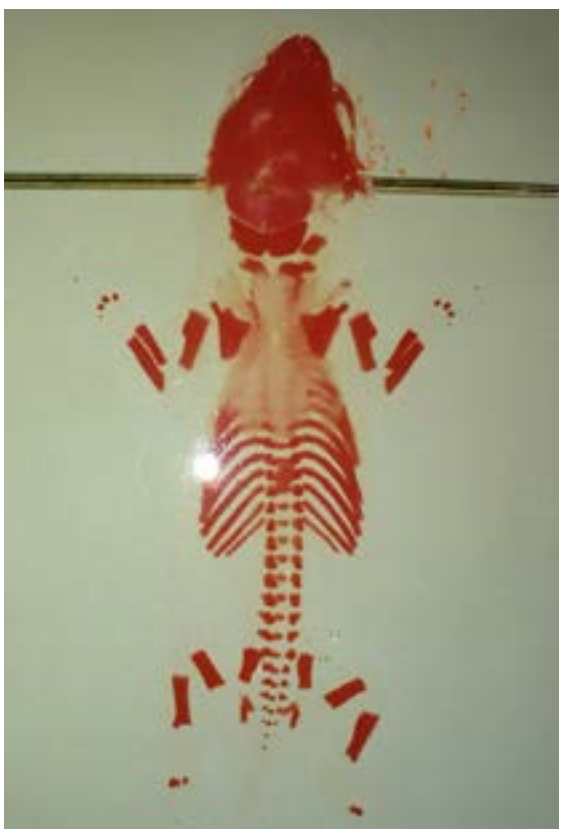

Fig. 2. Normal skeleton of a foetus delivered from a mother in the Control Group. Dowson method staining with red alizarin dye

Fig. 3. Skeleton of a foetus delivered from a mother treated with Adriblastin at dose of 16 mg/kg b.w. Apparent are: lack of 4 metacarpal bones, ribs fusion, decrement of thoracic vertebral bodies

Discussion

Teratogenic studies are part of the toxicological evaluation of all drugs in human use. Prior to any exposure in humans, especially in pregnancy, the drug or drug candidate must be evaluated in this aspect. Embryotoxic studies are recommended by WHO.[4,5] Nevertheless, it is widely agreed that we cannot exclude teratogenic effects in humans based on animal models, as no animal is identical to humans either in metabolism or placenta etc. Therefore, animal models may however support decisions in drug disapproval at early stages of drug development and help in selection of best candidates regarding safety profile. Expertise and experience in animal modelling, their reproductive processes are essential in those kinds of studies.[4,5]

Post-marketing surveillance of drugs is a worldwide standard as of today. Teratogenic effects are also collected from this source, and included in knowledge on

drug safety. A cautious assessment of both experimental animal studies and clinical (human use) drug safety data may improve the overall safety of medicinal products.[6,8,11,13,14] Embryotoxicity seems to be very specific to a given species, and not always translates to others in a simple manner.[6,15] Spontaneous anomalies occur quite frequently in mice at 0.4-18.6 rates, in pigs at 0.6-9.8%, in rabbits at 0.7-6.3%, but in rats only in 0.06-0.8%. Therefore, rats are much more reliable comparators than species mentioned earlier.[4,15]

In our experiment we focused on 3 most important features of teratologic techniques:

1. Phase specificity – meaning finding correlations between chemical substance exposure and severity of teratogenic effects
2. Drug specificity – differences between chemical substances themselves in teratogenicity
3. Dose specificity – differences in effects related to escalating dose of the same substance.

In our experiment females were exposed to drugs between the 8th and 15th day of gestation, which is the organogenesis stage in rats. We used drugs that are indicated in short, e.g. a few days, application in humans, not in chronic diseases.

425 fetal skeletal preparations by the Dowson method delivered substantial data on embryonic phase development, until 21st gestation day. In most skeletons exposed to active experimental drugs some defects were noted. Special attention should be paid to numerous defects in metacarpal bones and vertebral column, from 15.8% to 64.7%.

In our experiment, Bleomycin resulted in different defects as much as 47.9% of fetuses. In 57.9%, it caused premature deaths or blocked embryos implantation in wombs. Similar results were reported by Elis and DiPaolo for cytostatic antibiotics in animal models.[16]

Bleomycin is a cytostatic antibiotic, mixture of bleomycin type A2 and B2, produced naturally by Streptomyces verticillus strains or in chemical synthesis. It is a 6-aminoacid glycated peptide. It blocks DNA production in cancer cells not allowing thymidine to be linked in the DNA chain.[2,7,17,18] It is well absorbed and distributed in blood and quickly reaches all tissues. Unmetabolized drug is excreted by kidneys. It is widely used in treatment of melanoma, breast cancer, lung cancer, and lymphomas. It is well tolerated by patients, as compared to many other cytostatics. In higher doses, it causes pulmonary fibrosis.[12,19,20]

Adriblastin in our experiment caused 37.2% resorptions of embryos and defects in 59.4% of fetuses. The drug inhibits nucleic acid synthesis as well as some proteins, what is used in oncology and what also explains its teratogenic effects. Adriblastin interferes with mitochondria, lysosomes, and cell and organelle membrane transport. Therefore, Adriblastin presents a lot of serious and severe side effects, including vomiting, depression of bone marrow, and cardiotoxicity. The experiment females did not show visible symptoms or side effects, but over 50% of their alive fetuses at 21st day (decapitation) presented limited movements, were frail and limp.

Some evidence shows late and very late effects of Adriblastin. Mettler reported Adriblastin cardiotoxicity 4 to even 20 years after treatment in humans. In animal models, Adriblastin showed dose and time dependent cardiotoxicity in rabbits, mice, and rats.[4,20,21]

In necropsy of fetuses in our experiment we did not find macroscopic changes in hearts, but no microscopic data were available.

Conclusions

Bleomycin and Adriblastin proved to be embryotoxic to fetuses in our experiment. Bleomycin and Adriblastin influenced pregnant females, slowing and diminishing their weight gains. Both of tested substances may be used as a reference teratogenic substance to compare.

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ORIGINAL PAPER

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Retrospective analysis of reactive hyperplastic lesions in the oral cavity

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ABSTRACT

Introduction. Reactive hyperplastic lesions of the oral cavity are non-neoplastic lesions that result from low-grade chronic irritation of the oral mucosa.

Objectives. The aim of this study was to present the epidemiological characteristics of reactive lesions.

Materials and methods. The study was a retrospective analysis of the medical records of 116 patients with reactive lesions. The tissue specimens were obtained by biopsy. 115 patients underwent an excisional biopsy, whereas in one case an incisional biopsy was performed.

Results. The most frequently encountered lesion was inflammatory fibrous hyperplasia (IFH) (n=37, 31.9%), followed by irritation fibroma (IF) (n=36, 31%), pyogenic granuloma (PG) (n=15, 12.9%), fissured granuloma (FG) (n= 14, 12.1%). The lesions were more commonly observed in females (n=70, 60.3%) than in males (n=46, 39.7%) with a ratio of 1.5:1, respectively. The buccal and labial mucosa were the most prevalent sites of reactive lesions. Most of the lesions were between >5 mm and ≤10 mm in diameter except for FGs, which were much bigger.

Conclusions. Early detection and elimination of all potentially causative factors and irritants is a crucial matter, especially in the case of the vestibule of the oral cavity, which is the most susceptible area of the oral cavity.

Keywords. inflammatory fibrous hyperplasia, fissuratum granuloma, peripheral giant cell granuloma, irritation fibroma, pyogenic granuloma

Introduction

Reactive lesions are one of the most frequently encountered lesions in the oral cavity, varying in size from 0.5 cm to over 2 cm in diameter.[1] They emerge as a result of underlying systemic diseases, drug-induced stimuli, dental plaque and local iatrogenic factors. Even though clinically they may resemble benign tumors, they are in fact non-neoplastic proliferations appearing in response

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to irritation or injury caused by maloccluded teeth, ill-fitting dentures, orthodontic appliances, cracked teeth, overhanging dental restorations and other mucosa-irritating factors.[2,3] Furthermore, they may occur as a result of body-focused repetitive behaviors (BFRB), such as biting on lips or cheeks, leading to chronic inflammation of mucosa and subsequent hyperplastic growth of cells. In the case of peripheral giant cell granuloma (PGCG), inflammatory or developmental reactions in the periosteum or periodontal ligament have been proposed as potential etiologic factors.[4] Clinically, the reactive lesions are sessile or pedunculated masses covered with smooth or injured mucosa, bleeding easily when touched, and varying in color from bright pink to red.[5] It is worth mentioning that the lesions are painless, and therefore easy to be ignored for a long time, which allows them to attain greater size and compromise the ability to chew, speak, or maintain oral hygiene. Surgical excision of the tissues involved is a treatment of choice, and subsequent histopathological analysis is mandatory to confirm the initial diagnosis. Most of the hyperplastic lesions have a specific age and gender distribution, and preferential locations in the oral cavity; consequently, this study may help the practitioners to correctly diagnose the lesions and offer optimal treatment to the patients.

This study aims to present the epidemiological, clinical and histopathological characteristics of reactive lesions on the oral mucosa.

Material and methods

The retrospective study group comprised of the medical records of 116 patients, including 70 women (60.3%) and 46 men (39.7%) aged 18-91 years (average: 55.74 years), who demonstrated reactive lesions on the oral mucosa. The inclusion criteria were based on the final histopathological examination. The initial clinical diagnosis was not the basis of inclusion of these lesions to the study. The study included all the patients admitted to the Clinic and Department of Oral Surgery and Periodontology in the afore mentioned timeframe with a final histopathological diagnosis of: PGCG, pyogenic granuloma (PG), angiofibroma (AF), lipofibroma (LF), irritation fibroma (IF), granuloma fissuratum (FG), inflammatory fibrous hyperplasia (IFH) or inflammatory papillary hyperplasia (IPH). All the reactive lesions were classified according to the classification proposed by the ICD-DA (International Classification of Diseases to Dentistry and Stomatology) and by the WHO classification of tumors (2005).[6] The exclusion criteria included benign and malignant tumors of connective and epithelial origin, inflammatory changes of odontogenic and bone origin, pre-cancerous conditions of the oral mucosa, and cystic changes of the minor salivary glands. The data were collected from the archives of the Clinic and Department of Oral Surgery and Periodontology, Poznan University of Medical Sciences, Poland. Data from patient records from January 2013 to December 2017 were analyzed to identify the gender and age of the patients, the location of the lesions, the final histopathological diagnosis, the treatment selected and the histopathological image. Clinical data were collected from a comprehensive medical and dental examination. An examination of the mouth was performed and medical history was elicited from every patient. Tissue specimens for histopathological analysis were obtained by biopsy. 115 patients underwent complete excision of the lesion, whereas in one case an incisional biopsy was performed. Incomplete registered records and missed pathologic slides were excluded from the study. Repeated biopsies of already diagnosed lesions were also excluded. All specimens were assessed by an experienced pathologist.

The incidences of the data obtained were analyzed. Descriptive statistics were used to evaluate the data using IBM SPSS Statistics software (v. 23.0, Chicago, IL).

This study was performed in accordance with the ethical standards laid down in an appropriate version of the World Association Declaration of Helsinki. Written informed consent was obtained from every subject before any study procedure was carried out. Our study was a retrospective analysis and the additional consent of the Bioethics Committee was not necessary. We did not perform any additional research procedures.

Results

IFH was the most prevalent lesion (31.9%), followed by IF (31.0%), PG (12.9%), FG (12.1%), PGCG (8.6%), AF (2.6%) and LP (0.9%). The distribution of histopathological diagnosis was demonstrated in Table 1.

There was a clear predilection for females in each entity, except IFH, AF and LF, where females and males were affected to nearly the same degree. The gender distribution in all groups of reactive lesions was different, but showed a slight female predilection compared to males (Table 2). The majority of lesions appeared in the fifth and seventh decades.

The buccal mucosa was the most commonly affected site of occurrence (25.0%) followed by the lower lips (22.4%), marginal gingiva (15.5%) and vestibular mucosa (15.5%). Thorough distribution of the sites involved is presented in Table 3.

The majority of reactive lesions were seen in the maxilla followed by the mandible. IFH and IF were more frequent on the cheek mucosa and lower lip mucosa, respectively. The most common site of PG and PGCG was the anterior marginal gingiva with a predilection for the mandible in relation to PG. PGCG showed no predilection for mandible or maxilla. FG was also more prevalent in the anterior aspect of the mouth, but affected the vestibular mucosa beyond the gingiva.

Table 1. Distribution of histopathological diagnosis of reactive lesions

Reactive lesion	Frequency (n)	Percentage (%)	Valid percentage (%)	Cumulative percentage (%)
AF	3	2.6	2.6	2.6
LF	1	0.9	0.9	3.4
IFH	37	31.9	31.9	35.3
FG	14	12.1	12.1	47.4
IF	36	31.0	31.0	78.4
PGCG	10	8.6	8.6	87.1
PG	15	12.9	12.9	100.0
Total	116	100.0	100.0	

AF – angiofibroma, LF – lipofibroma, IFH – inflammatory fibrous hyperplasia, FG – granuloma fissuratum, IF – irritation fibroma, PGCG – peripheral giant cell granuloma, PG – pyogenic granuloma

Table. 2. Distribution of reactive lesions according to gender, denture user and diameter

Reactive lesions	Female/male ratio (n)	Dentures Yes/ No (n)	Diameter/size ≤5 mm (n)	Diameter/size >5mm≤10 (n)	Diameter/size >10mm≤20 mm (n)
IFH	19/18	10/27	10	18	9
IF	22/14	7/29	9	20	7
PG	10/5	1/14	5	9	1
FG	9/5	8/6	0	5	9
PGCG	7/3	2/8	3	5	2
AF	2/1	2/1	1	2	0
LF	1/0	0/1	0	1	0

Table 3. Distribution of the sites of reactive lesions

Location	Frequency (n)	Percentage (%)	Valid percentage (%)	Cumulative percent (%)
Floor of the mouth	1	0.9	0.9	0.9
Marginal gingiva	18	15.5	15.5	16.4
Maxillary tuber	1	0.9	0.9	17.2
Tongue	7	6.0	6.0	23.3
Hard palate	9	7.8	7.8	31.0
Buccal mucosa	29	25.0	25.0	56.0
Vestibular mucosa	18	15.5	15.5	71.6
Lower lip	26	22.4	22.4	94.0
Upper lip	2	1.7	1.7	95.7
Alveolar process	5	4.3	4.3	100.0
Total	116	100.0	100.0	

The diameter of reactive lesions ranged from a few mm to up 20 mm and was classified into three groups: (diameter ≤5 mm), >5 mm ≤10 mm >10 mm ≤ 20 mm. Most of the reactive lesions had a diameter ranging between 5 mm to 10 mm except the group of FG, where sizes of above 10 mm predominated (Table 2).

Discussion

In our study, IFH was the most common lesion, accounting for 31.9% of all lesions. Similar results were obtained in other studies.[4,5,7,8] These findings are not in agreement with Naderi et al. who reported a higher occurrence of PGCG.[1] In our study, IFH, also known as spurious fibroma, is a formation of excess connective tissue forming in response to irritation of oral mucosa which is not defined histopathologically. Chronic trauma can induce an inflammation which augments the production of the granulation tissue with endothelial cells, then the production of chronic inflammatory cells and subsequently increases the proliferation of fibroblasts, causing an overgrowth called reactive hyperplasia.[7] The age of the patients in the present study ranged from 22 to 83 years, with a strong predilection for the group of 70-80 years of age. The age predilection differed substantially from other studies, which reported IFH mainly in the fourth decade of life.[4,7,8] No gender predilection in this group was seen in our study. The differences in age, gender, histopathological diagnosis and anatomic location in comparison with other studies are mainly due to different classifications and terminology of lesions and the number of cases. The IFH was more prevalent on the buccal mucosa resulting from injury in the line of occlu-

sion.[9] The lower lip was the second most common site of occurrence without an essential difference in value. In our study, reactive hyperplasia was the most common histopathological image, as the fibroblasts were the most vulnerable to chronic irritation. The histopathological image of keratosis disorder, excessive connective tissue proliferation, granulation tissue formation, chronic inflammation and fibrosis predominated in our study.[8]
IF was the second most common lesion with an approximate frequency of 31%, which is similar to IFH. Fibroma is a well-defined lesion the color of oral mucosa, sessile or pedunculated, with a smooth non-ulcerated surface that is soft or firm in consistency.[10] The distribution of the most common occurrence site is similar to IFH, but it has a greater predilection for females, which is in accordance with the previous study by Hunasgi et al.[8] The age of patients ranged from 18 to 80 years with a peak incidence between the fifth and the sixth decade of life. The main differences between IF and IFH are the inflammatory cells. IF is merely a response to a chronic irritation without an inflammation and keratosis disorder. In our opinion, the development of these reactive lesions can be associated with different duration periods, an individual predisposition and the general health of the patient or the presence of other factors that modify the histopathological image, showing both epithelial and connective tissue involvement. We suggest that IFH and IF, especially in the same location, can be the same lesions at the different stages of histological maturation. PG was the third most frequent reactive lesion, which comprised 12.9% of all lesions. Clinically, it manifests itself as an exuberant, smooth or lobulated lesion with small, red papules on a pedunculated or sessile base, and is usually hemorrhagic. Inflammatory and vascular components, as well as granuloma tissue formation, were the typical features of PG.[4,11] In our opinion, a strong predilection for marginal gingiva both in the present study and the other studies provides evidence that periodontium was the primary source of PG. Periodontal ligament, periosteum and connective tissue are the origin of PG. Therefore, it seems that the evident prevalence of these lesions in gingival can be meaningful. PG is formed in response to local irritation from calculus, defective restorations or hormonal factors. In our study, there was a strong predilection for the gingiva in the anterior aspect of the mouth, especially in the mandible, in females because of the effect of female hormones such as estrogen and progesterone on the gingiva.

All reactive lesions were more common in females than in males, with a strong predilection in IF, FG, PGCG and PG groups. Similar results were presented in other studies in which there was a similar female/ male ratio 1.5:1.[5,7,12] This finding could suggest that greater diligence is required with respect to the aspect of dental care and the role of hormones in female patients.

GF due to epithelial and fibrous hyperplasia resulting from the trauma caused by the border of an ill-fitted removable denture constituted 12.1%. The medical records indicated that each of the patients in our study was using a denture, therefore we qualified removable prosthodontic appliances as the major factor in the etiology of FG. Removable dentures were the most common causative factor in the formation of GF. The primary location of GR was vestibular mucosa. GF had a bigger diameter in comparison to other reactive lesions. There was a slight predilection for females, and in the majority of cases, it occurred in the anterior vestibule proportionately in the mandible and maxilla.[9,13]

In our study, PGCG comprising 8.6% of all reactive lesions originates from the connective tissue of the periosteum or the periodontal ligament, hence the gingiva was the only site of occurrence. The lesions displayed a distinct predilection for females and the anterior portion of the mouth.[14] As in the previously reviewed studies, PGCG was the least common reactive lesion, not taking LF and AF into consideration. This contrasts with published data, where PGCG was the most commonly occurring lesion.[1,4,7,8]

In the majority of cases, the peak incidence of the appearance of reactive lesions was from fifth to the seventh decade of life in contrast to some previous studies and with an agreement with some other studies.[4-7,12,15-17] The older patients were the most significantly affected group of all because of the increasing awareness of young people regarding teeth alignment, and the necessity of restoring decayed or missing teeth. The elderly also have an oral mucosa which is less resistant to harmful factors. Our females: males ratio of 1.5:1 was in accordance with other studies.[5,7,12]

We found that the most commonly affected oral sites were, in descending order, the buccal mucosa, lower lip, vestibular mucosa and marginal gingiva. In general, the vestibular compartment of the oral cavity is vulnerable to the development of reactive lesions because of the exposure to mechanical irritation, injuries and trauma.[18,19,20] The most critical issue is the elimination of all potentially harmful and traumatic factors. On the other hand, this location is more accessible for early detection for both patients and doctors and also reachable for the complete excisional biopsy.

Our study is the first Polish epidemiological description of reactive lesions. It seems appropriate to compare our research on the Polish population with other medical centers as our results can reflect specific Polish epidemiological and population features. The limitations of this study arise mainly from the lack of the age diversity in the study group. Different dental and medical problems are typical for different age groups and in our study, there was a definite predominance of elderly patients. In many cases, the real causative factor of reac-

tive lesions was not determined. The differences in the clinical characteristics of reactive lesions were probably related to different methods of categorizing various benign oral soft tissue masses.

Conclusions

The most common lesion in the present study was fibrous hyperplasia and irritation fibroma. This is mainly due to the injury being the chief factor provoking the oral mucosa to chronic inflammation resulting in hyperplasia. In the management of reactive lesions, vigilance and an adequate initial diagnosis followed by a histopathological confirmation are crucial along with the complete excision and the elimination of local irritants - especially from the vestibule of the oral cavity which is the most vulnerable area in the oral cavity. The algorithm for reactive lesions includes fast detection and elimination of all potentially harmful habits, local irritants and parafunctions prior to all surgical procedures in order to minimize the future risk of recurrence.

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ORIGINAL PAPER

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Risk factors and the incidence of overweight and obesity in pre-school children from the southern part of Poland

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ABSTRACT

Introduction. In recent years there has been a significant increase in the prevalence of overweight and obesity in humans. It turns out that the problem is not limited to adults; excessive body weight is occurring in children more often.

Aim. The main purpose of this work was to determine the prevalence of overweight and obesity in preschool children from the Rzeszów district, and to determine risk factors for occurrence.

Materials and survey method. The study was conducted among 200 preschool children (3 - 6 years of age) from the Rzeszów poviat area. Measurements of height, weight, and determination of BMI were performed and these values are standardized according to the WHO centile grids appropriate for each age group.

Survey results. Normal weight was observed in 58% of the respondents, 11% were overweight, and 10.5% were obese, whereas 20.5% of children had undernourishment. Obese children were the largest group among 6-year-olds. Among 4 year old children, abnormal body weight were more frequent in boys. On the other hand, in children aged 5 years, undernourishment or overweight was found more frequently in girls.

Conclusions. The study did not confirm a significant relationship between gender, place of residence and socio-economic situation of respondents, and the prevalence of overweight or obesity. The results of this study indicate that the problem of excessive body weight refers to the increasing number of children.

Key words. obesity, overweight, undernourishment, preschool children

Introduction

According to the World Health Organization (WHO), obesity is a condition when the body accumulates too much body fat, which can negatively affect health.[1] Correct body mass is determined with respect to age, sex and race. Body Mass Index (BMI) and Waist-Hip Ratio (WHR) are commonly used to diagnose and assess obesity. World Health Organization (WHO) centile charts were used for children in this study. According to the latest WHO recommendations, overweight is diagnosed

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when BMI value falls within the range of the 85-95 percentile (cc) and obesity when this value is above 95 cc.[2]

Obesity in children is classified according to etiopathogenesis. The following types of obesity can be distinguished:

– Simple obesity, also called primary (monosymptomatic) is one of the most common forms of obesity (90% of obesity cases).
– Secondary (pathological) obesity is associated with endocrine diseases, genetic defects, diseases of the nervous system or is a consequence of long-term treatment.

Obesity in adolescence is associated with a slower growth rate, which reduces the need for energy, the child's appetite clearly increases, in order to accumulate fat, which will become the main source of energy in the maturation phase.[3,4] In pre-school children, one of the factors predisposing development of overweight or obesity are biological e.g. genetic.[5,6] Social and environmental factors that predispose overweight in children are mainly mass media and advertisements displayed by them, where the average calorific value of advertised food products is from 1700 – 33,000 kcal per day.[7,5] One of the most important determinants is the social environment in which the child lives and develops. Parents influence the availability of food products at home, shaping the children's eating habits and the time devoted by children to physical activity.[8,9] Consequences of overweight or obesity may include endocrine, cardiovascular, gastroenterological and pulmonary complications and disorders of the motor system.[10] Another discussed effect of obesity is the metabolic syndrome, which is characterized by carbohydrate or fat metabolism disorders and hypertension.[6] Consequences of obesity may also be low self-esteem, anxiety or depression.

Preventing the occurrence of obesity and overweight in children should start from the first day of life through the introduction of breastfeeding. In later stages of life, a child should be given the chance to decide what amount of food to eat with respect to hunger experienced.[11]

The main form of prophylaxis of overweight and obesity is nutritional education in terms of changing eating behavior.[12] Children should also be encouraged to become more physically active.[13] The immediate surrounding of the child plays an important role in promoting and caring for the right amount of physical activity. Proper nutritional behavior and high level of physical activity should accompany children from the early age, thus conditioning their proper development.

Aim

The aim of this study was to determine the prevalence of overweight and obesity and their risk factors (age, sex, place of residence) in pre-school children from the Rzeszów district.

Materials and methods

This study was conducted in kindergartens across the Rzeszów district. The study lasted four months. The study group consisted of 200 children of pre-school age from 3 to 6 years. The boys constituted 51.5% of the study group, and girls 48.5%.

The first stage of the study was to obtain parental written consent to conduct measurements (height and weight) in children and to fill in a child nutrition questionnaire.

A Child Feeding Questionnaire (CFQ) was used, which is a subjective assessment of beliefs, attitudes and parenting practices concerning child nutrition with an emphasis on the tendency to obesity or overweight in children. This questionnaire consists of 7 domains, 4 of them relate to the beliefs of parents affecting the tendency to childhood obesity, and the remaining three groups concern attitudes, practices and parental control in the field of child nutrition.

The study consisted of performing a measurement of height and weight in children three times. The body height was measured with a SECA 213 stadiometer three times. The height was measured with 5 mm accuracy. Measurements were made in a standing, upright position and without shoes. The average value from three measurements was used for further analyzes. The base of the stadiometer was disinfected after each measurement. Body mass measurement with 100 g accuracy was also performed in triplicate using a Tanita BC-418 MA body composition analyzer. The measurement values were read from the printout from the analyzer. The body mass index (BMI) was calculated based on the measurements using the formula: body weight (kg) / height (m^2). These values were standardized by WHO percentile grids for each age group, respectively (Fig. 1).

Children came mostly from four-person families (42.5% of the respondents), but also often from three-person families (23%) or five-person families (24.5%). About 40% of the mothers had secondary education, 36.0% had higher education, and 24% had primary, lower secondary or a vocational education. Accordingly, 22% of the fathers had secondary education, 13% had higher education, and 65% had primary, lower secondary or vocational education.

The criteria for inclusion in the study were the age and place of residence of the children and the written consent of the parents to carry out the measurement tests.

The results of the study were subjected to statistical analysis using the Pearson chi-square test. Statistical analysis was performed using Statistica 10.0 software.

Results

Overweight was found in 14.6% of 3-year-olds, 17% of 4-year-olds, 7.8% of 5-year-olds and 5.5% of 6-year-

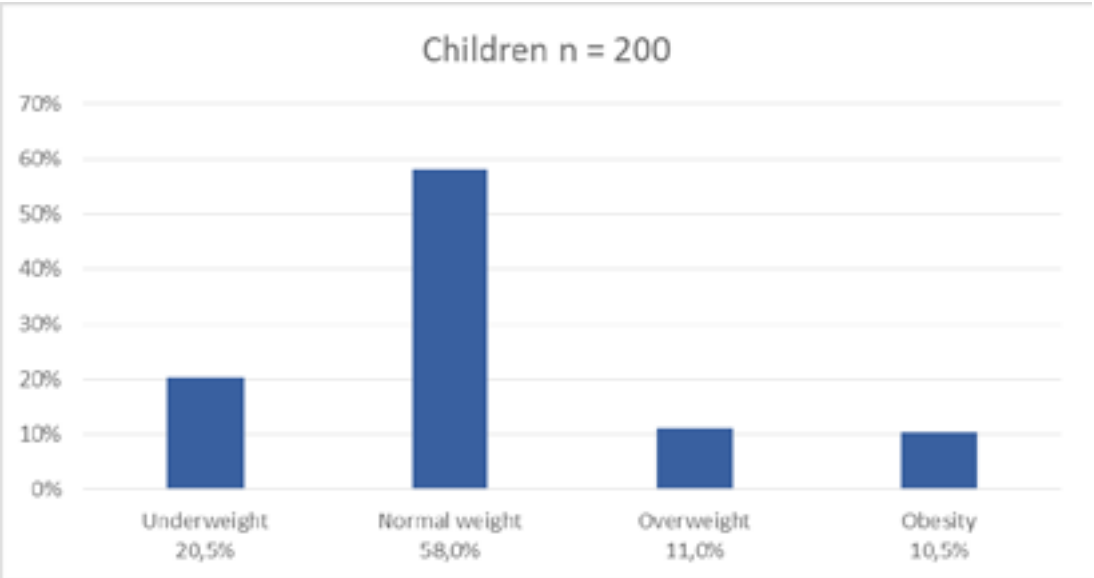

Fig.1. A graph of BMI standardized by WHO percentile grids for each age group from the children sampled in this study

Table 1. Age of the children with respect to BMI

		Underweight	Normal weight	Overweight	Obesity	*p*
(n)		41	116	22	21	
Age	3 - years old	7	23	6	5	0.1544
		17.1%	56.1%	14.6%	12.2%	
	4 - years old	11	31	9	2	
		20.8%	58.5%	17.0%	3.8%	
	5 - years old	12	32	4	3	
		23.5%	62.7%	7.8%	5.9%	
	6 - years old	11	30	3	11	
		20.0%	54.5%	5.5%	20.0%	

Table 2. BMI in 4 and 5-year-old boys and girls

		Underweight	Normal weight	Overweight	Obesity	*p*
4 - year old	Boys	25.9%	40.7%	25.9%	7.4%	
	Girls	15.4%	76.9%	7.7%	0.0%	0.0422
5 -year old	Boys	10.7%	78.6%	0.0%	10.7%	
	Girls	39.1%	43.5%	17.4%	0.0%	0.0027

olds. Obesity was found to occur in 12.2% of 3-year-olds, 3.8% of 4-year-olds, 5.9% of 5-year-olds and 20% of 6-year-olds. Children with normal body mass dominated in each age group (Table 1). There were no statistically significant changes.

In the group of 4-year-olds, there was a statistically significant difference in the value of BMI among girls and boys ($p = 0.0422$). Normal body mass was more frequently observed in girls (76.9%) among 4 year olds. Obesity was not found in the same group in girls. Boys, more often than girls, were underweight (25.9%) or overweight (25.9%) in the 4 year old group. In this group, there were also boys who were obese (7.4%). These dependencies were statistically significant.

Also statistically significant difference at the level of $p < 0.01$ ($p = 0.0027$) was confirmed in the BMI value among girls and boys aged 5. Normal weight was significantly more frequently found in boys (78.6%) than in girls (43.5%). A similar relationship was found with respect to obesity: boys - 10.7%, girls - 0.0%. Girls, more often than boys, showed abnormal body mass, such as underweight, which accounted for 39.1%, compared to boys where 10.7% were underweight and 17.4% overweight. In the group of 5-year-old boys no overweight boys were found (Table 2).

Underweight children more often live in urban areas (23.4%) in comparison with children who live in the countryside (18.7%). The difference is also observed in children with obesity, which is associated with the place of residence: the city - 6.5%, the village - 13%.

Over 1/3 (37.5%) of parents never allow a child to eat a large amount of fast food. Typically, about 35% of

parents offer their child their favorite foods in exchange for good behavior; 27.5% of parents use this practice occasionally. Likewise, around 35% of parents usually offer sweets to children as a reward for good behavior. About 17.5% of parents keep some products out of reach of the child; 22.5% of the respondents do it usually and about 1/3 (30%) do this often. In the survey, we found that 20.5% of children usually do not eat high-fat products and 13% of children do not eat them at all. The statement "the child does not eat too much sweets" was fully accepted by only 14.5% parents and 33% agreed with it as usually the case.

Regarding the incidence of overweight or obesity, it is important that parents monitor children's eating habits. Parents usually (44%) pay attention to how much sweets the child eats. Over 2/3 of the respondents watch how many snacks the child eats. On the other hand, 9.5% of the respondents rarely pay attention to the consumption of snacks by children, which may later be manifested by overweight or obesity.

Discussion

Currently, both overweight and obesity are a global problem. Epidemiological studies show that the age of the overweight or obese population has been declining in recent years. The problem of abnormal body weight concerns increasingly younger generations. According to studies conducted worldwide, the number of children who were found to have excessive body mass increased threefold in the last decade of the 21th century.[14]

A meta-analysis conducted from data spanning the years 1980-2013 by Marie Ng et al. shows that there has been a significant increase in overweight and obesity among children, not only in developed countries, but also in developing countries, which indicates that this is a global problem.[15] A similar study was published in 2017 by the Lancet describing the increase in the incidence of overweight and obesity worldwide.[16] Studies carried out in this paper showed overweight in 10.7% of boys and 11.3% of girls, while obesity was found in 13.6% of boys and 7.2% of girls.

In the studies by Mazur et al. from 2008, over 9.9% of boys were overweight and 9.1% of girls, whereas obesity was found in 8.4% of boys and in 7.2% of girls. The authors draw attention to the high incidence of obesity among 3-year-old girls (19.1%) and 6-year-old boys (11.9%). In turn, researchers most often found overweight in 6-year-old girls (14.1%) and in 3 and 6-year-old boys: 12.5% and 12.9% respectively. Based on these results, the authors suggest the incidence of "rebound obesity" in these children, which increases the risk of excessive body weight in adolescence and adulthood.[17]

In studies conducted by Weres et al. in 2016, overweight was recorded in every age group in boys and girls. However, obesity was not reported in 3-year-old, 4-year-old and 6-year-old girls and in 3-year-old boys.[18] When comparing the results obtained by the author with the results of this study, it is worth noting that the results obtained by us also did not show obesity in 4-year-old girls.

In the research of the Children's Memorial Health Institute, Warsaw, Poland conducted in 2010 - 2012, overweight and obesity was found in a group of 3-year-olds in 9% of boys and 12.6% of girls, and in 14.8% of boys and 18% of girls in a group of 6-year-old children. Moreover, as in our study, correlation between place of residence and the risk of overweight or obesity was not found in these age groups.[19] However, in the studies conducted by Mielnik-Błaszczak et al., rural children were more likely to eat sweets compared to urban children, which may predispose them to abnormal (excessive) body weight.[20] Ligenza et al. in research carried out in 2010 found excessive body mass in 24% of children living in a big city and in 10.8% of children from a smaller town. However, obesity was observed in 12% of the examined children from a big city and 6.2% of children from a small town, respectively. In addition, the researchers showed that only 10.6% of the parents notice the problem of excessive body weight in their children, while overweight or obesity was found in over 20% of pre-school children.[8] Studies on the incidence of overweight and obesity in pre-school children from the Lublin area were carried out in 2013 under the supervision of Kostecka M. Due to the diversity of indicators applied, overweight and obesity cannot be compared with the results of our study. A total of 21% of the study group were children with excessive body mass.[21]

In the studies of Kułaga et al. among pre-school children, overweight or obesity was found in 12.2% of the examined boys and in 10% of girls according to the definition of overweight / obesity determined by WHO. The study also compared the incidence of abnormal body weight in Polish children and their American and Norwegian peers. Obesity was more frequently observed in Polish boys than in Norwegian. Among girls, excessive weight was observed in Polish women more often than in American women.[22] Similar results were obtained in our study. Overweight was found in 11% of the children, and obesity in 10.5% of the subjects.

In the years 2011-2012, nationwide studies were conducted in Poland regarding the incidence of overweight and obesity in children aged 3 to 5 supervised by H. Weker and Z. Chwojnowska. Overweight was found in 12.1% of children, and obesity in 8.1%. In addition, excessive body weight was more often found in children living in rural areas, which is also confirmed by the results of our study. Weker and Chwojnowska's studies also showed a more frequent incidence of excessive body mass in children from families in which mothers had basic or vocational education. We found that the

higher the mother's education, the lower the percentage of children with normal body weight.[23]

Mazur et al. in their research, did not confirm a significant relationship between the material situation of the family and the incidence of overweight or obesity in children.[9] Likewise, no significant relationship was found between the economic status of the family and BMI of children in our study.

The results of research developed for the purposes of this paper, as well as reports from the studies cited above, suggest the need for prevention and prophylaxis of obesity or overweight in the youngest. One should also focus on research determining risk factors for excessive body mass among pre-school children from all over Poland, because this is not a problem related only to the Podkarpacie region.

All these activities will allow us to develop a detailed plan to prevent the incidence of excessive body mass in children. In addition, such a procedure will help to minimize risk factors conducive to this phenomenon, and, above all, will enable the implementation of individual programs promoting a healthy lifestyle.

Conclusions

On the basis of the results presented above, the following conclusions were drawn:

1. Overweight was found in 11% and obesity in 10.5% of the respondents. 58% of the examined children have normal body mass, while underweight was found in 20.5% of the subjects.
2. A statistically significant difference in the BMI value was found among girls and boys in the group of 4-year-olds.
3. A statistically significant difference was also confirmed in the BMI value among girls and boys aged 5, where girls were more often underweight. In contrast, boys were more likely to have normal body mass.

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ORIGINAL PAPER

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The comorbidity of papillary thyroid carcinoma and the primary hyperparathyroidism

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ABSTRACT

Introduction. The prevalence of papillary thyroid carcinoma (PTC) in patients with primary hyperparathyroidism (PHPT) is low, it can be estimated around 2 to 4%. For unknown reasons it is higher than the prevalence of PTC in the overall population. The authors analyse the comorbidity of PTC with PHPT on patients treated in their institution.

Material and method. The analysis covered medical records of 885 patients subject to the thyroid resection procedure and 95 patients operated for PHPT, the procedures were performed in years 2005-2014.

Results. In the above-mentioned period there were 121 patients operated due to a malignant thyroid tumour and there were 95 patients that had surgery for PHPT. There were 4 cases of comorbidity of PHPT with papillary thyroid cancer.
Prevalence of PTC at the patients with PHPT was 4.2%.
In two out of the four cases, both diseases were diagnosed prior to the procedure and the single appropriate surgery i.e. total thyroidectomy and excision of parathyroid adenoma was performed.
In the other two cases false positive localisation of parathyroid adenoma occurred due to metastatic cancerous lesions in cervical lymph nodes. The diagnosis of PTC was made postoperatively based on surgical specimen examination. Second surgical procedure appropriate for this diagnosis was necessary in both cases.

Conclusions. The comorbidity of PHPT and PTC is clinically important and should be taken into account in the case of patients with PHPT and thyroid tumours. There is the possibility of false positive localization of parathyroid adenoma in the case of metastatic cancerous lesions in cervical lymph nodes.

Keywords. thyroid carcinoma, primary hyperparathyroidism, parathyroid adenoma

Introduction

Primary hyperparthyroidism (PHPT) affects around 0.1-0.5% of population and the clinical symptoms of it are: hypercalcemia, often urolithiasis and osteopenia.[1] It may occur as a part of Multiple Endocrine Neoplasia syndromes.

According to the Polish National Cancer Registry, thyroid neoplasms are 0.5% of neoplasm cases in men

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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 2.6% in women. They occur predominantly for people in their thirties and fifties, more often in women than men. Papillary thyroid cancer (PTC) and follicular thyroid cancer are 90-95% of all cases of thyroid cancer.

The comorbidity of PHPT and thyroid diseases is frequent and occurs in 20-60% of cases.[2–5] The comorbidity of thyroid and parathyroid diseases was described for the first time in 1947 and it concerned a case of comorbidity of hypothyroidism and hyperparathyroidism.[6]

In the genetic Multiple Endocrine Neoplasia-2A syndrome (also known as Sipple's syndrome), the medullary thyroid cancer and PHPT often co-occur.[7] On the contrary, the comorbidity of PHPT and PTC is rare (2-4%), but it is more frequent than in general population.[8-25] The reasons for higher prevalence of PTC in patients with PHPT are yet to be discovered.[24]

Aim of the study

The purpose of the paper is to assess the comorbidity of PTC and PHPT in patients treated in the authors institution.

Material and method

Based on the computer database containing the medical records of Clinical Hospital No. 2 in Rzeszow, we identified all patients admitted since January 2005 to April 2014 who had the thyroid resection procedure and/or patients subject to surgical treatment for PHPT at the Department of General Surgery

In this period, there were 885 thyroid procedures performed. In 341 cases, it was the complete removal of the thyroid; in 314 cases, it was the removal of a thyroid lobe and isthmus; in 217 cases, it was the subtotal removal of the thyroid; and in 13 cases, it was the partial removal of the thyroid (Fig. 1). All patients operated due to thyroid malignancy were identified and compared with the list of patients operated for PHPT.

In all patients with the thyroid pathology, the neck ultrasonography (USG), Fine Needle Aspiration Biopsy (FNAB) of the thyroid tumours suspected for malignancy, laryngological examination as well as levels of FT3, FT4, TSH in blood serum were performed. In the case of PHPT, the levels of parathormone, calcium and phosphates in blood serum were routinely checked; the neck scintigraphy 99m Technetium-sestamibi ('sestamibi') dual-phase and USG neck scan were performed.

Results

There were 121 patients operated due to a malignant thyroid tumour, which is 13.7% of all thyroid resections performed.

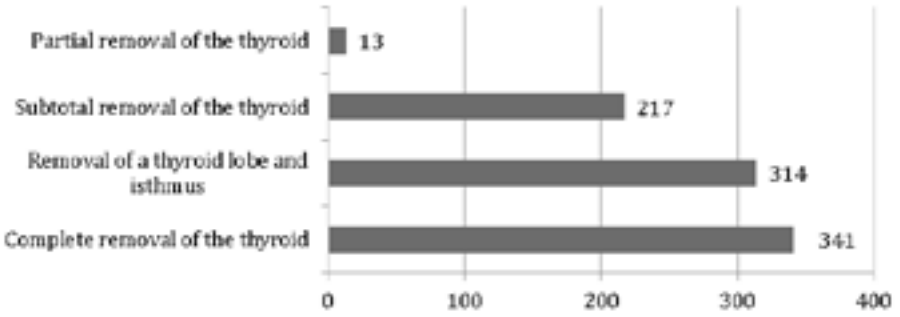

Fig. 1. Thyroid resection procedures performed from Jan 2005 to Apr 2014

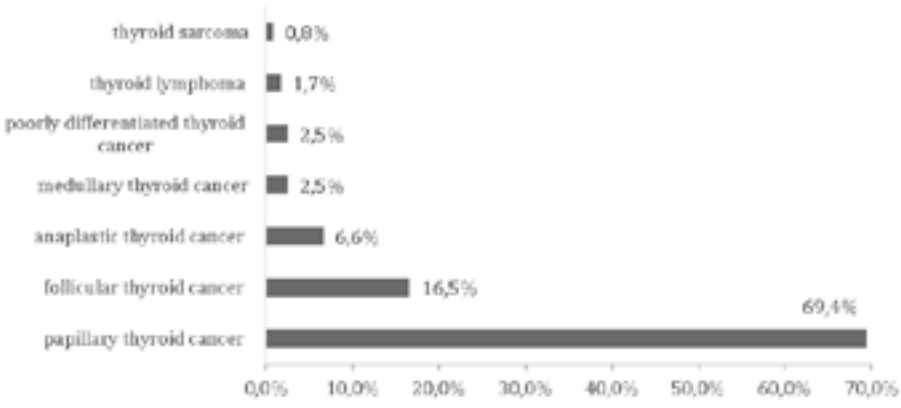

Fig. 2. Thyroid carcinomas identified during the histopathological examination after thyroid resection procedures from Jan 2005 to Apr. 2014

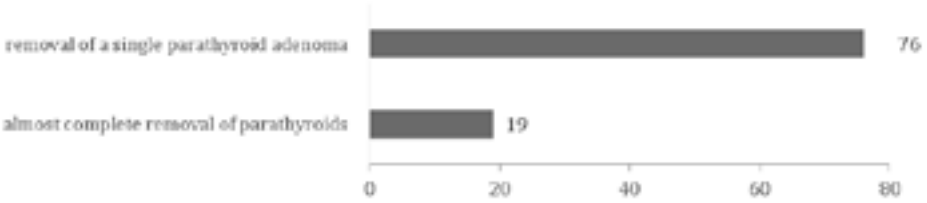

Fig. 3. Parathyroid resection procedures performed from Jan 2005 to Apr 2014

There were 84 patients operated due to PTC (69,4%), 20 patients operated due to follicular thyroid cancer (16.5%), 8 patients operated due to anaplastic thyroid cancer (6.6%); 3 patients due to medullary thyroid cancer (2.5 %), 3 due to poorly differentiated thyroid cancer (2.5 %), 2 due to thyroid lymphoma (1.7%), 1 due to sarcoma (0.8%) (Fig. 2).

In the studied period, 95 procedures of parathyroid resection were performed.In 19 cases, it was almost complete removal of parathyroid glands (20%), and in 76 cases it was the removal of a single parathyroid adenoma (80%) (Fig. 3).

Gender and age of patients with PHPT and PTC is presented in Tab. 1.

In four patients, the comorbidity of PTC and PHPT was determined, which is 4.2% of PTC cases in patients operated due to the PHPT. All four patients have diagnosis of primary hyperparathyroidism based on metabolic evaluation and elevated serum parathormone level.

Table 1. Gender and age of patients with PHPT and PTC

	women	men	womens age	mens age
PTC	69	15	18-87 avg 53	20-76 avg 48
PHPT	75	20	20-84 avg 60	22-75 avg 55

The diagnosis of PTC before surgery was made only in two out of four patients (50%) by FNAB. The other two patients had diagnosis of PTC based on surgical specimen microscopic examination after first surgery.

Parathyroid adenomas localization by ^{99m}Tc-MIBI dual-phase planar imaging was correct in one patient out of four patients. In the next case there were no gathering of marker outside thyroid gland and in two other cases scintigraphy showed metastatic lymph nodes while adenomas were not localised.

Clinical and surgical details of patients with the comorbidity of PTC and PHPT are summarised in Tab. 2, 3, 4.

Table 2. Diagnostic workup of cases with the comorbidity of PTC and PHPT

	gender	age	PTH serum level	Calcium serum level	Diagnosis before surgery
Case 1	W	62	239.8 pg/ml (std. 15-65pg/ml)	elevated	PHPT- adenomas of right parathyroids, Right lobe thyroid tumor
Case 2	M	71	elevated	elevated	PHPT – adenoma of right lower parathyroid, nodular goiter
Case 3	W	66	elevated	elevated	PHPT- adenoma of left lower parathyroid, PTC
Case 4	W	27	130.5 pg/ml (std. 15-65pg/ml)	12.2 mg/dl (std. 8.8-10.6 mg/dl)	PHPT, left lobe PTC

Table 3. Imagining examinations and FNAB of cases with the comorbidity of PTC and PHPT

	FNAB of thyroid tumor	USG scan of the neck	CT of the neck	Scintigraphy of the neck
Case 1	benign lesion	Right thyroid lobe tumor, two tumors outside the right thyroid lobe	Right thyroid lobe tumor, two tumors outside the right thyroid lobe	Intensified gathering of marker in lesions outside thyroid
Case 2	benign lesion	Nodular goiter, Right lower parathyroid adenoma	none	Right lower parathyroid adenoma
Case 3	PTC	Nodular goiter, lesion suspected for malignancy in isthmus	none	Left lower parathyroid adenoma
Case 4	PTC	Nodular goiter, lesion suspected for malignancy in left lobe	none	No localization of parathyroid adenoma

Table 4. Surgical treatment of cases with the comorbidity of PTC and PHPT

	First surgical procedure	Specimen microscopic examination	Second surgical procedure	Results
Case 1	Right thyroid lobectomy Removal of two tumors located near right thyroid lobe	Right lobe PTC with metastases to lymph nodes	Completion thyroidectomy, right cervical lateral lymphadenectomy, left lower parathyroid adenoma removal	Supplemented treatment with radioiodine, Euthyroidism on levothyroxine, normal function of parathyroids
Case 2	Removal of enlarged lymph node below lower pole of right thyroid lobe, Excision of parathyroid adenoma in mediastinum	PTC metastasis to lymph node, parathyroid adenoma in mediastinum	Total strumectomy, no primary PTC focus in thyroid	Suplemented treatment with radioiodine, Euthyroidism on levothyroxine, normal function of parathyroids
Case 3	Total strumectomy, paratracheal lymhadenectomy, excision of left lower parathyroid adenoma	Bifocal PTC of the thyroid, left lower parathyroid hyperplasia, No metastases to lymph nodes	none	Euthyroidism on levothyroxine, normal function of parathyroids
Case 4	Total strumectomy, paratracheal lymhadenectomy, excision of right upper parathyroid adenoma	Left lobe PTC, no metastases to lymph nodes, right upper parathyroid adenoma	none	Euthyroidism on levothyroxine, Planned Supplemented treatment with radioiodine, hypoparathyroidism

Discussion

The incidence of PTC in patients with PHPT is rare. It is higher than the incidence of PTC in general population. It can be estimated as 2-4%.[11,21–23] In our series it was 4.2% and was comparable to the results described in the literature. It does not differ from the incidence of PTC at patients operated due to the nodular goitre.[21,25] So far, it has not been determined whether a factor causing the higher incidence of PTC at patients operated due to PHPT exists.[11]

Due to the high prevalence of the thyroid gland pathology in patients treated surgically for PHPT, estimated around 20-60%[2–5], it is advisable to perform standard preoperative USG scan of the neck with a possible fine needle biopsy of the focal thyroid lesions and/or lymph nodes suspected for malignancy.[4,26]

In our material, the cervical USG scan and 99m Technetium-sestamibi dual-phase scintigraphy of the neck were routinely performed at patients qualified for the surgical treatment due to PHPT, which are currently standard techniques of parathyroid imaging.[27–30]

Despite performing these tests, the thyroid cancer was diagnosed before surgery in only two out of four cases with comorbidity of PHPT and PTC. This indicates difficulties in differentiation of malignant tumors and parathyroid adenomas using the above methods.

Similar diagnostic problems using sestamibi dual-phase scintigraphy and USG of the neck descibed K.L.Whitcroft. False identification of thyroid PTC as parathyroid adenoma resulted in surgical bilateral neck exploration to find real localisation of parathyroid adenoma, and second surgery for completion thyroidectomy due to PTC, which was unsuccessful due to extensive postoperative fibrosis.[31]

Diagnostic problems in patients who had coincidence of thyroid and parathyroid pathology showed by Onkendi et al in their retrospective analysis of scintigraphy used in 374 patients. A false-positive rate of parathyroid localization in 22% of patients with benign thyroid disease and 45% with malignant thyroid disease was reported.[32]

Inadequate diagnosis before surgery makes two problems. First, inappropriate localization of parathyroid adenoma can cause failure of PHPT treatment. Second, misinterpretation of scintigraphy can result in lack of diagnosis of PTC before surgery. Both problems can lead to second surgery with increased risk of complications such as recurrent laryngeal nerve palsy or postoperative parathyroid insufficiency. Increased risk is caused by inflammation and/or fibrosis after original operation.

In our series, one adequate surgery for both diseases was performed in patients with correct diagnosis made before surgery.

The next two cases were misdiagnosed, metastatic lymph nodes were wrongly identified as parathyroid adenomas. Correct diagnosis was based on postoperative surgical specimen examination which revealed metastasis of PTC to the lymph nodes removed.

Both cases were scheduled to total thyroidectomy, and second surgery was performed uneventfully. In one

of the patients removal of earlier missed parathyroid adenoma was also performed

Similar cases was described by Lee JK and co-authors[33] and by Polyzos SA et al.[34] The thyroid cancer was diagnosed based on examination of the metastatic lymph node removed along with the parathyroid adenoma; in the thyroid removed during second surgery the focus or foci of papillary thyroid cancer was found.

In our patients with second surgery performed, there were two foci of PTC in one patient and no focus of PTC in the second.

The last patient may be similar to cases described by Yamamoto T and co-authors.[35] They reported the metastases of PTC in 3 out of 148 patients subject to the cervical lymphadenectomy due to squamous cell cancer of the oral cavity. The patients were subjected to observation, and the progression of thyroid cancer was not found in 3-5 years.

The detection of parathyroid adenomas by ^{99m}Tc-MIBI dual-phase planar imaging in our series was correct in one patient out of four. In the next case no gathering of marker outside thyroid gland occurred and in two other cases scintigraphy showed metastatic lymph nodes and adenomas were not found.

To improve the accuracy of preoperative diagnosis in primary hyperparathyroidism, secondary hyperparathyroidism, thyroid lesions or metastatic lymph nodes newer diagnostic methods like ^{18}F-fluorocholine hybrid positron emission tomography/X-ray computed tomography (FCH-PET/CT) or ^{99m}Tc-MIBI single-photon emission computed tomography associated with computed tomography scintigraphy (^{99m}Tc-MIBI SPECT/CT) were proposed.

FCH-PET/CT detects a significantly greater number of abnormal parathyroid glands than USG and it is at least as sensitive as ^{123}I/^{99m}Tc-sestaMIBI dual-phase scintigraphy without reducing specificity.[36]

^{99m}Tc-MIBI SPECT/CT has significantly higher sensitivity in the detection of PHPT lesions than does ^{99m}Tc-MIBI dual-phase planar imaging (87.8% *vs.* 75.6%, $P < 0.05$) and it also can detect a greater number of other abnormal lesions, particularly hyperplastic lesions.[37]

On the other hand Nagar and coauthors recommend a novel interpretation techniqe of SESTAMIBI SCANS combined with USG of the neck to increase the detection of parathyroid adenomas. Additional lenght of the thyroid lobe on sestamibi compared to the lobe lenght on USG was considered a positive finding. They increased sensitivity of adenoma detection to 93.8% in comparison to the traditional SESTAMIBI image (68-87%).[38]

Conclusions

PHPT and PTC occur together rarely with the prevalence of 2-4%. Diagnosis of the comorbidity of both diseases makes possible treatment during one surgery. Thus, such a comorbidity should be considered in patients with PHPT and focal thyroid lesions. Despite performing imaging tests like cervical USG and SESTAMIBI SCANS on routine basis, a correct diagnosis before surgery may not be easy. Metastatic cervical lymph node may be wrongly assumed as parathyroid adenoma.

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ORIGINAL PAPER

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Analysis of the correlation between body composition, construction and aerobic capacity in teenage team sport training

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ABSTRACT

Introduction. Research results indicate that a low level of physical fitness is associated with a high percentage of fat in the body and low levels of physical activity. The aim of this work was to assess the relationships between selected morphological indicators, and the level of aerobic capacity in adolescents attending the schools with team sports.

Material and methods. The studies covered students participating in sports in middle school and high school; the study group included 90 boys aged 13-19 years.

Body height and weight were measured as well as waist and hip circumference. Body weight components were assessed by using a Tanita Body Composition Analyzer. A Fitnessgram® test battery was used to assess physical fitness.

Results. Research results indicates systematic increase of somatic characteristics such as weight, body height and waist and hips circuits with age. Taking into account the results obtained with the PACER test indicate a very good aerobic capacity of tested boys.

Conclusions. Age is a factor that improves motor fitness of physically active adolescents. There is no linear relationship between BMI and fitness among the boys who regularly train team sports, but both lower and higher BMI values seem to be connected with lower physical fitness.

Keywords. fitnessgram, adolescents, cardio respiratory fitness

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Introduction

Research results indicate that a low level of physical fitness is associated with a high percentage of fat in the body with low levels of physical activity.[1–5] In the most highly developed countries, for the last 20 years, there is an epidemic of sedentary lifestyle.[6] The results of the observation of changes in physical activity in studies of Polish teenagers conducted in the 90s of the last century leads one to believe that levels of physical activity decreases with age and is constantly lower than recommended.[7] One can estimate that only 30% of children and adolescents, and 10% of adults practices physical forms of activity in which the intensity of the load effort meets the physiological needs of the organism. Moreover, it is reported that cardiopulmonary efficiency is more strongly associated with cardiovascular risk factors than objectively measured physical activity components in children and adolescents.[8] As physical fitness is an important part of metabolic health as well as a strong independent predictor of premature mortality, identification of changes level of physical fitness among children in the age of "the obesity and sedentary lifestyle epidemic" may indicate the need for implementation of actions aimed at improving physical fitness in this group.[9–14]

Aim of this study

The aim of this work was to assess the prevalence of the relationships between selected morphological indicators, and the level of aerobic capacity in adolescents attending the schools with a sport profile.

Materials and methods

Studies were carried out within the framework of the project of the Student Research Society of Diagnostics in Sport and Health Training titled "Sport talented children and teenager assessement of the Podkarpacie region" in Secondary School number 2 in Rzeszów. The study group included 90 boys aged 13-19 years who are students of Middle and High Schools that participate in sports. Most participents trained football (85.7%), and rest of the group trained in handball. The criteria for inclusion to the test group were: being a student of V High School named of Krzysztof Kamil Baczyński, Sports Championship School or the Sports Gymnasium in Rzeszów and a valid sports medical examination. Exclusion criteria were: lack of a valid medical sport result or injury precluding performance of fitness tests and a lack of mentors or coach consent for student testing.

The first anthropometric assessment was carried out in accordance with the protocol recommended by the International Society for the Advancement of Kinanthropometry (ISAK).[15] Body height (BH) was measured using a Martin type Anthropometer; body weight (BW) and its components was assessed by using a Tanita Body Composition Analyzer type TBF 300. It is a tool recommended for this purpose by The Cooper Institute which developed the Fitnessgram® test battery. In addition, waist circumference (WC) of the subjects was measured at half the distance between the last touchable rib and the top edge of the iliac crest; hip circumference (HC) was measured at the most rearward points of the buttocks (at the widest point of the hips). Measurement circuits were made with the help of Gulick anthropometric tape to the nearest 1 mm.

Next, a physical fitness assessment was performed in accordance with the protocol described in the guide to the Fitnessgram® test battery.[16] Evaluation of aerobic capacity was carried out using a standardized 20 meter shuttle run (Progressive Aerobic Cardiovascular Endurance Run - PACER) with progressive intensity for indirect assessment of maximal oxygen consumption (VO_2 max [ml/min/kg]). The test was carried out in a gym and was based on shuttle 20 meters running distances with increasing rate of the signal generated by computer program "beep test ver. 4_1", up to the denial (fatigue) of the test, or the decline in pace in accordance with given signal.[17] The test enabled the participation of a higher number of people, and the coefficients of reliability and repeatability of results make it a recommended and used tool in population studies of children and adolescents ($r = 0.89$).[18] Muscular strength and endurance was rated by three tests: Curl Up, Trunk Lift and Push Up. Curl Up test is based on performing at the same rate (according to the sound file playing by teacher) the largest number of curl-ups in order to lie back with knees flexed and feet unanchored. The maximum number of sits predicted by the authors of the test was 75 assays. The result of assays is the number of full cycles. A trunk Lift test was performed from prone lying position with arms along the body (hands slipped under the hips). The mattress includes a point that should be addressed by sight during time of the test. The tested person slowly lifts the body as high as possible and keeps it in order to measure distances of chin from the floor. The result is given in centimeters. In the 90° Push Up, the student being tested assumes a prone position on the mat with hands placed under or slightly wider than the shoulders, fingers stretched out, legs straight and slightly apart, and toes tucked under. In this way tries to perform as many repetitions as possible. The pace of implementation is served by a teacher playing an audio file. The result of the assay is the number of complete motor cycles made until, when the tested person after the second time does not maintain the recommended speed. Flexibility was assessed using the Sit-and-reach test where the student removes his or her shoes and sits down at the test appa-

ratus. One leg is fully extended with the foot flat against the face of a box. The other knee is bent with the sole of the foot flat on the floor. Tested person performs this four times with progressively deeper slope. The result of the trial is to measure the distance from the point of the seat back of the foot of the platform given in centimeters. The test is performed twice with a straight right foot, followed by the left.

On the basis of the measurements, waist to hip ratio was calculated (WHR) as waist circumference divided by the hip circumference and waist to height ratio (WHtR) as waist circumference divided by height, where all variables are expressed in centimeters. Body mass index (BMI) was also calculated by dividing body weight in kilograms by the square of the body height in meters and the Rohrer Indicator (RI) by multiplying by 100 the result of dividing the body weight in grams by body height, expressed in centimeters and raised to the power of three. The value of VO_2max was calculated on the basis of the model proposed by Boiarskaia.[19]

$$\mathbf{VO_2max = 32.57 + (laps \times 0.27) + (3.25 \times (sex)) + + (0.03 \times (age))}$$

Statistical analysis was performed with the use of SPSS statistical analysis software. The normality of the distribution of the analyzed variables were evaluated using the Shapiro-Wilk test. The differences between the group characteristics of somatic and physical fitness due to age have been assessed using Kruskal-Wallis test due to failure to comply with the assumptions of a normal distribution of the analyzed features. In all analyses, statistically significant results were found with $p < 0.05$.

Results

Research results indicates a systematic increase of the somatic characteristics such as weight, body height, waist and hip circumference with subject age. The results obtained with the PACER test indicate a very good aerobic capacity of the boys in the study group. The biggest intra-group variation of these parameters was observed in the youngest study group. This dependency has been shown by statistical significance (Tab. 1). By analyzing the WHR, it was concluded all age groups had an average value of 0.8 level. WHtR had values between 0.42 and 0.44. According to the BMI analysis, one can note that the results of the study group fall within the limits of 19.2 and 22.2. The Rohrer indicator in all age groups accepted the value of 1.2. As in the case of indicators: WHR, WHtR and BMI, so in Rohrer indicator, the largest intra-group variation was observed in the youngest study group. Taking into account the calculated anthropometric indicators, statistical significance was noted only in the case of BMI. Body weight composition analysis showed that the highest content of fat in the body, at 12.1%, was found in boys at the age of 16 years. A similar level of body fat (11.8%) was noticed at the age of 17 years. The smallest percentage of the test parameter was noted among 15 year-olds. Results of the study shows that the value of fat free mass and total body water increases with subjects age. The value of the variation coefficient for each body composition param-

Table 1. Differences in the structure and composition of the body weight in different age groups

Age		The structure and composition of the body											
		BH [kg]	BW [cm]	WC [cm]	HC [cm]	WHR	WHtR	RI	BMI	FAT [%]	FAT [kg]	FFM [kg]	TBW [kg]
14 years (N=17)	*M*	53.5	165.7	73.3	86.5	0.8	44.3	1.2	19.2	10.8	6.2	47.3	34.6
	sd	14.3	10.8	7.9	8.3	0	4.4	0.2	3.2	5	4.6	10.8	7.9
	V	26.8	6.5	10.7	9.6	4.6	9.9	14.3	16.5	46.1	73.7	22.8	22.8
15 years (N=15)	*M*	59.6	172.6	72.9	90.2	0.8	42.2	1.2	20	9.9	6.1	53.5	39.2
	sd	7.4	4.9	4.6	5.7	0	2.3	0.1	1.7	3.1	2.5	5.5	4
	V	12.4	2.9	6.4	6.4	4.9	5.5	7.7	8.6	30.8	40.7	10.2	10.3
16 years (N=22)	*M*	68.8	178.5	76.3	93.7	0.8	42.8	1.2	21.6	12.1	8.6	60.3	44.1
	sd	8.5	6.1	5.3	5.2	0	2.7	0.1	2	3.8	3.5	6.2	4.5
	V	12.3	3.4	6.9	5.5	4.6	6.4	9.2	9.3	31.5	40.4	10.3	10.3
17 years (N=28)	*M*	72.1	180.3	78.1	94.7	0.8	43.3	1.2	22.2	11.8	8.9	63.1	46.2
	sd	11.7	6.2	6.8	6.3	0.1	3.7	0.2	3	4.8	4.8	7.8	5.7
	V	16.2	3.5	8.7	6.7	6.4	8.6	13.7	13.6	40.9	53.3	12.4	12.4
18 years (N=23)	*M*	73.2	181.5	78.3	95.2	0.8	43.1	1.2	22.2	10.8	8.2	65	47.6
	sd	10.1	6.2	5.5	5.5	0	2.5	0.1	2.2	3.3	3.6	7.3	5.3
	V	13.8	3.4	7.1	5.8	5.1	5.9	9.4	10	30.5	43.7	11.3	11.2
Test K-W	p	**0.0001**	**0.0001**	**0.0053**	**0.0004**	0.1129	0.6695	0.1823	**0.0004**	0.3807	**0.014**	**0.0001**	**0.0001**

M – arithmetic average, *sd* – standard deviation, v – coefficient of variation [%], BH – body height, BW – body weight, WC – waist circumference, HC – hip circumference, WHR – waist to hip ratio, WHtR – waist to height ratio, RI - Rohrer indicator, BMI – body mass index, FFM – fat free mass, TBW – total body water

eter indicate that the biggest intra-group variation was noted in group 14-year olds. At the same time, it has been observed statistical significance between the age of the study group and the mass of the body fat, fat free mass and total body water (Tab. 1).

Table 2 shows the numeric characteristics of individual tests that comprise the assessment of physical fitness of study group in terms of age. Analysis showed that the largest number of 20 meter distances (LAPS) in the PACER test of was noted in the group of the oldest tested participants (M = 93.0). A similar high score, at the level of 92.5 distances, was noted among the age of 16 years participants. The weakest result during this attempt was recorded in the group of 14-year boys (M=68.5). At the same time in this age group it was observed the biggest variety in terms of intra-group test parameter. In addition, there was noted statistical significance between the age of the study group and the number of distances according to PACER test (Table 2).

After analyzing the strength and endurance of the abdominal muscles, it is concluded that the results recorded in the group of 18-year-olds and men at the age of 15 years are at a similar level with a small advantage in the oldest group. The results obtained by 16- and 17-year-olds are also similar (16 years - 66.2; 17 years - 66.4). Studies have also shown that the smallest strength of ab-

Table 2. The differences of individual components of physical fitness in different age groups

		Physical Fitness						
Age		PACER Laps [n]	Curl Up [n]	Trunk Lift [cm]	Push Up [n]	Sit-and-reach R[cm]	Sit-and-reach L [cm]	VO2max
14 years **(N=17)**	*M*	68.5	57.7	23.6	20.9	24.2	23.8	54.7
	sd	17.6	21.7	6.9	7.6	4.3	3.9	4.7
	V	25.6	37.5	29.3	36.1	17.6	16.4	8.7
15 years **(N=15)**	*M*	84.7	70.5	22.1	22.6	29.8	28.6	59.1
	sd	19.8	9.6	3.7	6.5	6.4	7.6	5.4
	V	23.4	13.6	16.9	29	21.6	26.4	9.1
16 years **(N=22)**	*M*	92.5	66.2	22.9	26	28.7	28	61.3
	sd	17.9	18.1	8	13.4	7.7	7.6	4.8
	V	19.4	27.4	34.9	51.4	26.9	27	7.9
17 years **(N=28)**	*M*	89.4	66.4	24.1	26.4	30.6	30.9	60.4
	sd	20.6	16	6.7	7.7	4.9	5.1	5.6
	V	23	24.1	27.8	29	16	16.5	9.1
18 years **(N=23)**	*M*	93	71.2	27	27.7	31.7	31.3	61.5
	sd	20.9	10.3	5.1	8.9	5	5.4	5.7
	V	22.5	14.5	18.8	32.3	15.8	17.4	9.2
Test K-W	p	**0.0013**	0.2416	**0.0107**	0.0605	**0.0018**	**0.0015**	**0.001**

M – arithmetic average, *sd* – standard deviation, v – coefficient of variation [%]

Table 3. The differences of individual components of physical fitness in terms of quartile BMI groups

		Physical Fitness						
Age		PACER LAPS [n]	Curl Up [n]	Trunk Lift [cm]	Push Up [n]	Sit-and-reach R [cm]	Sit-and-reach L [cm]	VO2max
Q1 (N=28)	*M*	80.8	64.8	21.8	21.9	25.6	25.2	58.1
	sd	22.4	17.3	6.6	6.7	6.2	6	6.1
	V	27.8	26.8	30.1	30.6	24.3	24	10.5
Q2 (N=26)	*M*	95.8	66.7	23.9	25.6	30.4	30.4	62.2
	sd	18	18	5.3	9.4	5.7	6.1	4.9
	V	18.8	27	22.2	36.7	18.8	20.1	7.8
Q3 (N=25)	*M*	88.9	73.4	23.9	28	30.6	30.1	60.3
	sd	18.6	6.9	6.5	10.7	4.7	5.8	5.1
	V	21	9.4	27.1	38.2	15.3	19.3	8.4
Q4 (N=26)	*M*	82.1	61.9	26.9	25.5	31	30.3	58.5
	sd	21.9	17.7	6.8	10.1	6.4	6.4	5.9
	V	26.7	28.6	25.1	39.4	20.7	21.3	10.1
Test K-W	p	**0.027**	**0.025**	**0.0619**	0.134	**0.0063**	**0.007**	**0.027**

dominal muscles have men at the age of 14 years (M = 57.7). In this case, the greatest stability in terms of this parameter was noted among 15 year-olds (V = 13.6%). In the next stage of the research, results of the Trunk Lift test were analyzed as an assessment of back muscle strength. As is apparent from the figures contained in Table 2, the highest values of the test parameter again were presented by men at the age of 18 years (27.0 cm).

The results recorded in the other age groups are on similar levels with a small margin 17- year olds (24.1 cm). As in the case of distance, there was reported statistical significance between the strength of the spine muscles and the age of study group. Analyzing the value of the coefficient variation of the attempts results in the Push Up test, it is noted that the largest variety is characterized by a group of 16-year-olds (V = 51.4%). While the greatest stability in terms of the test parameter has been among 15 to 17-year olds. At the same time, it is noted that the youngest group recorded the weakest results of Push Up, and a group of 18-year-olds had marked the highest level of the test parameter (M = 27.7). In addition, a correlation between age of study group members and number of Push Up attempts (Tab. 2) has not been demonstrated. Research shows that the best test result of Sit-and-reach for the right and left leg were reported among 18-year-olds (right leg 31.7 cm; left leg 31.3 cm). Similar results, although slightly lower, were found in men about a year younger (30.6 cm; left 30.9 cm). The results of a similar level during the Sit-and-reach test for both legs were also noted in a group of 15-and 16-year-olds with a slight predominance of group at the age of 15 years. The average value of the Sit-and-reach test for the right and left legs was 29.8 cm and 28.6 cm among 15- olds and 28.7 cm and 28.0 cm in the test group at the age of 16 years. As is apparent from the figures included in Table 2, there is statistical significance between the age of the study group and the value of the Sit-and-reach test for both the right and the left leg (Tab. 2). The last analyzed parameter was VO_2max. As is apparent from the data contained in Table 2, average result of VO_2max in a group of 15-, 16-, 17-and 18 year olds are similar in value. The highest level of endurance was noted among the oldest test group (VO_2max = 61.5 ml/kg/min) and the lowest VO_2max was noted among a group of 14-year-olds (VO_2max = 54.7 ml/kg/min). In addition, it is noted that the biggest intra-group variations in terms of the test parameter represent the men at the age of 17 and 18 years of age. The greatest stability of VO_2max is observed among 16-year-olds. In addition, statistical significance was found between age of subjects and the value of the VO_2max (Tab. 2).

For further analysis, the study group was divided into four equal quartile parts. For the criterion of allocation, body mass index was taken into account, marking them appropriately Q1, Q2, Q3 and Q4. The Q1 group included persons with the lowest, while Q4 with the highest value index BMI. Analyzing the physical fitness of tested men, taking into consideration quartile BMI groups, it is concluded that the greatest amount of 20 meters distance recorded the study group belonging to the Q2 (M = 95.8). The second in order of the best result reached the men of the group Q3 (M = 88.9). The worst result recorded Q1 group at the level of 80.8. In this group was observed the largest diversity of intra-group in terms of the test parameter. In addition, the study showed that there is a statistically significant difference between groups of Q1 and Q2 (Tab. 3).

From the data contained in Table 3, it is also clear that the group belonging to the Q3 have the greatest strength of the abdominal muscles (M=73.4). Results in the remaining quatrain BMI group are at similar levels with a clear predominance of group from Q2, who recorded during the test result on the level of 66.7. During this test, the worst result reached group Q4 (M=61.9). At the same time the greatest stability in terms of the test parameter was noted in group Q3. Another analyzed sample was the Trunk Lift. As it is clear from the research results that Q2 and Q3 groups during the trunk raise attempts have adopted the same values (23.9 cm). The members of group Q1 reached a similar result - 21.8 cm, however, the best one was Q4, whose members reached the result at 26.9 cm. For each group of quartile BMI, there has been high coefficients of variation, but the biggest variation in intra-group is evident among Group Q1. In addition, between Q1 and Q4 there was statistical significance. Analyzing the test of Push Up, it is concluded that the biggest amount of repetitions were done by quatrain Group Q3 (M = 28.0). Results of Q4 groups (M = 25.0) and Q2 (M = 25.6) are on the same level with a slight predominance of study group Q2. The worst result during this attempt belonged to the men of the Group Q1 (M = 21.9). The biggest intra-group variations in terms of test parameter is observed in the Group Q4 (V= 39.4%). Further analysis showed that there is a statistically significant variation between Q1 and Q3 and Q4 during the Sit-and-reach test for the right and left legs. The results recorded in this test in group Q2 (M=30.4 cm), Q3 (M=30.6 cm) and Q4 (M=31.0 cm) adopt similar values, with a slight predominance of test group belonging to the Q4 Group. In this case, the largest intra-group variety is characterized by group Q1. A similar phenomenon has been observed during the Sit-and-reach test for the left leg. Also group Q1 was characterized with a great high coefficient of variation (V=24.0%). As with previous attempts, the results in groups Q2, Q3 and Q4 are on similar levels with a small margin of the group Q2 (M=30.4 cm). In addition, as is apparent from the figures contained in Table 3, recorded results showed statistical significance between Q1 and Q2, Q3 and Q4. The last parameter to

be analyzed in terms of quartile groups BMI is VO_2max. The analysis show that the greatest stability in terms of the test parameter is characterized by group Q2, which recorded the lowest coefficient of variation (V=7.8%). The best VO_2max have the study group Q2 (VO_2max=62,2 ml/kg/min). The second best result was VO_2max at level of 60.3 ml/kg/min, which obtained the men from Group Q3. Statistical significance was noted only between groups of Q1 and Q2 (Table 3).

Discussion

Analysis of the research carried out indicates a systematic increase with age of somatic characteristics such as weight, body height, waist and hip circumference, which can be justified in the natural physical development of children in this age range. The average body weight and height and calculated on this basis BMI among tested boys in all age groups was between 50 and 75 centile according to growth chart, based on data representative for the population of children and youth in Poland, prepared within the framework of the OLAF project and centile charts developed by Dobosz.[20–23] The value of BMI among tested boys also point to the standard according to International Obesity Task Force.[24] Average waist and hip circumference among tested boys similar to BMI, was between 50 and 75 centile according to OLAF growth chart.[22] In the same centile range there were counted values calculated on the basis of the above measurements indicators WHR and WHTR both for Polish and Greek standards.[25,26] Analysis of Rohrer's indicator allows to pass the test group to those with slim body structure (classification according to Wanke). The percentage of body fat in all study groups of boys significantly deviates from the above classification. Referring to international standards (FAT% value in the study group) there are some with less than the 2 centile and this indicates at the same time less than the correct fat percentage in study group.[27,28] So low fat content in the body may be associated with high physical activity of group, who attended the school with sport profiles.

Taking into account the results obtained with the PACER test indicates a very good aerobic capacity of tested boys. According to the international standards for this test, average results obtained for 14 year olds are between 70 and 80 centile. The best results were reported in a group of 16 year olds and place them between 90 and 95 centile, other age groups ranged between 80 and 90 centile of the above standards.[29] Due to the lack of international or Polish standards relating to other physical fitness tests, the obtained results can only refer to the standards for the American population elaborated by Cooper Institute. The creators of the test to assess the physical fitness of study group refer the individual test results to the so called Healthy Fitness Zone (HFZ). According to this classification, the strength and endurance of the abdominal, back and shoulder griddle muscles and the flexibility of the surveyed boys are located in standard known as the HFZ.[30] Statistically significant differences in fitness between age groups, such as in somatic characteristics, one can explain by the process of physical development that can directly influence the results in fitness assays. Similar results were presented by Migasiewicz and Milanese who carried out an assessment of selected anthropometric characteristics and physical fitness in 152 children aged 6-12 years.[31,32] Also in the work of Mota et al., there was demonstrated a correlation between the status of sexual maturity and fitness on the basis of test covering 494 children aged 8-16 years.[33]

A linear correlation between body construction and fitness among the boys who regularly train team sports has not been demonstrated. In study group with the lowest value of BMI, there was found the weakest results from almost all fitness tests; the exception was Curl Up test, in which the weakest results were recorded in Q4 group and thus people with the highest BMI. The best results of the aerobic capacity reported in Q2 group, while the greatest strength of the abdominal muscles and the shoulder girdle in the Q3 group. These results seem to confirm the idea that not only the potential obesity, but also underweight can adversely affect physical performance. The lack of a linear relationship between BMI and cardiorespiratory fitness was also recorded in work by De Araujo et al, who tested 288 students aged 10 to 14 years old using the fitnessgram® test battery.[34] The lack of reliance between BMI and fitness was also noted in the work of Milanese et al. Authors of that work suggest that subcutaneous fat is a better predictor of physical fitness than BMI or waist circumference.[31] Also in work by Ortega et al., a correlation between waist circumference and the level of cardio respiratory fitness was shown.[35] Similar results were obtained Tomaszewski et al., who carried out an assessment of selected anthropometric characteristics and International Fitness Test among 308 boys at the age of 9 years. The results of their work have shown significant difference in body weight, waist circumference and the size of the body in groups of different physical fitness.[36]

Conclusions

Motor fitness of physically active adolescents tends to improve with the subjects age. There is no linear relationship between BMI and fitness among the boys who regularly train team sports, but both lower and higher BMI values seem to be connected with lower physical fitness.

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REVIEW PAPER

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Effects of obesity on health condition with an emphasis on bone tissues disorders

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ABSTRACT

Introduction. Obesity is today one of the most dangerous and the fastest growing civilization diseases in the world. The number of overweight or obese people is continually increasing. Obesity is defined as abnormal fat accumulation in an organism that may cause health impairment. Obesity may be conducive to an increased risk increase for occurrence of cardiovascular diseases as well as stroke, some types of cancer, endocrinal disorders, osteoarthritis and other bone disorders. Some studies have demonstrated that high body mass index (BMI) is protective against the development of osteoporosis and osteoporotic fractures in men and women. In slim people with a lower BMI than normal, weight loss is associated with low bone mineral density (BMD). On the other hand, obesity in childhood may lead to fragility fractures and may lead to early development of osteoporosis in adulthood. Currently, we have numerous methods for measurement of obesity such as dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), total body electrical conductivity (TOBEC) as well as magnetic resonance imaging (MRI) and computed tomography (CT). These methods are useful for diagnosing obesity and bone tissue disorders such as osteopenia with sarcopenia or osteoporosis, in particular in perimenopausal women and men after andropause.
Aim of the study. The purpose of the study was review the literature on obesity and bone tissue disorders and their interrelations.
Material and method. Analysis of literature.
Keywords. fat tissue, bone, obesity, osteosarcopenic obesity, osteoporosis

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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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Introduction

The World Health Organization (WHO) published alarming data showing a continual increase in the number of overweight or obese people. In recent years, the prevalence and corresponding ratio of obese people increased significantly. In 2008, about 35% of adults were overweight and 10% of males and 14% of females of the world population were obese.[1] The percentages increased after eight years and in 2016, 39% of people suffered from overweight, 11% of males and 15% of females suffered from obesity worldwide.[2]

Obesity is a state of excess storage of body fat resulting from a chronic imbalance between energy intake and energy consumption as well as a lack of physical activity. This is also a complex condition causing more serious health problems affecting virtually any age and all socioeconomic groups. In the pathogenesis of obesity, main roles are played by genetic and environmental factors, as well as social and cultural factors and the hormonal state of the organism. The results of epidemiologic studies are very upsetting. Over the last few years, a progressive decline of the age threshold of subjects with these disorders has been observed; the problem of excessive body mass increasingly embodies the adolescent population. The results of relevant published studies are inconsistent. Some of them showed a protective role of body fat for skeletal health, whereas others point out a negative effect of adiposity on bone mineral density (BMD) and bone turnover. Obesity can also increase the risk for occurrence of type 2 diabetes, cardiovascular diseases as well as stroke, some types of cancer, endocrinal disorders, osteoarthritis, and premature death worldwide.[2,3,4,5]

Obesity in childhood can lead to fragility fractures and result in the early development of osteoporosis in adulthood. On the other hand, in postmenopausal obese women, the increase of BMD compared to slim women has been reported. Fat tissue, as the largest endocrine organ, secretes numerous adipokines such as the hormones leptin, adipokine visfatin, and cytokines (e. g. IL-1, IL-6, TNF-α) and their direct osteotropic effect is still under discussion. Moreover, adipokines affect bone tissue indirectly, by the controlling the action of other hormones, playing a key role in bone physiology (GH-IGF-I and 1.25 (OH)2D3). Additionally, an obese person has higher levels of serum estrogen and parathyroid hormone (PTH) and lower 25-hydroxyvitamin D (25OHD), sex hormone - binding globulin and lower 1.25 dihydroxyvitamin D_3; all of them have an important osteotropic effect. Obesity is also associated with higher levels of pancreatic hormones such as amylin and insulin which have anabolic properties in relation to bone.[6,7]

Diagnostics of obesity

Body mass index (BMI) is a simple index of weight for a given height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). The WHO definition is: a BMI greater than or equal to 25 is overweight and a BMI greater than or equal to 30 is obesity.[3]

According to the proposed classification, overweight occurs when the value of BMI is in the range 25.0 – 29.9 compared with the norm 18.5 – 24.9. When its value increases over 30.0, it indicates obesity which can be classified and attributed in corresponding degrees. First degree obesity (I degree) is characterized by a BMI value in the range 30.0 - 34.9, II degree in the range 35.0 - 39.9 and the highest III degree obesity is indicated by a body mass value equal or larger than 40.0.[1,2] Due to a large risk of error, other methods of obesity qualification have been proposed. At present, the most popular is the method based on the location of the largest amount of fat tissue in the organism. Using this method, central obesity also called ventral there is distinguished where the waist measurement is taken into account. However, the waist measurement values are differentiated according to race. Therefore, three subpopulations (American, European and Asian) are distinguished for comparison. The second type is hip and thigh obesity where the measure of obesity degree is hip and thigh circumference. It is assumed that the ratio obtained from the measurement of waist, hips, and thigh circumference after some calculations should not exceed 0.8 in woman and 0.9 in men. Special attention should be paid to the fact that the above methods can be applied for adults but not to children and youth during growth and development and differences in the metabolism of young and mature individuals. The composition of the body in children differs significantly from that in adults when the growth is ceases. An error is often made in diagnostics of obesity when children and youth are examined using the methods attributed to adults. Such a type of classification is allowed for youth in some cases when growth can be assumed to be nearly complete. Published centile charts can be used for diagnosis of obesity in children and youth.[1,3,8]

Currently, numerous methods of measurement of obesity such as dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), total body electrical conductivity (TOBEC) as well as magnetic resonance imaging (MRI) and computed tomography (CT) are applied. [9] Dual-energy X-ray absorptiometry (DXA), the measurement of the transmission of X-rays through the body at high and low energies, is a means of measuring bone mineral density (BMD). The additional capability of DXA to differentiate between bone mineral, fat tissue, and lean tissue has contributed to its

emergence as a popular tool to assess body composition. The DXA method allows determining such parameters as percentage of total fat content (Total Fat %), percentage ratio of fat tissue content in relation to soft tissue (Soft Tissue Fat %) and percentage ratio between the total skeleton BMC and the fat-free mass of the body (%TBMC/FFM).[10,11] The bioelectrical impedance analysis (BIA) is a method of assessing body composition, the measurement of body fat in relation to lean body mass. This non-invasive test simply involves the placement of two electrodes on a person's right hand and right foot. The flow of the current is affected by the amount of water in the body. The device measures how this signal is impeded through different types of tissue. Tissues that contain large amounts of fluid and electrolytes, such as blood, have high conductivity, but fat and bone slow the signal down. As BIA determines the resistance to the flow of the current as it passes through the body, it provides estimates of body water from which body fat is calculated using selected equations.[12,13] The total body electrical conductivity (TOBEC) was introduced as a rapid, safe, and noninvasive method suitable for the estimation of fat- free mass. The instrument (TOBEC) operates on the principle that organisms placed in an electromagnetic field perturb the field to a degree that depends on the amount and volume of distribution of electrolytes electrolytes.[14] Magnetic-resonance imaging (MRI) is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines physical and chemical properties of atoms or the molecules in which they are contained. This phenomenon of nuclear magnetic resonance can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. Magnetic-resonance imaging is used in human adults and children to obtain measures of total body fat mass (FM) with high precision. Magnetic- resonance imaging and computed tomography scanning provide accurate data on adipose tissue distribution, but remain limited to the specialist use and are unsuitable for the routine clinical application. Additionally, CT involves exposure to high levels of radiation.[15,16,17]

Obesity development and bone tissue

Accessible methods for measuring obesity and control of its treatment allow determining contents of an individual fraction of fat tissue in the organism. Due to close relations between the fat tissue and the bone tissue as well as metabolic interactions between them, location and content of fat in the organism should be taken into account. The largest reservoir is the subcutaneous fat tissue whose contribution can be up to 70%, constituting the largest reserve of energy in the organism due to the ready acquisition of fatty acids which can be used in metabolic transformations.[4,6,8] The second fraction with respect to the amount is the visceral fat tissue and its content does not change greatly during the individual life as in the case of subcutaneous fat tissue but its contribution is sexually differentiated. This constitutes about 20% of the total fat content in men's body and 8% in women. Thus the visceral fat tissue is a source of triglyceride as well as LDL cholesterol contributing largely to the development of atherosclerosis and adipocytokines taking a direct part in bone tissue metabolism. The remaining contribution comes from the fat tissue dispersed throughout the organism as intermuscular fat surrounding the nervous tissue or articulations.[18,19]

As follows from published studies, an increase in the fatty tissue content and growing obesity result in hormonal economy disorders with respect to estrogen and androgen sex hormones. Their role in bone metabolism is enormous as they stimulate osteoblasts to synthesize many factors responsible for intensification of bone formation and inhibit synthesis as well as release proresorption factors. The function of estrogens consists in inhibition of bone tissue resorption. In turn, androgens have the function of stimulating bone formation.[20,21] Central obesity in men causes loss of endocrine equilibrium reducing the level of testosterone and proteins binding it. The favorable effect of testosterone results in stronger lipolysis in the visceral fat tissue and reduces capture of lipids in the blood. A different character of obesity effect is observed in women where obesity is accompanied by a decrease in sex hormone binding proteins which leads to the increase of estradiol in blood.[21] Therefore, in menopausal women that are overweight or have first-degree obesity, the fat tissue possessing protective properties against osteoporosis or related fractures seems to be advantageous. Moreover, it was shown that the loss of body mass during the menopausal period and a BMI below the norm leads to a decrease of BMC and BMD of the whole bone system and also a decrease the mechanical properties of bone.[22,23]

Fat tissue and osteopenia with sarcopenia

Besides the positive effects of the increased content of the fat tissue and muscle mass, obesity can affect the bone tissue negatively. Osteosarcopenic obesity (OSO) is a description with discrimination of the bone tissue quality which was defined recently. This condition is characteristic of postmenopausal women where, with reduction of bone mineral density (BMD), there is an observed decrease in the muscle mass and strength with an increased content of body fat typical of obesity.[24] Unfortunately, when it is necessary to bear a larger body mass, muscle strength can be reduced. In this disease, there is observed a larger content of fat tissue which results in movement restrictions and difficulties with locomotive faculty thus enhancing the risk of falling down and fractures.[25] As follows from the investigations of

BMD of thigh and neck bone diminishes with fat tissue contribution exceeding 33% but in the case of BMD of lumbar vertebra it exceeds 38%.[26] Also, proinflammatory cytokines produced by fat tissue (above-mentioned IL-1, IL-6, TNF-α) affect OSO development as they intensify the bone loss process changing the architecture of the bone tissue and increasing risk of fracture.

Defined OSO allows to estimate fracture risk in people which so far has not taken into account overweight patients. It was believed that one reason that obesity could protect against fractures is due to the larger loading of the skeleton which could stimulate bone formation.[27] Despite this, Zhao et al. showed that increased body mass cooperates negatively with the quality of bone tissue after determination of bone stimulation effect through mechanical loading by means of dynamic tests.[28] In turn, another factor which suggests a positive effect of obesity is the fact that the fatty lining can act as a buffer against the force acting on the bone tissue during the fall thus protecting against fracture. Moreover, it was demonstrated in many papers that increased body mass and BMI correlate with increased BMD and BMC and that a drop in body mass results in deterioration of bone tissue structure contributing to a larger number of fractures in people with a very small body mass.[29,30]

Fat tissue and osteoporosis

Numerous literaturę reports are contradictory with regards to the effect of fat tissue and/or muscle tissue content on the development of osteoporosis. Lau et al. showed that intensification of bone tissue resorption through an increase of bone turnover in postmenopausal women leads to BMD reduction and lower body fat.[31] In turn, Christensen et al. showed that with the increase of fat tissue content in an organism, some fluctuation in the BMD and BMC is observed compared to control group patients.[32] The changes in the bone tissue with age depends on several factors connected with the fact that when the peak bone mass (PBM) resorption processes is reached and prevails over bone formation in postmenopausal women, they are predisposed to the occurrence of osteoporosis.[33] An increased body mass at BMI above 25kg/m^2 in elderly people exerts osteoprotective activity persons of lower BMD value which was demontrated by the studies of Zhao et al.[28] A different character of changes was presented by Greco et al., who found lower BMD values of thigh bones in overweight people compared to those with proper BMI values[21] which indicates that increased body mass is not indifferent as regards bone tissue metabolism. Moreover, in women who are postmenopausal overweight, studies with pQCT showed higher values of volumetric BMD of the tibia and brachial bones compared to the women of the same age with the regular body mass. [34]

Besides the percentage content of fat tissue, that of muscle tissue is more and more often taken into account in diagnostics of obesity and bone disorders. Low muscle mass near to or during the menopausal period is correlated with lower values of BMD and the positive effects of increased body mass in counteracting osteoporosis depends mainly on increased muscle mass but not the percentage content of the fat tissue.[35,36] Larger body mass in the elderly contributes to a smaller risk of osteoporotic growth by the increase in the mechanical loading of bones. The gain in long bone resistance results from intensification of osteosynthesis processes and inhibition of osteoblast apoptosis which contributes directly to BMD value growth [37] which is contrary to the studies by Zhao et al. who showed an opposite dependence.[28]

The positive influence of adiposity on bone tissue can also be a consequence of increased mechanical loading of the skeleton. Body mass is directly associated with bone mineral density and a low body mass index (BMI) is an important risk factor for lower BMD in aged patients. Lean mass and fat mass are both independent determinants of bone mass. In postmenopausal women, low muscle mass is associated with low BMD, and positive effects of a higher body mass on bone occurs only when it is primarily composed of lean mass. In conclusions, the quality of bone tissue depends on numerous factors. The level of growth hormones, cytokines, vitamins as well as calcium intake and calcium absorption play the main role in maintaining of bone mineral density (BMD) and content (BMC). However, the role of fat tissue in the physiology of bone is still under consideration and further studies are necessary. [28,34-36]

Conclusions

Given the divergence in the literature with regards to the effect of fat tissue, it is difficult to state its explicit influence on the organism including the bone system. Both obesity and undernutrition, quality and quantity of consumed nutritional components as well as lipid economy can have a modulating effect on the bone parameters. During the diagnostic of bone distemper, individual fractions of fat tissue and their percentage contribution in the organism should be taken into account. Moreover, the proportions between the fatty and muscle tissues should be considered in diagnostic of OSO or osteoporosis as both fat mass and lean mass play a key role in the development of these osteoporotic diseases.

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REVIEW PAPER

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Singlet oxygen lifetime and diffusion measurements

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ABSTRACT

Introduction. Photodynamic therapy (PDT) is considered to be a promising antitumor methodology due the cytotoxicity of singlet oxygen ($^1O_2$).

Aim. To present singlet oxygen which is highly reactive and decomposes to the ground state rapidly.

Material and methods. Analysis of literature.

Results. This review presents techniques to measure lifetime and diffusion of $^1O_2$.

Keywords. photodynamic therapy, singlet oxygen, diffusion

Introduction

One cancer treatment method that has developed significantly in recent years is photodynamic therapy (PDT). In this method, a patient is given a photosensitizing dye that is intended to accumulate in the target tissue. In the next step, the tumor site is irradiated with light in the visible or near-infrared range. When the photosensitizer (PS) is exposed to light, its molecules are excited and transfer excitation energy to ground state oxygen and singlet oxygen ($^1O_2$) is generated. The resulting singlet oxygen leads to necrosis of the tumor tissue.[1]

PDT is characterized by high efficacy and relatively minor side effects compared to such therapies as radiation therapy or chemotherapy.[2] In the case of PDT therapy, its region of application is very important. In this technique, it is important to provide the sensitizer as close to the cancer cells as possible, which are then selectively destroyed. High reactivity of $^1O_2$ leads to the destruction of healthy cells when it is generated directly in them or at a sufficiently close distance.[3] Due to the short lifetime of $^1O_2$, long-distance travel is impossible.[4] An important role in the diffusion of $^1O_2$ is played by the environment in which it is generated.[5] The most preferred solution would be the selective generation of $^1O_2$ by delivery of light to a specific cellular domain. Currently, in PDT, in addition to diseased cells, their close surroundings may also be illuminated (healthy cells). Therefore, information on the degree of diffusion of $^1O_2$ in cells during its lifetime is very important. That is why it is so important to develop a method that allows measuring and monitoring $^1O_2$. Experiments to determine the value of the intracellular

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lifetime of $^1O_2$ have significant value for the PDT method. Previous studies have established that the intracellular lifetime of $^1O_2$ and the distance traveled by $^1O_2$ during this period is severely limited due to intracellular viscosity that hinders translational movement.[4] In this review, examples of lifetime and singlet oxygen diffusion in different media are presented.

Measurements of lifetime and diffusion of singlet oxygen

A $^1O_2$ measurement lifetime measurement is difficult due to its very short lifetime and requires the use of advanced equipment. By measuring the lifetime of singlet oxygen we are able to determine its diffusion distance.[6] The range d, in which $^1O_2$ can exist, is limited by its lifetime τ, which correlates with its diffusion coefficient D (Equation 1).[7]

$$d = \sqrt{2\tau D}$$

Equation 1.

Table 1. Lifetimes of $^1O_2$ in various solvents

References	Solvent	$^1O_2$ lifetime (μs)
Rodgers M. A. J.[9]	H_2O	4 ± 2
Adams D. R. & Wilkinson F.[12]	D_2O	30 ± 10
Merkel P. B. & Kearns D. R.[10] and Long C. A. & Kearns D. R.[11]	H_2O:CH_3OH, 1:1	3.5
	D_2O:CH_3OH, 1:1	11
	$CHCl_3$	60 ± 15
	CS_2	200 ± 60
	$CDCl_3$	300 ± 100
	C_6F_6	600 ± 200
Adams D. R. & Wilkinson F.[12]	$(CD_3)_2CO$	640
Merkel P. B. & Kearns D. R.[10] and Long C. A. & Kearns D. R.[11]	CCl_4	700 ± 200
	CCl_3F / (Freon 11)	1000 ± 200

Data available in the literature determine the range of travel of $^1O_2$ in water at room temperature at d = 125 nm.[8] The lifetime of $^1O_2$ in cells is shortened by reaction with cellular molecules. Redmond and Kochevar have shown in their research that chemical reactions of singlet oxygen with protein amino acids, nucleic acids or with unsaturated lipids reduce the lifetime of singlet oxygen.[8] The generation of singlet oxygen can take place in various types of solvents. The solubility of the substrate and sensitizer as well as the properties of the solvent used has a significant effect on the life expectancy of $^1O_2$. Table 1 shows lifetimes of $^1O_2$ for various solvents.

In PDT, it is the lifetime of $^1O_2$ that determines how excited the oxygen molecule can diffuse from the place where it was created. For live cells, the *D* value for molecular oxygen is around $1.4 \times 10^{-5}\,cm^2s^{-1}$, hence the diffusion length under these conditions is in the order of nm.[13] Currently available data on the oxygen diffusion coefficient indicate that subcellular domains that tend to be quite viscous have a significant impact on its average/apparent value.[14] Table 2 presents examples of studies on the diffusion of singlet oxygen in cells.

Induction of minimal damage that arises during PDT is often tolerated by healthy cells located in close proximity to the site of $^1O_2$ formation; it may even lead to stimulation of their growth. The photosensitizers used play an important role in this case.[13] This shows that the diffusion distance of $^1O_2$ is very important in the case of PDT. A study conducted by Ogilby et al. in 2006 involving the detection of singlet oxygen from single cells showed that the lifetime of $^1O_2$ may be longer than commonly thought. It also means that singlet oxygen can diffuse over long distances, including through the cell membrane to the extracellular environment.[21] Similar results were obtained by Hatz et al. in studies measuring the lifetime of singlet oxygen in a single cell.[22] These studies show that in a living, functioning cell containing water, the lifetime of singlet oxygen is about 3μs.[22] In turn, in a study conducted by Kuimova et al. monitored the time-resolved luminescence decay of $^1O_2$ after production by the sensitizers chlorin (Chl) and 5,10,15,20-Tetrakis(N-methyl-4-pyridinio)-21H,23H-porphine (TMPyP) that were localized in different domains of the living cells.[6] The obtained data indicated that both the lifetime and rate constant for $^1O_2$ quenching depends on the photosensitizer. In addition, these studies have shown that despite the relatively long intracellular lifetime, due to the heterogeneity of the cell, $^1O_2$ does not diffuse at long distances from the place of its production. The authors of this study also pointed out that high intracellular viscosity has a significant impact on this.

A diagram showing the operation of PDT in clinical use is shown in Figure 1. Under the influence of laser light, we observe the production of $^1O_2$, which in addition to destroying cancer cells, may diffuse into neighboring cells causing healthy tissue damage.

Studies related to the effects of singlet oxygen on lipid membranes are often reported.[23,24] In many studies, diffusion distances of $^1O_2$ are calculated based on studies carried out on model lipid membranes that are protein-free. The studies conducted by Pooler aimed to check whether there are differences in $^1O_2$ diffusion in the case of lipid domains and band protein 3.[25] The results obtained showed that the diffusion of singlet oxygen from both locations is at the same level and no significant differences in the results were ob-

Table 2. Measurements of diffusion distance of singlet oxygen in different media

References	Materials and Methods	Media	Distance
Hatz S. *et al.*[4]	the single cell experiments were performed using a microscope with the focused output of a pulsed fs laser as the excitation source; O_2 (a^1 Δg) was monitored using a cooled photomultiplier tube operated in a photon counting mode; the sensitizer used in these experiments was 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP)	cells	60–159 nm
Moan J. & Berg K.[15]	NHIK 3025 cells were incubated with Photofrin II (PII) and/or tetra (3-hydroxyphenyl)porphyrin (3THPP) and exposed to light at either 400 or 420 nm	cells	10 - 20 nm
Redmond R.W. & Kochevar I.E.[8]	decay kinetics and diffusion distance of singlet oxygen in aqueous solution	water	~ 125 nm
Egorov S.Y. *et al.*[16]	luminescence component with a lifetime of about 1μs in yeast cells,	yeast cells	≤ 0.07 μm
Sokolov V. *et al.*[17]	aluminum phthalocyanines were adsorbed to only one interface of planar lipid bilayers	lipid membrane	~ 100 nm
Krasnovsky A.A.[18]	diffusion distance from the generation site in chloroplas thylakoids	chloroplas thylakoids	~ 5.5 nm
Skovsen E. *et al.*[5]	in the nucleus of the cell, experiments were performed in which the cell was exposed to bovine serum albumin (BSA); in D_2O-based experiments, the medium surrounding the cell contained 0.77 mM BSA; the distance traveled by singlet oxygen in 6 μs was measured	cells	~ 268 nm
Baier J. *et al.*[19]	incubated the HT29 cells with Photofrin or ATMPn, the photosensitizer molecules were located in the cellular membranes (fluid, heterogeneous mosaics of proteins and lipids)	cellular membranes	~ 3 μm
Dysart J.S. *et al.*[20]	in vitro experiments were performed in which MatLyLu (MLL) cells were incubated in Photofrin	cells	~ 0.05 μm

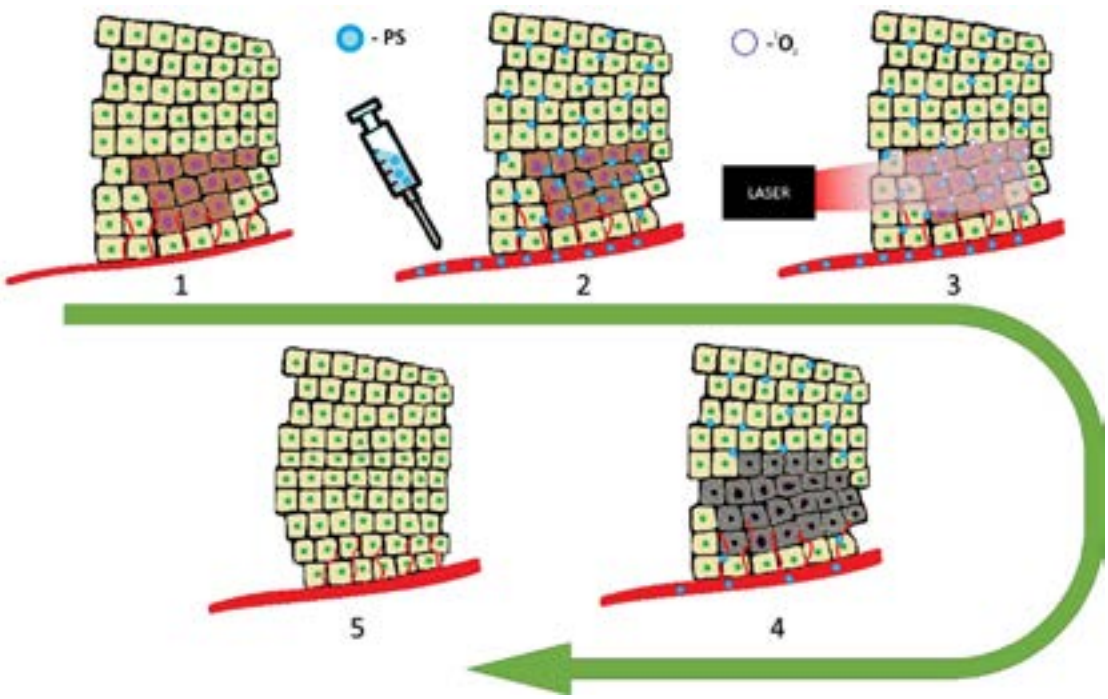

Fig. 1. A schematic illustration of photodynamic therapy. 1 – normal and cancer cells; 2 – PS administration and accumulation in cells; 3 – irradiation and $^1O_2$ production; 4 – tumor necrosis and damage to neighboring healthy cells; 5 – tissue regeneration

served. Moan et al. arrived at completely different conclusions and discovered that $^1O_2$ was unable to diffuse at sufficient distance to trigger the effect of Photofrin molecules in cell membranes, despite the fact that the calculations showed that the distance between adjacent molecules was small enough for this effect to occur.[26] These studies suggest that proteins shorten the lifetime of $^1O_2$, resulting in a reduction in diffusion distances inside cell membranes.

Conclusion

This review includes studies on the lifetime and the diffusion of singlet oxygen. The lifetime of singlet oxygen and its diffusion distance are interrelated. Diffusion $^1O_2$ depends not only on the photosensitizers used, but above all on the medium in which ROS production is to take place. All studies have one conclusion that is a critical aspect in photodynamic therapy which is that the photosensitizer should be as close as possible to the cancer cell.

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REVIEW PAPER

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Biological properties of *Cistus species*

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ABSTRACT

Aim. This paper presents a review of scientific studies analyzing the biological properties of different species of Cistus sp.

Materials and methods. Forty papers that discuss the current research of Cistus sp. as phytotherapeutic agent were used for this discussion.

Literature analysis. The results of scientific research indicate that extracts from various species of Cistus sp. exhibit antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, cytotoxic and anticancer properties. These properties give rise to the possibility of using Cistus sp. as a therapeutic agent supporting many therapies.

Keywords. biological properties, Cistus sp., medicinal plants

Introduction

Cistus species (family *Cistaceacea*) are perennial, dicotyledonous flowering shrubs in white or pink depending on the species. Naturally growing in Europe mainly in the Mediterranean region and in western Africa and Asia.[1-3] These plants are capable of growing in difficult climatic and soil conditions.[4,5] For many years, plants of the genus *Cistus sp*. were used in folk medicine mainly in Mediterranean regions as infusions, extracts and as a resin Ladano in the treatment of many diseases.

Modern scientific research has focused on the isolation and identification of compounds present in extracts, and resins from various species of *Cistus*. Studies have also analyzed their biological and pharmacological activity which elicit healing properties. Phytochemical studies using chromatographic and spectroscopic techniques have shown that *Cistus* is a source of active bioactive compounds, mainly phenylpropanoids (flavonoids, polyphenols) and terpenoids.

These compounds determine the medicinal properties of *Cistus* such as anti-inflammatory, antibacterial, antifungal, antiviral, anti-allergic and strengthening the body's resistance and an analgesic effect which allows their use as therapeutic agents in a wide range of diseases.[6-19]

This article presents the biological and pharmacological properties of various species of Cistus sp., with

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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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Fig. 1. Dried herb leaves *Cistus incanus* (photograph by Agnieszka Ewa Stępień)

particular emphasis on *Cistus creticus*, *Cistus creticus subspecies cretenicus L., Cistus creticus subspecies creticus, Cistus creticus subspecies eriocephalus, Cistus incanus L., Cistus inacus, Cistus inacus subspecies creticus, Cistus inacus subspecies tauricus, Cistus monspeliensis L., Cistus libanotis, Cistus villosus, Cistus villosus L., Cistus monspeliensis, Cistus ladanifer, Cistus populifolius, Cistus salviifolius, Cistus parviflorus, and Cistus laurifolius.* Their antioxidant, antibacterial, antifungal, antiviral, cytotoxic and anti-cancer properties have been particularly emphasized.

Cistus sp. species are a rich source of natural compounds with antioxidant properties, mainly flavonoids and polyphenols. The ability of antioxidants to capture toxic oxygen free radicals is very important. Oxygen reactive forms such as peroxides, superoxide, peroxy and hydroxy radicals play an important role in oxidative stress contributing to the development of many diseases including diabetes and Alzheimer's disease.

It was confirmed that the species *Cistus laurifolius* is characterized by antioxidant properties.[20] In the extract from leaves and small branches of *Cistus laurifolius* the presence of 16 bioactive compounds was determined by ^{1}H and ^{13}C NMR techniques and EI-MS mass spectrometry. The following compounds from *Cistus laurifolius* have shown the ability to capture free radicals: 3-*0*-methyl quercetin (**1**), 3,7-*0*-dimethyl quercetin (**2**), ellagic acid (**8**), quercetin 3-*0*-α-rhamnoside (**10**), 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-α-L-rhamnopyranoxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**12**), olivil 9-*O*-β-D-xyloside (**13**), berchemol 9-*O*-rhamnoside (**14**) and (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 9'-*O*-α-L-rhamnoside (major isomer) (**16**).[20] It was observed that *Cistus incanus* and *Cistus parviflorus* both show high activity of scavenging free toxic radicals.[21]

In subsequent studies, it was observed that the content of antioxidant phenolic compounds in extracts of *Cistus populifolius* is higher than for *Cistus ladanifer*. Also, analysis of the ability of extracts to inhibit the formation of lipid peroxyl radicals confirmed that *Cistus populifolius* has higher antioxidant activity than *Cistus ladanifer*.[22]

It was also confirmed that other *Cistus* species including *Cistus incanus* L., and *Cistus monspeliensis* L., contain numerous compounds with antioxidant potential, among others polyphenols and flavonoids.[23,24] Their antioxidative capacity was also examined, due to the presence of phenols, flavonoids and tannins of the obtained ethanol, hexane and water extracts from the leaves of *Cistus monspeliensis* and *Cistus salvifolius*. The highest antioxidant activity was demonstrated by ethanol extracts from both *Cistus* species. The research results indicate that the proper selection of the type of solvent affects obtaining of an extract with a high content of antioxidants.

Research by Loizzo et al. (2013) indicated that the essential oils of the species *Cistus creticus*, *Cistus salvifolius*, *Cistus libanotis*, *Cistus monspeliensis* and *Cistus villosus* exhibit antioxidant properties.[16] They indicated that the greatest antioxidant properties have essential oils derived from *Cistus monspeliensis* and *Cistus libanotis*. They also analyzed their acetylcholinesterase and butyrylcholinesterase (BChE) inhibitory activity. *Cistus salvifolius* species were characterized by the highest activity against AChE, while *Cistus libanotis*, *Cistus creticus*, *Cistus salvifolius* had good inhibitory activity against BChE.There were 26 types of antioxidant present in the leaves and flower buds of *Cistus salviifolius*. The highest concentration of phenols and flavonoids were recorded in extracts from flower buds, while tanning agents and anthocyanins were found in leaf extracts. The obtained results indicated that the leaf extract was characterized by a higher inhibitory activity against the AChE enzyme as compared to the extracts from flower buds. This activity is probably due to the higher content of anthocyanins in the *Cistus salviifolius* leaf extract that may have a significant effect on the inhibition of this enzyme. The results of the above research work emphasize that extracts from the above *Cistus* species are a source of compounds with high antioxidant potential and can be used in therapy for many diseases caused by oxidative stress and may be helpful in the prevention and treatment of Alzheimer's disease.

It has been determined that polyphenol compounds with antioxidant properties present in extracts also have antiviral properties. Activity against the influenza virus from extracts derived from *Cistus incanus spp. tauricus* was found without negative side effects.[13]

Researchers also point to the valuable antibacterial and antifungal properties of *Cistus* species that result from their antioxidant activity, i.e. phenolic compounds.[18] Antibacterial and antifungal properties of leaf extracts of *Cistus villosus L. (= incanus)* and *Cistus monspeliensis L.* were determined against Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and the fungi *Candida glabrata*, *Candida krusei* and *Aspergillus fumigatus*. Extracts from *Cistus villo-*

sus showed higher activity than those from *Cistus monspeliensis* against *Staphylococcus aureus* and *Candida glabrata*. However, *Candida krusei* and *Aspergillus fumigatus* were characterized as the most resistant among all tested microorganisms for both extracts. The tests were carried out using chloramphenicol, amoxicillin and amphotericin B as standard antibiotics for comparison.[27]

In the extracts from the species *Cistus ladanifer*, the presence of a large group of ellagic acid with phenolic compounds punicalagin and gallate were determined.[12] The compound elagitanine is attributed to the effect on the strong inhibition of the growth of *Candida albicans*, *C. glabrata* and *C.parapsilosis*-inducing fungi causing infection in immunocompromised individuals.

It has been shown that methanolic *Cistus monspeliensis* flower extract has a more inhibitory effect than the leaf extract on the growth of Staphylococcus epidermis Gram-positive *Staphylococcus bacteria.*[28] Further phytochemical analysis of the extract obtained from *Cistus monspeliensis L.* leaves determined the structures of biologically active compounds by ^{1}H and ^{13}C NMR spectroscopy. It was determined that this inhibitory activity resulted from the interaction of clerodane (+)-19-acetoxy-cis-clerodan-3-ene-15-oic acid an isolated diterpene.[29] Phytochemical analysis of *Cistus creticus* essential oil showed the presence of ten different volatile diterpenes of the labile type by GC-MS.[30] In vitro studies of the impact of these diterpenes on *Borrelia burgdorferi sensu stricto (Bbss*) bacteria that cause borreliosis were carried out. It was found that the diterpenes manoyl oxide, 13-epi-manoyl oxide, 3-acetoxy-manoyl oxide, and the monoterpene carvacrol with 3-hydroxy-manoyl oxide determined the antibacterial effect of this oil. The interaction of aqueous extracts, hexane and ethyl acetate extracts from *Cistus* leaves was also analyzed, but only the aqueous extract did not inhibit the growth of microorganisms.

Barrajón-Catalán et al. in their studies determined that the aerial extract of *Cistus ladanifer* inhibits the growth of Gram-positive bacteria *Staphylococcus aureus*. The extract obtained from *Cistus populifolius* reveals a high growth inhibitory activity against Gram-negative bacteria strain *Escherichia coli*.[22]

Mahmoudiet et al. studied the activity of aqueous extracts obtained from *Cistus monspeliensis* and *Cistus salvifolius* leaves against pathogenic Gram-negative bacteria (*Escherichia coli ATCC 8739, Salmonella typhimurium NCTC 6017,* and *Pseudomonas aeruginosa ATCC 27853*), two gram-positive bacteria (*Staphylococcus aureus ATCC 29213* and *Enterococcus faecalis*) and two fungal/yeast species (*Aspergillus niger* and *Candida albicans*). Both extracts showed cytotoxic activity against each pathogen. The highest activity was demonstrated by *Cistus salvifolius* extract against *P. aeruginosa* and *A. niger*. In contrast, the extract from *Cistus monspeliensis* has demonstrated a high inhibiting ability against *E. coli, P. aeruginosa* and *C. albicana*.[25] Scientific research indicates that the extracts of *Cistus sp*. can be potentially used as an adjuvant for the treatment of inflammation caused by the undesirable effects of microorganisms.

Scientific research indicates and confirms the presence in many plants, including *Cistus sp*, of compounds with anticancer properties, among others *Cistus sp*. The anti-cancer compounds cause changes in the cancer cell cycle invoking a mechanism in the cell whose main element is the apoptotic pathway. The pathway involved the apoptotic process, i.e. the programmed death of a cell that causes cell contraction, changes in the cell membrane, and in chromatin resulting in the death of the apoptotic cell.[31,32] The observed changes are a decrease in the proliferation index (cytostatic effect), and a decrease in cell survival resulting from the induction of apoptosis (cytotoxic effect). The Dimas research team identified nine labdan diterpenes in the *Ladano Cistus creticus subspecies cretenicus* (L.) resin.[33] Subsequently, studies were conducted to assess their impact on human leukemic cell lines: T-ALL / lymphoblasts (CCRF-CEM), acute T cell lymphoid (MOLT3), T cell lines (H33AJ-JA13), T-cell lymphoma / lymphoblast (HUT78), lymphoma (H9), childhood B acute lymphoblastic (KM3), Burkitt's lymphoma (NAMALWA), Burkitt's lymphoma (DAUDI SDK), Burkitt's lymphoma (JIYOYE), acute lymphoblastic (CCRF-SB), promyelocytic leukemia / lymphoblast (HL60), CML / lymophoblast (K562) and monocytes (U937) indicating their cytotoxic activity. The test results showed cytotoxic activity of the compound (13E) -labd-13-ene-8a15-diol against 13 tested cell lines, while (13E) -labd-7,13-dienol was only active in the HL60 line. In the course of further research, Dimas et al. in 2001 determined the presence of other labile diterpenes in leaf and fruit extracts of *Cistus creticus subsp. creticus*. Isolated sclareol (1) and ent-3α-hydroxy-13-epi-manoyl oxide (2) belong to the labdane type diterpenes and the derivative of compound 2 thiomidazolidine (3), and their activity against human leukemic cell lines: T cell lines (H33AJ-JA1) and lymphoid (MOLT-3). They indicated that compounds 1 and 3 induce the death of apoptotic cells in human leukemia lines, disrupting their cell cycle.[8]

Isolated from the leaf extract *Cistus creticus subsp. eriocephalus* diterpenes of the labile type were the compounds: labd-13 (E) -ene, -8α, 15-diol (1) and labd-13 (E) -ene, -8α, 15-yl acetate (2) and 19-acetoxy-cis- clerodane-3-ene-15-oic acid (3) from *Cistus monspeliensis L*. The structure of these compounds was confirmed by the technique of ^{1}H and ^{13}C NMR and GC/ MS. These compounds were tested for cytostatic and cytotoxic properties in human leukemic cell lines: T-ALL / lymphoblasts (CCRF-CEM), acute T cell lymphoma / suspension (MOLT4), T-cell lymphoma (HUT78), myeloma / suspension. (RPMI 8226), promyelocytic leukemia / lymphoblast

(HL60), CML / lymophoblast (K562), camptothecin resistant: cross resistant to etoposide daunorubicin, doxorubicin (CCRF-CEM / C2), mitoxantrone resistant: cross resistant to etoposide and doxorubicin (HL60 / MX1, HL60 / MX2) mitoxantrone resistant: cross resistant to etoposide, daunorubicin and doxorubicin). It was determined that the highest cytostatic and cytotoxic activity among these diterpenes against the tested leukemic human cell lines was compound 1, followed by compound 2. In contrast, compound 3 showed no activity of this type. The least susceptible to cytostatic and cytotoxic compounds 1 and 2 were the HUT78 and K562 cell lines. Studies indicate that these diterpenes induce apoptosis in these tumor cell lines via a mechanism that regulates the c-myc gene, without affecting the expression of the anti-apoptotic protein bcl-2.[34]

Also, the extract from *Cistus creticus ssp. creticus* is characterized by cytotoxic activity in relation to cancer cells. Ethanol extracts of *Cistus creticus ssp. creticus* showed inhibitory effects on the development of line cell human cancer cervix (HeLa), breast (MDA-MB-453) and melanoma (FemX). It was determined that the effect of the labdan type of diastpenes in the Cistus extract is responsible for this effect on these cell lines.[35] In the extract of *Cistus incanus subsp. creticus* labd-14-ene-8,13-diol (sclareol) was identified by 1H NMr and GC/MS. The effect of sclareol against the MN1 (p53-expressing) and MDD2 (p53-defective) derived from the parental cell line MCF7 was evaluated. It has been shown that this sclareol was able to inhibit DNA synthesis in the cell apoptosis cycle independently of p53, and thus induce cell cycle arrest. The course of breast cancer cell apoptosis induced was assessed by detecting DNA fragments. It was also indicated that sclareol strengthens the activity of the anticancer drugs doxorubicin, etoposide and cisplatinum against human breast cancer cell lines MDD2. Sclareol is also now a certified drug used in cancer therapy on breast cancer.[36]

In subsequent studies, Dimas et al. in 2000 analyzed the cytotoxic and cytostatic activity of antioxidant flavonoids: 3,7,4 ', 5'-tetramethyl ether of myricetin (1) isolated from the hexane extract of *Cistus monspeliensis L.* and its 3', 5- diacetyl derivative (2) synthesized from 1 and myricetin (3). Their proliferation index (cytostatic effect) and cell survival (cytotoxic effect) were assessed against the human leukemic cell lines lymphoblasts (CCRF-CEM), lymphoid (MOLT4), T-cell lymphoma (HUT78), B lymphocyte (RPMI 8226), promyelotic (HL60), proerythrocytes (K562), multidrug resistant (MDR-CCRF-CEM / C2), and in mitoxantrone-selected HL60 (HL60 / MX1, HL60 / MX2). Compound 2 showed a higher inhibitory effect on the growth of all tested cell lines than compound 1. On the other hand, compound 3 showed lack of cytostatic and cytotoxic activity against these cell lines. This indicates that the acetylation of myceritin increases the cytostatic and cytotoxic effect of flavonoids. However, the lowest cytotoxic and cytostatic activity of compounds 1 and 2 were found in the K562 cell line.[37]

In in vitro experiments, extracts from *C. ladanifer* and *C. populifolius* have been analyzed for their cytotoxicity to human tumor cells. Extracts from *C. populifolius* and *C. ladanifer* have demonstrated the ability to inhibit pancreatic cancer cell proliferation (M220) and in breast cancer cells (MCF7 / HER2 and JIMT-1). The leaves of these plants are the source of water-soluble polyphenol extracts enriched with ellagitannins with antioxidant effect, and their effects of cytotoxicity against cancer cells deserves attention.[22] In the studies of Vitali et al., the effect of extracts of *Cistus incanus L.* and *Cistus monspeliensis* L on human tumor cell lines was determined. They showed activity against human prostate cells (PZ-HPV-7 and PNT1A) and lung fibroblast cell line (V79-4). Cytotoxic effects on these lines were observed, acting to inhibit their growth and significantly reduce cell viability. It indicates that the antioxidants present in the *Cistus incanus L.* and *Cistus monspeliensis L.* extracts may prove to be very helpful in the treatment of benign prostatic hyperplasia (BPH).[38] Human melanoma cell lines (A-375) introduced into cultures were compared to human breast cancer cells (MCF-7) extracts from *Cistus libanotis, C. villosus* and *C. monspeliensis*. The analysis of the results showed greater antiproliferative activity of these extracts against melanoma (A-375) than against breast (MCF-7).[39]

El Euch et al. in their studies analyzed the anticancer activity of leaf extract and flower buds from *Cistus salviifolius*.[26] They determined that the flower bud extract showed cytotoxic activity against ovarian carcinoma cell (OVCAR) and breast (MCF-7). And the leaf extract showed a lack of cytotoxic activity against both tumor lines. Antitumor activity results from the high content of polyphenols and flavonoids in the obtained flower bud extract. Subsequent researchers undertook a study to evaluate the antioxidant activity of *Cistus incanus L.* and pomegranate peel (*Punica granatum L.*) rich in polyphenolic compounds.[40] Incubation of human cancer line breast (MCF-7) and colon (LOVO) cultures with pomegranate and cistus extracts resulted in a slowing down of the growth of tumor cells of both lines.

Research indicates that purified extracts can complement human cancer treatment. However, it requires research to understand its effects and interact with the recommended drugs.

Summary

Species of the genus *Cistus* exhibit a number of medicinal properties resulting from the presence of compounds with biological activity. The presented research

results in our article indicate that the antioxidant properties of *Cistus sp*. affect its antibacterial and antifungal properties. This draws attention to the possibility of using extracts and biologically active compounds isolated from *Cistus* in the treatment of inflammation caused by pathogenic microorganisms and the strengthening of antibiotic therapy. The biological activity of the *Cistus* herbaceous plants against tumor cell lines indicates that they can be considered as potential therapeutic agents in the treatment of neoplastic diseases.

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Medicinal benefits from the use of Black pepper, Curcuma and Ginger

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ABSTRACT

Introduction. *Black pepper*, *Curcuma* and *Ginger* are three of the most popular and most frequently used spices. Due to their beneficial medical and pharmacological properties, they are increasingly appreciated phytotheraputic plants. Most of their actions are attributed to their biochemical compositions.

Aim. Our intention is to equip the reader with the information and knowledge necessary to understand the role of natural products in the drug discovery process and to enable the assessment of potential benefits and harms of plant-based medicines when advising patients who wish to use them.

Material and methods. Analysis of literature.

Results. In this paper, we reviewed the use of *Black pepper*, *Curcuma* and *Ginger* documented in the treatment of colds and flu, support for immunity, but also use in digestive ailments and beneficial effects on the cardiovascular system and immune system.

Keywords. medicinal plants, phythotherapy, *Black pepper*, *Curcuma, Ginger*

Introduction

Knowledge about plant-derived medicinal products is essential in all areas of healthcare, not only because these forms of treatments are popular in healthcare (often used as a self-medication) but because of their importance in many traditional medical systems globally. This article is not a guide to treatment but rather a presentation of the scientific principles summarizing traditional preclinical and clinical evidence underpinning the use of herbal and other plant derived medicine. Several hundred species of plants growing on the Earth are confirmed by science as having healing activity. Accord-

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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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ing to the latest reports of the World Health Organization (WHO), almost 7000 chemical compounds that are used in medicine are derived from plants. Based on other reports from the WHO, in Europe over 30-40% of medicines exist whose components come from plants and in the United States, preparations of vegetable origin constitute about 24% of all medicines. Herbs and other plants can be used in various ways. Usually, plant infusions are used in teas, extracts, syrups, etc. However, in order to expect effective therapy, we must be sure that we will use the right dose of specific medicinal substances (active). Such guarantees also give us the naming of preparations that are produced based on standardized plant extracts, i.e. those for which the dose of medicinal compounds is known.

Black pepper, *Curcuma* and *Ginger*

In recent years many medicinal plants have been studied in many recognized medical laboratories of the world, and it was recognized that some of them are irreplaceable in the treatment of immune-stimulating, or stimulating, human immune systems.

Scientific research in recent years has shown that plants such as *Black pepper*, *Curcuma* and *Ginger* increase the body's resistance to infections. These three spices contain substances with a broad spectrum of antimicrobial activity, mainly contained in the essential oils. They are also a rich source of natural antioxidants that neutralize free radicals. A diet rich in antioxidants reduces the risk of cancer, heart disease, degenerative diseases of the joints, and slows down the aging process.

Black pepper produced from unripe, dried and fermented fruits is native to southern India. The molecule piperine is responsible for the spicy characteristic taste of *Black pepper*. It is an organic compound located in the top layer of the peel of this popular spice. *Black pepper* was used in eastern medicine as a remedy for indigestion, various pain and infections. In addition, *Black pepper* has antiemetic and antipyretic effects. *Curcuma* is one of the strongest antioxidants with very strong anti-inflammatory, antiviral, antibacterial, cleansing, anti-cancer, antioxidant, antiseptic, radioprotective and cardioprotective effects. *Curcuma* has a purifying effect on blood, and supports the work of the pancreas and liver. Research is ongoing on the use of these substances in the prevention and treatment of diseases such as rheumatoid arthritis, diabetes, and Alzheimer's disease. In folk medicine turmeric is used, among others in the treatment of diseases of the gall bladder, kidneys and also in stomach ailments because of the fact that it supports metabolism and accelerates digestion. Various species of the *Curcuma* genus have been known in medicine since at least the 19th century. The main pharmacological activities of *Ginger* and compounds extracted from it's rhizome include immunomodulatory, anti-cancer, anti-inflammatory, analgesic, antihyperglycaemic and antiemetic activities. There are also great opportunities to use *Ginger* in the treatment of heart disease, and inflammation of joints and bones. The research also shows low toxicity of *Ginger*, which is related to the safety of its use. It is used in both raw and powdered form. From the rhizomes of G*inger* during the distillation processes, 0.6 to 3.5% of the essential oil is obtained, whose exact chemical composition depends on the place of origin of the plant. *Ginger* contains many nutrients that change slightly depending on the form consumed.

Figure 1 below presents total global production of *Black pepper* and *Ginger* and their consumption in Poland. The chart was made on the basis of data for 2013 and 2016 from The Food and Agriculture Organization of the United Nations site.[1]

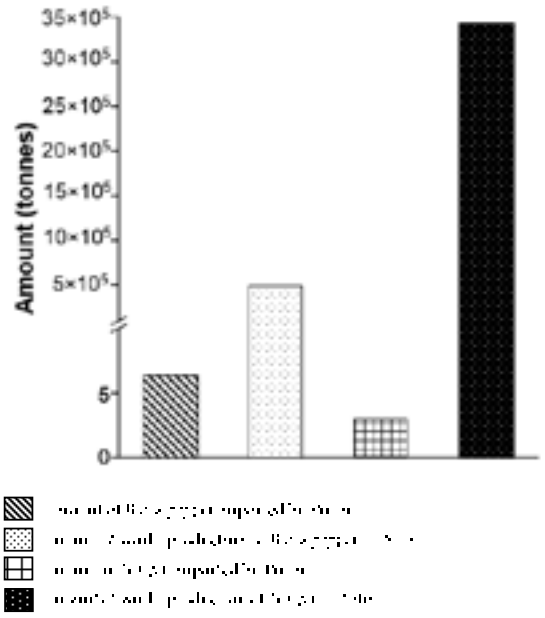

Fig. 1. *Black pepper* and *Ginger*: World production and consumption in Poland

Chemical composition

The composition of individual substances in *Black pepper* depends on the variety of the plant from which the raw material is obtained. *Black* p*epper* in its natural form contains about 2.6% essential oil, 13% water, 12% nitrogen compounds, 7% fats, in addition to starch, cellulose, alkaloids, other essential oils and about 7% piperine. *Black pepper* oil does not contain piperine, because it is a very volatile component. The main biologically active agents are acidic amides. *Black pepper* is a perennial plant originating in India grown in tropical areas, especially in southern India, Indonesia, the Malay Peninsula, Central America and the Philippines. The flowers of *Black pepper* are collected in spiky blooms. The *Black pepper* crop is significantly influenced by environmen-

tal factors that significantly affect its productivity. Yudiyanto et al. in their observations stated that the most important factor turned out to be the intensity of precipitation.[2] *Black pepper* is available in several forms: fresh, dried in the form of whole grains, or in ground form. Figure 2 below shows dried *Black pepper*.

Fig. 2. Dried *Black pepper*

Herbs and turmeric root have within their composition, among others: mineral salts (lime, iron, magnesium), fats, fiber, proteins, starch and essential oils. The *Curcuma* rhizome has an intense yellow color, derived from dyes, the so-called curcuminoids, which include compounds such as curcumin (diferulomethane, makes up about 70%), demethoxycurkumin (about 15%) and bis-dimethoxycurmarine (about 3%). The Figure 3 below shows *Curcuma* powder.

Fig. 3. *Curcuma* powder

It should be noted that the chemical composition and microstructure is influenced by the rate and method of freezing. Singha & Muthukumarappan on the basis of microscopic examination found that structural damage was more pronounced in slower frozen rhizomes than fast frozen ones. In addition, degrees of fruition significantly affected the composition and color of *Ginger*.[3] Table 1 below shows selected ingredients of rhizomes and powder of *Ginger*.

Table 1. Selected ingredients of rhizomes and powder of *Ginger*

Rhizomes		
Compound	**Quantity**	**Ref**
zingiberene	37.9%	(Koch *et al*. 2017)[4]
sabinene	13.5-38.0%	(Verma *et al*. 2018)[5]
(E)-1-(3′,4′-dimethoxyphenyl) buta-1,3-diene (DMPBD)	20.6-35.3%	
terpinen-4-ol	9.0-31.3%	
γ-terpinene	1.1-4.8%	
β-phellandrene	1.0-4.4%	
6-gingerol	(268.3 mg/kg)	(Koch *et al*. 2017)[4]
potassium	0.98 ppm and 1.38 ppm (white and yellow types)	(Ajayi *et al*. 2013)[6]
calcium	0.68 ppm and 0.41 (white and yellow types)	
***Ginger* powder**		
Compound	**Quantity**	**Ref**
potassium	43.963 mg/kg of dry mass	(Koch *et al*. 2017)[4]
manganese	758.4 mg/kg of dry mass	
calcium	1-1.5%	(Uma Pradeep *et al*. 1993)[7]
iron	54-62 mg/100 g	

Figure 4 below shows rhizomes and powder of *Ginger*.

Fig. 4. Rhizomes and powder of *Ginger*

Black Pepper

Black pepper is most often used in the form of ground powder. In the scientific literature there are many reports on the method and conditions during the milling process. Ghodki et al. in their work described the cryo-

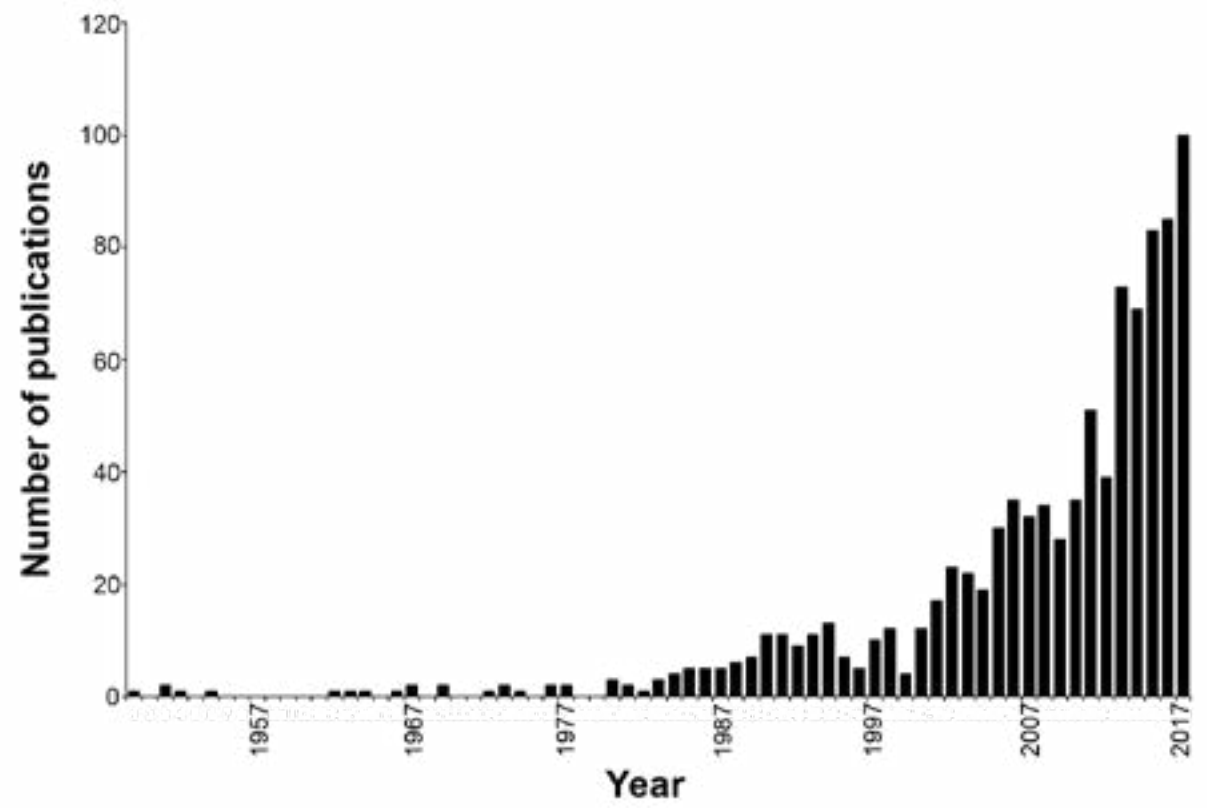

Fig. 5. Number of publications regarding *Black pepper* collected from Library of National Center for Biotechnology Information (NCBI) PubMed Data Base

genic grinding method, which is used to ensure the best quality of spices. The study looked at the physicochemical characteristics of ground *Black pepper* grains at temperatures of -120°C, -80°C, -40°C, 0°C and 40°C. The content of the mineral content of ground *Black pepper* increases with the reduction of the crushing temperature.[7] Whereas Ghodki & Goswami in their research found that the best f way to grind *Black pepper* is cryogenic grinding with a maximum grinding temperature -21.27°C.[9] In the literature, there are many reviews on the subject of crops, chemical composition, applications, health and therapeutic benefits of various herbs and descriptions of antioxidant, antimicrobial, anti-inflammatory, gastro-protective and antidepressant properties of *Black pepper*.[10,11] Figure 5 represents the increased interest in applications of *Black pepper*.

In addition, works have described the biological role of *Black pepper*.[17] The main healing agent in *Black pepper* is *piperine*, which is a powerful antioxidant. It demonstrates a strong action against free radicals. It helps in the protection of the circulatory system, the liver and protection against DNA damage, showing anticancer activity. In addition, aging processes are slowed. There are many reports in scientific publications confirming the beneficial effects of eating *Black pepper*. [18] The de Souza Grinevicius group investigated the association of overproduction of reactive oxygen species (ROS), DNA fragmentation, cell cycle arrest and apoptosis induced

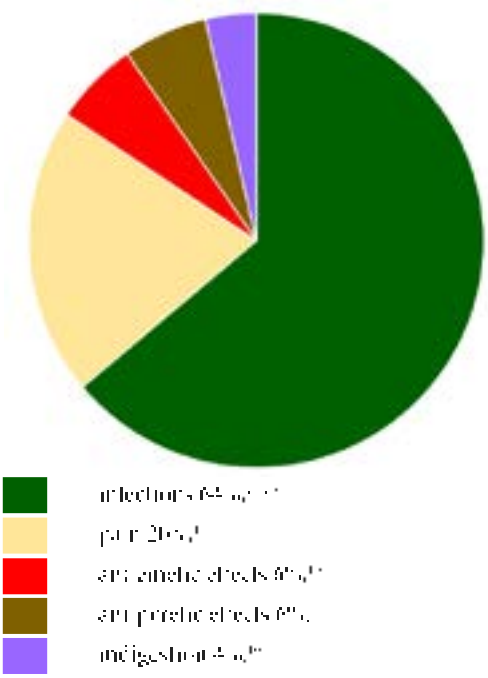

Fig. 6. Application of *Black pepper*

by *Piper ethanolic nigrum*. As a result of the administration of the solution, cytotoxic and antiproliferative effects were found on MCF-7 cells in *in vivo* studies. The demonstrated antitumor activity is most likely associ-

ated with ROS overproduction, causing oxidative stress affecting the key proteins involved in G1/S cell cycle arrest and triggering apoptosis.[19] The Deng et al. group came to a similar conclusion in its scientific research, which also evaluated the anti-cancer action of *piper nigrum* in animal studies. The results of the research show contributions to the generation of reactive forms of ROS oxygen, which results in anticancer effects.[20] Whereas Gunasekaran et al. in the study looked at the effects of *piperine* against hepatocellular carcinoma. The study showed that *piperine* may be a pro-oxidant that alleviates hepatocellular carcinoma.[21] In addition, the Guineensin extract found in black and long *Black pepper* has anti-inflammatory activity, inhibiting the uptake of endocannabinoids by the cells. The Reynoso-Moreno group evaluated guineensine in mouse models of acute and inflammatory pain and endotoxemia. The strong pharmacological action of guineinin may be responsible for the antiinflammatory effects of *Black pepper*.[22] Grains of *Black pepper* and the essential oil obtained from them contain compounds that have antimicrobial activity. It is a source of natural antioxidants that stop rancid fats, which is the task of a natural preservative.[23] In addition, a diet rich in *Black pepper* antioxidant substances can be helpful in reducing the likelihood of cancer, heart and blood vessel diseases and degenerative joint disease, and can also help to slow down the aging process. Ahmad et al. in their research investigated the effect of *Black pepper* extracts on cultures of different bacteria and activity against toxin producing metabolites (*Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, Bacillus subtilis, Bacillus cereus, Staphylococcus aureus and Candida albican*s). Based on these studies, it has been found that the majority of generally available extracts of *Black pepper* have activity against pathogenic microorganisms.[24] In addition to antimicrobial activity, the *piperine* contained in *Black pepper* positively affects the digestive processes. Its main advantage for health is the secretion of gastric juices, which contributes to the improvement of digestion, thereby increasing appetite. Strong warming properties improves the blood supply to the entire digestive system, which contributes to a positive effect on the absorption of nutrients contained in food, improves the absorption of certain substances. In addition, the extracts of the *Black pepper* act on the body in a diuretic and slightly laxative way, which allows you to quickly get rid of harmful products of metabolism from the body. *Black pepper* in traditional folk medicine has long been used as a drug for stomach problems such as diarrhea, nausea, bloating or digestive disorders, because its consumption influences the production of saliva and digestive enzymes. In addition, *Black pepper* soothes inflammation. The research group of McNamara et al. characterized the action of *piperine* on the human vanilloid receptor TRPV1, where *piperine* showed pronounced agonist activity. The results show that *piperine* is mediated by TRPV1 mediated gastrointestinal function.[25] *Black pepper* also has antibacterial properties, helpful in the treatment of intestinal diseases caused by various types of bacteria. In addition, *Black pepper* can contribute to the breakdown of fat cells, due to the presence of nutrients in the outer layer of the grains. Ebihara et al. in their studies determined the effect of olfactory stimulation with volatile *Black pepper* oil on risk factors for pneumonia. The study concluded that the use of nasal inhalation with BPO *Black pepper* oil may contribute to the activation of the isletic or orbital-frontal cortex, which improves the reflex swallowing movement.[26] It should be noted that the use of *piperine* may exert an immunotoxic effect, which has been confirmed in studies in mice by several scientists. Dogra et al. concluded that a dose of 1.12 mg *piperine* per kg body weight does not have any immunotoxic activity and can be considered an immunologically safe dose.[27] When it comes to the use of piperine extract with drugs, it is possible to increase the effect of drugs. Rao et al. investigated piperine effects on liver function in animal studies. There are more and more studies describing the impact of *Black pepper* and *piperine* on drug metabolizing enzymes.[28] Based on the results of Rao et al. high-dose *piperine* extract have significant impact on liver damage.[29]

Curcuma

Parveen et al. extracted the ethereal oil from the leaves of the *Curcuma longa L*. Kasur cultivar bred in Pakistan. The antimicrobial properties of *Curcuma longa* leaves were then verified using the disk diffusion method. Several different human pathogens were selected among eight fungi and five bacterial strains. The essential oil showed maximum resistance to *Fusarium miniformes*, followed by *Bacillus subtilis* and showed the least resistance to *Fusarium oxysporium*. The results showed that the essential oil shows significant inhibitory activity on the test organisms.[39] Katsuyama et al. studied Curcuminoids isolated from *Curcuma longa*. Two additional type III polyketide synthases, named CURS2 and CURS3, have been identified and characterized, which are able to synthesize curcuminoids. *In vitro* analysis showed that CURS2 preferred feruloyl-CoA as the starter substrate, and CURS3 preferred both feruloyl-CoA and p-coumaroyl-CoA. These results suggest that CURS2 synthesizes curcumin or demethoxicurumin, and CURS3 synthesizes curcumin, bis-thyroxycurcumin and demethoxycurcumine.[40,41] Krishnaraju in his research produced a new demethylated cobrologic composition (DC) containing at least 95% of all demethylated kamkinoids (67.8% bis-methyl cletumin, 20.7% demethlonodimethoxocycloin, 5.86% bis-dimethoxycurmarine, 2.58% demethyl sucrose) (PCT) IN05 / 00337, dated 13 October 2005),

starting from *Curcuma longa* extract containing 95% of all curcuminoids (C95). The DC composition is characterized by better neuroprotective and anti-inflammatory efficacy in comparison. In addition, it is safe to use.[42] *Curcuma*, an antioxidant found in the spice, inhibits carcinogenesis in animal models and has been shown to be an antiinflammatory agent.[43] In the search for reagents that inhibit NO production and study the chemical composition of natural food of plant origin, *Curcuma* chemical ingredients used as a spice were studied. As a result of this study, 2 new terpenoids and 14 known analogues were isolated. It has been found that all compounds have NO inhibitory activity in murine microglial BV-2 cells. The discovery of two new compounds in this chemical study further revealed the chemical composition of *Curcuma* and a biological test suggested that the natural *Curcuma* food seasoning containing terpenoids with NO inhibitory activity could potentially be a promoter for humans health.[44] Kurkimin, contained in the root of turmeric, is a very strong, natural antioxidant. As a result, turmeric has anti-cancer, anti-inflammatory, antibacterial and cleansing properties. The use of turmeric can be useful in the treatment of various types of cancer inter alia, skin, esophagus and tumors of the abdominal cavity and in the prevention of their formation.[45,46] Turmeric is capable of causing cancer cells to self-destruct through the process of apoptosis. *Curcuma* helps in stopping tumor growth, metastasis and spread of cancer cells at the molecular level.[47] In addition, the chemical compounds contained in the curcumin block the formation of alpha-toxins and nitrosamine - two very carcinogenic substances. Hong et al. checked the effect of turmeric root oil on anti-proliferative activity against some human cancer cell lines (MCF7, Ca Ski, A549, HT29 and HCT116). Strong cytotoxicity was demonstrated for HT29 cells (IC 50 value 4.9 ± 0.4 μg / ml), weak cytotoxicity to A549, Ca Ski and HCT116 cells (with IC 50 values 46.3 ± 0.7, 32.5 ± 1.1 and 35.0 ± 0.3 μg / ml, respectively) and no inhibitory effect on MCF7 cells.[48] Strong cytotoxicity was demonstrated for HT29 cells (IC 50 value 4.9 ± 0.4 μg / ml), weak cytotoxicity to A549, Ca Ski and HCT116 cells (with IC 50 values 46.3 ± 0.7, 32.5 ± 1.1 and 35.0 ± 0.3 μg / ml, respectively) and no inhibitory effect on MCF7 cells.[49] The HP CR-SR essential oil showed more significant cytotoxicity on tumor cell lines than on individual herbs of *Curcuma Rhizoma* and *Spargania Rhizoma*. In summary, the oil from HP CR-SR differs from any of the *Curcuma* Rhizoma and Sparganii Rhizom, or simply their superposition, and the HP CR-SR oil presented a more significant anticancer and antioxidant effect compared to the *Curcuma Rhizoma* and *Sparganii Rhizoma* oils.[50] Huang et al. looked at TNBC breast cancer, which because of its weak sensitivity to conventional therapies is extremely difficult to cure, and the impact of curcumol on the development of cancer cells. It was found that curcumol contained in curcumin inhibited the growth of MDA-MB-231 cells and triggered apoptosis-independent apoptosis mediated by the p73A-PUMA/Bak signaling pathway.[51] Based on these results, it can be concluded that the development of new drugs on TNBC may involve the use of extracted curcumol. *Curcuma*, due to its choleretic properties and the secretion of gastrin, secretin and pancreatic enzymes, has mild anti-inflammatory, antibacterial and antispasmodic activity. Turmeric ingredients curcumin and essential oils stimulate the secretion of bile necessary for digestion of fats. Because of this it works to help in digestive disorders and ailments such as bloating, stomach flu, diarrhea and irritable bowel syndrome. It is also used in the treatment of stomach ulcers caused by H. pylori, which *Curcuma* affects. In addition, turmeric has an important property of cleansing the liver from toxins, especially associated with alcohol consumption and the use of drugs and strong drugs. Turmeric naturally stimulates the production of enzymes responsible for the metabolism of toxins. It is used in conditions of liver damage and insufficiency, also in inflammatory conditions of the liver and bile duct. In addition, it has protective properties on liver cells and is used as an auxiliary in its regeneration. In addition, curcumin prevents the accumulation of adipose tissue, purifies the blood and reduces cholesterol. Samuhasaneeto et al. in a study in rats found that curcumin could prevent the activation of NF-κB, which affects genetic hepatitis. *Curcuma* also prevented the activation of kappa B factors in rats administered alcohol.[52] In subsequent surveys on the shrubs of Tranchida et al. found that the administration of *Curcuma longa* extract increases some of the defense mechanisms, acting on choline metabolism, preventing the development of fatty liver.[53]

The complete chloroplast genome (cp) of *Curcuma* flaviflora, a medicinal plant in Southeast Asia, has been sequenced. The size of the genome was 160 478 bp, with a content of 36.3% GC. A pair of inverted repeats (IR) of 26 946 base pairs was separated by a large single copy (LSC) of 88008 bp and a small single copy (SSC) of 18.578 bp. The cp genome contained 132 annotated genes, including 79 genes encoding the protein, 30 tRNA genes, and four rRNA genes. And 19 of these genes were duplicated in the inverted regions of the repetitions.[54] In rat studies, Tranchida et al. in 2015, she stated that curcuminoids contained in C*urcuma* can positively affect fatty acid metabolism, hexosamine biosynthesis pathway and alcohol oxidation. Supplementation with *Curcuma longa* extract seems to be beneficial in these metabolic pathways in rats.[55] Dall'Acqua et al. showed that supplementation with *Curcuma* extract for healthy animals causes changes in urine composition by reducing the concentration of allantoin. On this basis, it

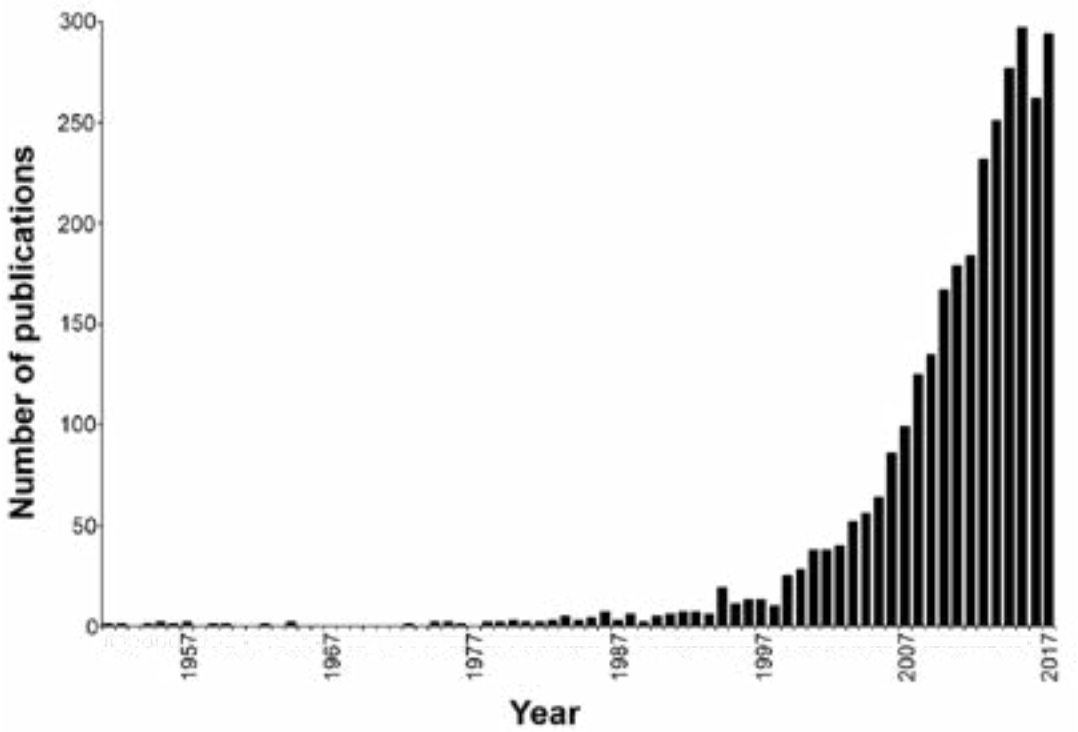

Fig. 7. Number of publications on *Curcuma* collected from Library of National Center for Biotechnology Information (NCBI) PubMed Data Base over the years starting from 1945

can be concluded that the extract has an effect on oxidative stress in animals in *in vivo* studies.[56] The impact of high ambient temperature has a negative effect on poultry production in many countries. One of the most practical ways to eliminate these effects is to modify your diet. Grupa Akbarian et al. studied herbal extracts and their effect on reducing the side effects associated with increased temperature in broiler chickens. Turmeric extract from xantho- rhohydra at a dose of 400 mg / kg, has positively affected the alleviation of some changes in the blood composition.[57] In addition, Ramkissoon et al. showed positive effects on aging, diabetic complications and diseases related to oxidative stress.[58]

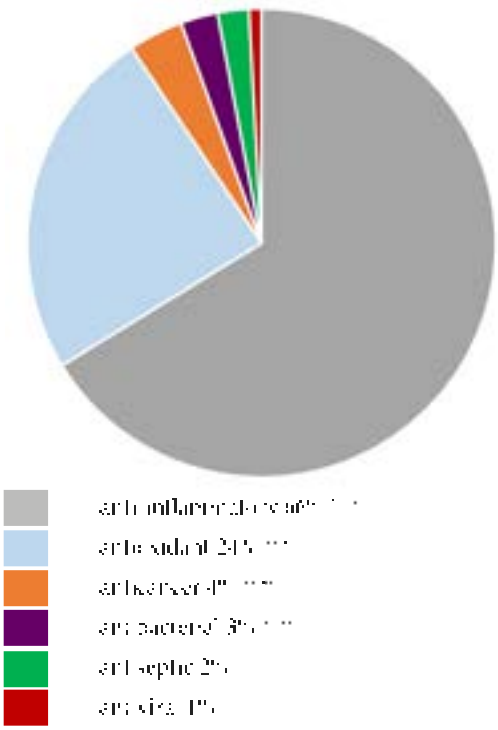

Fig. 8. Application of *Curcuma*

Figure 7 presents the number of papers regarding the use of *Curcuma*.

Ginger

Ginger spice has a calcium content of 1-1.5% and an iron content of 54-62 mg/100 g.[7] Both root and powdered G*inger* have warming properties, improve blood circulation and support the natural cleansing of the body. Due to the content of G*inger* that increase the body's resistance, it is often used to treat colds. The nutrients contained in G*inger* are very easily absorbed by the body, which makes it effective. Sebiomo et al. published a study comparing the effectiveness of G*inger* and a conventional antibiotic on two selected pathogenic bacteria (Staphylococcus aureus and Streptococcus pyogenes). The plant extracts were prepared by weighing the plant leaves and root (20, 40, 60, 80 and 100 g) into 100 mL of water and ethanol (at g/100 ml) to determine the extract concentrations. The study concluded that G*inger* has much stronger antibacterial properties than antibiotics.[59] Awad & Awaad found in their research that G*inger* results in a significant strengthening of the immune system of fish in prevention and control of microbial diseases. The mechanism of action of medicinal plants

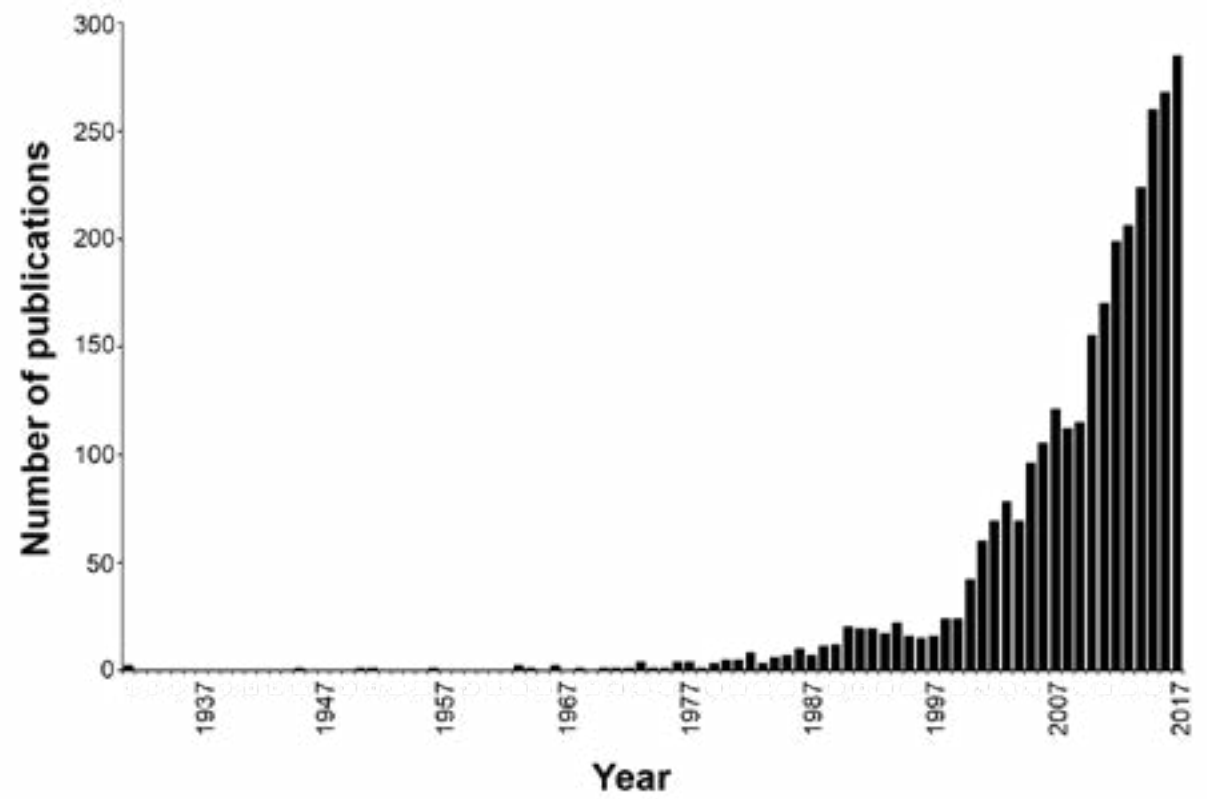

Fig. 9. Number of publications on *Ginger* collected from the National Center for Biotechnology Information (NCBI) PubMed Data Base

consisted in the stimulation of the cellular and humoral immune response, which was monitored by raising immunological parameters.[60] Figure 9 and 10 present the number of publications in studies of ginger and its applications respectively.

Ginger extract, intensely consumed as a spice in food and beverages around the world, is an excellent source of many bioactive phenols, including non-volatile acute compounds such as gingerole, paradole, shogaole and *Ginger*. Strong anti-inflammatory and analgesic properties can be used for joint and muscle pain thanks to anti-inflammatory substances that are used in various types of ointments and warming patches. In addition, *Ginger* oil can bring relief to sore muscles. Grzanna et al. reported that *Ginger* inhibits prostaglandin synthesis by inhibiting cyclooxygenase-1 and cyclooxygenase-2. An important extension of this early work was the observation that *Ginger* also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase. This pharmacological property distinguishes *Ginger* from non-steroidal anti-inflammatory drugs. The pharmacological characteristics of *Ginger* entered a new phase with the discovery that the G*inger* extract from Zingiber officinale (Zingiberaceae family) and galanga alpina (Zingiberaceae family) inhibits the induction of several genes involved in the inflammatory response process.[69] Altman & Marcussen, on the basis of research, found that Sida cordifolia L. and Zingiber officinale had a protec-

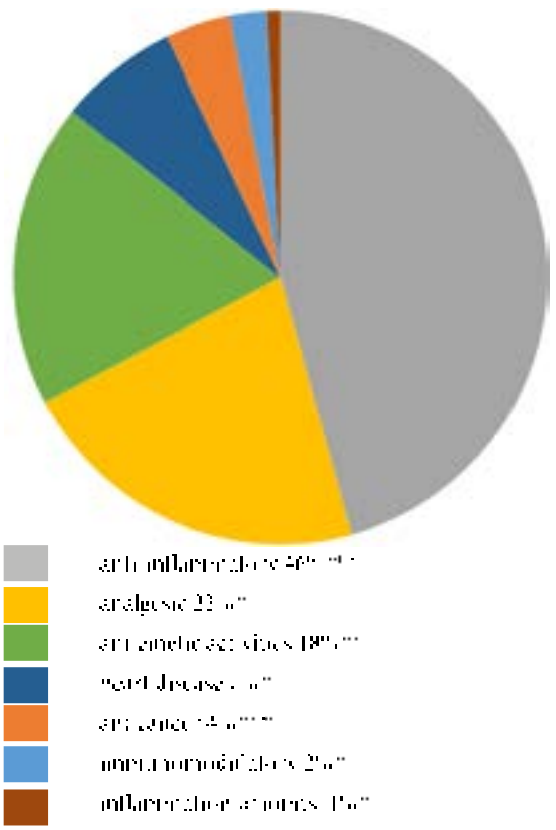

Fig. 10. Main areas of ginger use from the National Center for Biotechnology Information (NCBI) PubMed Data Base

tive effect on the cartilage. *Ginger*, due to its anti-inflammatory properties helps to combat knee pain and osteoarthritis.[70] Black & O'Connor investigated the effect of *Ginger* on muscle pain, inflammation and dysfunction caused by eccentric effort. It was found that 2 g of G*inger* can alleviate the daily progression of muscle pain.[71] In addition, G*inger* is effective in relieving menstrual pain. In the study by Ozgoli et al., people from the G*inger* group took 250 mg of G*inger* powder capsule four times a day, while people from another group were taking 250 mg mefenamic acid or 400 mg ibuprofen, respectively. They found that *Ginger* is as effective as mefenamic acid and ibuprofen in relieving pain in women with primary dysmenorrhea.[72] Metronidazole (MTZ) is the drug of choice in the treatment of lambliosis; its chemical composition has serious hazards and becomes less sensitive. The aim of this study was to look for natural extracts alternative to MTZ. In-vivo effects of dichloromethane extracts of G*inger* and cinnamon in doses of 10 and 20 mg / kg / day divided into 6 groups (5 rats each). The potential therapeutic effect of G*inger* and cinnamon extracts on G. lamblia infection in albino rats as a promising alternative therapy for commonly used antiplatelet agents has been confirmed.[73] The oil contained in the G*inger* rhizome positively affects digestive work, which may be helpful in digestive disorders, food poisoning or indigestion, because it exhibits choleretic and diastolic effects and may be helpful in stimulating gastric juices. In addition, *Ginger* is known for the relief of nausea and antiemetic (Zingiberis rhizoma). According to traditional Chinese medicine, nausea is one of the commonly used herbs for *Ginger*. The efficacy of *Ginger* in the treatment of nausea and vomiting was studied in the Ernst and Pittler study which looked more closely at six previous studies. It was reiterated that the positive effect of *Ginger* is greater than the placebo effect (*Ginger* and placebo groups for *Ginger* (1g) taken before operation (absolute risk reduction 0.052 (95% confidence interval -0.082 to 0.186)).[74] In addition, Borrelli et al. found that *Ginger* can be an effective way to treat nausea and vomiting during pregnancy. In efficacy studies, dosages ranged from 500 to 1,500 mg per day and the duration of treatment ranged from 3 days to 3 weeks. The comparisons included placebo and vitamin B6.[75] Pharmacological studies in humans require confirmatory testing to exclude the interaction of *Ginger* preparations with platelet aggregation. Preclinical safety data do not exclude potential toxicity, which should be monitored especially after ingestion of *Ginger* for a long time.[76] *Ginger* is also used in the weight loss process. *Ginger* has a sensitizing effect on glucose and stimulates the gastrointestinal tract. In the study of Mansour et al. evaluated the effect of a hot *ginger* beverage on energy expenditure, the feeling of appetite and satiety, and metabolic risk factors in overweight men. The results show enhanced thermogenesis and reduced hunger with consumption of *Ginger*, suggesting the potential role of *Ginger* in weight management. Additional studies are needed to confirm these findings.[77] In addition, studies indicate that *Ginger* facilitates the digestion of fat. The generation of heat in the body has a positive effect on the stimulation of the metabolic system. Some studies have shown antitumor activity of *Ginger* on cancer cells *in vitro* and *in vivo*. *Ginger* is not only a large amount of antioxidants that fight free radicals and show cytotoxicity to cells. About 3% of the weights of Ginger are very aromatic essential oils. Padama et al. in their studies, they checked the *in vitro* cytotoxic activity of the salt extract obtained from the *Ginger* extract on the HEp-2 cell line. The present results show that the extract exerts a dose-dependent suppression of cell proliferation; the involvement of free radicals has been confirmed by increasing the production of superoxide, reducing the formation of nitrates and depleting glutathione in cells treated with *Ginger*. Further screening of active ingredients by means of gas chromatography and mass spectrometry analysis revealed the presence of clavatol, geraniol and pinostrobin in the extract. The results of this study suggest that *Ginger* may be useful as a potential anti-cancer agent.[78] Research group Jeong et al. suggests that gingerol a natural component of G*inger*, has anti-inflammatory and anti-cancer activity.[79] Numerous studies show a positive effect of *Ginger* in combating breast, ovarian, prostate and intestine cancer, without affecting the development of healthy cells. *Ginger* in addition to anti-inflammatory, antioxidant and anti-proliferative activity, which indicates properties as a chemotherapeutic agent. Karna et al. in studies, it shows that *Ginger* extract (GE) exerts significant growth-inhibiting and death induction effects in the prostate cancer cell spectrum.[80] *Ginger* was reportedly used in folk medicine to treat and prevent arterial hypertension and other cardiovascular diseases. This suggests that a possible mechanism by which *Ginger* induces its antihypertensive properties may be by inhibiting ACE activity and preventing lipid peroxidation in the heart.[64] *Ginger* is a powerful antioxidant and can alleviate or prevent the formation of free radicals. It is considered a safe herbal remedy with minor side effects/side effects. Further research is needed on animals and humans regarding the kinetics of *Ginger* and its components and the effects of their consumption for a long time.[81] Essential oils from *Ginger* root can also be used as a good natural preservative in fish food, due to their antioxidant and antibacterial properties.[82] Gurbuz & Salih at work evaluated the potential impact of various sumac seeds from sumac (*Rhus coriaria L.*) and *Ginger* (*Zingiber officinale*) seeds on fatty acids in egg yolks and cholesterol in blood and yolks from hens. However, dietary supplementation with sumac and *Ginger* powder decreases cholesterol and blood cholesterol

levels in laying hens. Supplementation of sumac and *Ginger* affected with HDL showed a significant effect ($p < 0.05$) in the treatment groups. The results of this study suggest that feeding sumac and G*inger* has a tendency to reduce cholesterol levels in both yolk and blood in hens.[83] Chitra et al. in their research, they used Poloxamer 188 polymer and plant extract Z. officinale to prepare silver nanoparticles (AgNP) by green synthesis and to study the anti-bacterial activity of AgNP using three human pathogens Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus. AgNP protected poloxamer 188 inhibits bacterial growth more efficiently than pure Z. officinale and AgNP extract from Z. officinale extract.[84] In the Yuan & Gao study, they re-hatred and analyzed Bacillus pumilus bacteria causing rhabdominal rot disease, which allowed for a better understanding of the genetic diversity of phages.[85]

Ginger cultivation, which also affects its chemical composition, is also important in this respect. Grupa Ghasemzadeh et al. noted that the increase in CO_2 concentration in the atmosphere due to climate change and agricultural practices may have an impact on biotic changes resulting in plant growth, allocation and chemical composition.[86]

Conclusion

The content of a large amount of essential oils in Imbirze gives it a characteristic burning taste and aroma with a refreshing note. Medicinal *Ginger* is not present in the wild state, it is a cultivated plant. Its morphological structure includes a creeping rhizome from which flower shoots grow. The root of *Ginger* has valuable properties that are used in herbal medicine and have a positive effect on health. It has antibacterial, antiviral, antiparasitic and antioxidant effects.

Ginger contains bioactive ingredients that have prohealth properties, which certainly makes it a plant with a high therapeutic potential. Regardless of the choice of the *Ginger* variety or the form consumed, the root contains many nutrients, minerals, amino acids that can be used in lacustrine or as food supplements.

Black pepper in the kitchen has been popular for many years. In addition to the taste, due to the content of *piperine*, it can exert a positive effect on human health. The most valuable substance contained in the grains of pepper is the piperine, which has a positive effect on the body, however, in the *Black pepper* its content is small and it is difficult to expect satisfactory effects from consumption. It should also be emphasized that excessive consumption of *Black pepper* can irritate the digestive system.

Curcuma has a bile-forming, choleretic and antimicrobial effect, thus preventing infections in the bile ducts. It is often used in indigestion and digestive disorders. As an auxiliary, it can be used in inflammation of the bile ducts and gall bladder. However, it is not recommended for use during pregnancy and breastfeeding or in children under 12 years of age (contains alcohol). So far, no side effects associated with the use of turmeric in the diet have been observed

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REVIEW PAPER

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Military candidate health qualification and adjudication

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ABSTRACT

Introduction. This study reviews the link between personal health and military qualifications. It was found that there is evidence of a strong link between obesity levels across young individuals and military qualification adjudication.

Aim. The purpose of the study was to review the literature about significance of the rules for adjudicating on the ability to perform active military service and analysis of the literature regarding the health condition of Polish citizens subject to perform obligatory military service.

Materials and method. Analysis of foreign and Polish literature

Keywords. legal regulations, civilians subject to military qualifications, health status, army

Introduction

In most nations in the world, as in Poland, every citizen is obliged by the law to defense of their country. The Act of November 21, 1967 on the Universal Defense of the Republic of Poland defines the duties of a citizen of the Republic of Poland in relation to the Homeland.[1] According to article. 4 par. 1, all Polish citizens who are of appropriate age and are able to perform this duty depending on their state of health are subject to universal defense. As part of the universal defense obligation, Polish citizens are obliged to:

1. military service,
2. performing duties resulting from assigned crisis assignments and mobilization allocations,
3. to provide work as part of employee mobilization allocations,
4. serving in civil defense,
5. education for safety,
6. participating in self-defense of the population,
7. doing exercises in units intended for militarization and serving in militarized units,
8. performing defense services

- on terms and within the scope specified in the Act

In this article, we will consider the obligation of military service for citizens of the Republic of Poland. Article 59 of the cited Act clearly defines who is a soldier in active military service. They are people who perform the following duties:

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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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1. essential military service;
2. military training;
3. territorial military service;
4. military exercises;
5. preparatory service;
6. periodic military service;
7. military service in the event of mobilization for war.

However, in order for a Polish citizen to be able to perform one of the above-mentioned types of military service, it is necessary - before appointment to serve - to define the category of the capacity for active military service, referred to in art. 30a para. 1 of the Act on the general obligation to defend the Republic of Poland. The decision on the inclusion of a given person in one of the categories is determined by competent medical boards, on the basis of a medical examination of the physical and psychological abilities of this person for the appropriate type of military service, including the results of specialist tests and, if necessary, hospital observation. Such decisions are issued by poviat and voivodeship medical commissions, appointed annually by voivodeships in consultation with the heads of provincial military staffs. These commissions are issued on the basis of the ordinance of the Minister of National Defense in the matter of adjudicating on the capacity of active military service and the procedure for the conduct of military medical commissions in these matters .[2] This regulation sets out in detail:

1. the mode of referral to military medical committees;
2. detailed conditions for adjudicating by military medical commissions on the capacity for active military service, including the ability to perform this service in particular types of troops, as well as on individual positions and military functions requiring particular health predispositions;
3. detailed conditions for adjudicating by military medical committees on the ability to perform active military service outside the state borders;
4. the manner in which military medical committees establish a relationship of illness, infirmity and death with active military service;
5. the procedure of adjudication by military medical commissions on the ability to enter active military service and to serve this service outside the state borders and to determine the relationship of illness, infirmity and death with active military service;
6. the manner of adjudication by military medical committees concerning the need to grant health leave to a soldier engaged in active military service;
7. a list of diseases and disabilities taken into account when adjudicating on the ability to perform active military service and to provide this service outside the state borders;
8. a list of diseases and disabilities taken into account when adjudicating on the ability to undergo active military service in particular types of troops and services, as well as on individual positions and military functions requiring specific health predispositions.

The ordinance in the annex contains a detailed list of diseases and disabilities of individual human and organ systems.

According to art. 32 para. 1 of the Act, men who, in the given calendar year end are nineteen years of age are required to appear at a specified date and place for military qualification. Volunteers, including women, may also volunteer by the end of the calendar year at twenty-four years of age, regardless of their qualifications and education, and if they are at least eighteen years of age. Volunteers who have entered military qualifications, on the day of their appearance, are subject to the obligation of active military service. Women with qualifications useful for active military service and women who receive education in order to obtain those qualifications that graduate or are university students or graduates in a given academic or academic year may be subject to the obligation to appear for determination of military qualifications, starting from January of the calendar year in which they finish nineteen years. For the above qualifications, they are recognized as education or professional qualifications required to perform medical, veterinary, marine and air occupations, as well as professions such as: psychologists, physiotherapists, radiologists, laboratory diagnostics, IT specialists, teleinformatics, navigators and translators. Military qualifications are called for by permanent residence or temporary stays lasting over three months.

Becoming qualified for military service involves appearing in front of the commune head or mayor (president of the city), the poviat medical commission and the military commander of the supplements. As part of the military qualification, related activities are to:

1. checking the identity of persons subject to military qualifications;
2. determining the capability for active military service of persons subject to military qualifications;
3. the initial destination of persons subject to military qualifications to particular forms of the general duty of defense of the Republic of Poland and acceptance of applications for substitute service;
4. assumption or updating of military records and processing of data collected in this record;
5. issuing military personal documents;
6. transfer of persons subject to military qualification to the reserve and issue, at their request, certificates of regulated relation to military service;
7. preparation of military recruitment for voluntary forms of military service.

Activities to determine the abilities for active military service belong to the poviat medical commission. The remaining activities belong to the commune head or mayor (city president) or an authorized employee of the commune (city) office, as well as to the military commander of the supplements or his authorized representative.

The military qualification is carried out by the voivodeship with the participation of heads of voivodeship military staffs, military commandants of supplements and starostas, as well as mayors or mayors (city presidents). The staroste (city president) is responsible for the military qualification in the poviat (city with poviat rights). In order to carry out military qualifications, the voivodeship appoints poviat medical committees annually, which specify the date of action and the territorial scope of the activity. The poviat medical commission consists of one physician with the right to practice as a doctor and having at least 1st degree of specialization in the field of general surgery or 1st degree of specialization in internal medicine, at the same time, a chairman of this commission, secretary and one employee of middle medical staff are appointed to this commission.

A person becoming qualified for military service presents medical documentation to the poviat medical commission, including the results of specialist tests carried out in the period of twelve months before the date of entering military service. The Medical Committee documents the examination of the health of people in the medical certificate book, which records the results of the examination, the results of specialist tests, including psychological or hospital observation, and medical information that arise from the medical records submitted by the medical commission. The chairman of the poviat medical commission directs the person for specialist examinations, including psychological or for hospital observation, in the case when, after conducting the tests and assessing the health condition of that person, fitness for active military service cannot be determined. The Medical Committee, specifying the person's ability to perform active military service, includes:

1. results of the examination of the health status of a person carried out in the course of military qualification;
2. results of specialist tests, including psychological tests, if the person was referred to such tests;
3. results of hospital observation, if the person was referred to such an observation;
4. medical information contained in the medical documentation if such documentation has been presented to the commission.

The head of the poviat medical commission decides on a one-man basis. In the judgment, the poviat medical commission defines a person becoming qualified for military service one of the following categories:

1. category A – capable of active military service, which means the ability to perform a specific type of active military service, as well as the ability to serve in civil defense and substitute service;
2. category B – temporarily incapable of active service military, meaning a transient impairment of general health or acute or chronic illnesses which, up to twenty-four months after the date of the survey, indicate a recovery of military capability;
3. category D – incapable of active military service during peacetime, with the exception of certain service posts intended for territorial military service;
4. category E – permanently and completely incapable of active military service, during peace and in the event of mobilization and during the war.

The decision is served on the person becoming qualified, who is entitled to appeal against the decision within 14 days from the date of delivery. The ruling is also received by the military commandant of the supplements, is recognized in the military register referred to in art. 49 of the Act of November 21, 1967 on the general obligation to defend the Republic of Poland.

In conclusion, the details on the health status of persons subject to military service, determined on the basis of medical commission decisions, are conducted in military commands of supplements, voivodeship military headquarters and at the level of the Ministry of National Defense. The collected data give the possibility of making various kinds of analyses of the health condition of people covered by military records.

Youth health and the ability to active military service

The current specifics of the operation of the army forces candidates to a high level of physical fitness and good health. Thus, emphasis is also put on the work of medical committees deciding on the state of health in terms of the ability to perform active military service.

Research on a group of recruited civilians are few, and are often referred to as a physical body.[3,4,5] It is worth noting that in the majority of countries around the world the prevalence of overweight and obesity among children, adolescents and adults indicates a growing trend. [3,6,7,8,9,10] According to data from the World Health Organization (WHO) in 2016, over 1.9 billion adults in the world were overweight, while 650 million were obese. The global obesity rate in the years 1975 - 2016 has tripled.[11]

Recent research on military services indicates that this population is also experiencing a rising BMI weighting trend, reflecting the situation in the open population. In recent decades, there has been an increase in the prevalence of overweight and obesity among civilians recruited to the army[3,12–18] and because they are derived from the open population, neg-

ative trends regarding BMI body mass index observed in many countries may have an impact on the capabilities of military organizations to recruit healthy and efficient military personnel. The physically demanding nature of military service imposes on the recruits a requirement of good health and physical fitness, which is why BMI, as one of the components of physical fitness, is an important factor predicting the effectiveness of military service as well as military operations. Recent studies indicate that 80% of physical readiness tests are failed due to overweight or obesity.[19] On the other hand, there are recent reports indicating an increased risk of musculoskeletal injuries in young military adepts with the lowest BMI values.[5] Thus, changes in the BMI index may not only influence the prevalence of overweight and obesity in the open population, but also reflect the effectiveness of military services. An important health problem for people applying for military service is arterial hypertension.[3,20–24]

A study consisted of screening tools including self-administrated questionnaire, general physical examination, anthropometric measurements, and assessment of blood pressure of 1238 Saudi military active duty service personnel was conducted in the military units of Taif region, western Saudi Arabia. Multivariate logistic regression in this study performed during four months showed that obesity as measured by body mass index [odds ratio (OR)=2.71, confidence interval (CI): 1.39-5.28], positive family history (OR=1.46, CI: 1.03-2.06), ever smoking (OR=1.45, CI: 1.05-2.02), and increased waist circumference (OR=1.04, CI: 1.02-1.06) were the significant predictors of hypertension among military active duty personel.[25] Implications for recruitment and retention of defense force personnel were reviewed by McLaughlin and Wittert.[26] These studies were used electronic database and identified 17 research paper about why individuals are suitable or not for employment in the military. A review of cardiovascular risk factors in in younger age groups US military personnel was provided by McGraw and coworkers.[27] The increase in BMI is a serious factor in non-infectious diseases such as ischemic heart disease, diabetes, musculoskeletal disorders or some cancers.[28–30] Diseases are a cause of over 56 million deaths.[31] In Poland, the mortality rate resulting from non-communicable diseases amounted to 470/100000.[32]

A report, which was published in 2016, showed that at least 12% of European children under 5 years old were overweight, and the trend was continuing to rise.[33] An earlier survey, performed in 2010 in 13 European countries, showed that the prevalence of overweight including obesity ranged from 10.8% in six-year-old Belgian boys to 45.1% in nine-year-old Greek boys using the IOTF definition.[34] In the same report, the prevalence of obesity ranged from 2.8% in six-year-old Belgian boys to 14.7% in in nine-year-old Greek boys also using IOTF definitions.[34] Both percentages from prevalence of overweight including obesity and prevalence of obesity show a contrast between northern and southern countries in Europe.[34,35] The same trend was shown by the Identification and Prevention of Dietary and Lifestyle Induced Health Effects in Children and Infants study[36] which estimated that the combined prevalence of overweight and obesity ranged from more than 40% in southern Europe to less than 10% in northern Europe and the overall prevalence of overweight and obesity was higher in girls (21.1%) than in boys (18.6%).[36]

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CASUISTIC PAPER

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Comparison of two suicide attempts with long-acting insulin – The rare way to commit suicide

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ABSTRACT

Introduction. In previous years, the number of suicide attempts has increased in Europe. Intoxication with hypoglycemic drugs, including insulin is a rare a tool for attempting suicide that may lead to a severe patient status.

Aim. The aim of the study was to assess the severity of insulin poisoning with examples of two patients.

Methods. The analysis of clinical history of patients and review of available literature.

Results. A 22-year-old patient was hospitalized in the Department of Toxicology and Cardiology due to a suicide attempt in the way of insulin poisoning; time of poisoning was unknown, and the level of glucose was indeterminable. The patient was treated with intensive specific pharmacotherapy. After hospitalization, which lasted 5 months, the patient's condition had been stabilized but with no verbal contact and quadriplegic paralysis. Another patient was a 41-year-old woman hospitalized two times in the Department of Toxicology and Cardiology due to the insulin poisonings. In each case of hospitalization of this woman, severe recurrent hypoglycemia was observed up to 25 mg% until the fifth day of hospitalization and the treatment used improved the patient's condition and there was no development of serious complications.

Conclusion. Normally effective treatment at the right time can recover the patient completely.

Keywords. suicide attempt, insulin, intoxication, toxicology

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not make enough insulin or when the body cannot use the insulin despite effective production. Insulin is a hormone that regulates blood sugar. The lack of successive insulin production leads to excessive amounts of glucose in the blood. Hyperglycemia, along with the duration of the disease, leads to serious damage to many systems of the human organism, especially the nervous and cardiovascular systems. Hypoglycemia is as dangerous as hyperglycemia. Early symptoms of hypoglycaemia are: anxiety, nervousness, weakness, pallor of the coatings, and increased sweating. In the later stage, following the insufficient supply of central nervous system glucose, symptoms such as orientation disorders, confusion, amnesia, convulsions and even coma occurs.

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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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The WHO report from 2014 indicates that in the above year 422 million people had diabetes and the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014.[1] The increase in people suffering from diabetes and those who treat it can results in increase in the number of poisonings with hypoglycemic agents. Accidental overdose of insulin is much more common than intentional poisoning.[2] Non-accidental suicidal insulin overdose is rare among people who are not treated due to diabetes. There are described cases, which show that it seems to be more common among people working in medical professions.[3] In recent years, we have seen an increase in the number of suicide attempts and the suicide rate death is much higher in Poland than the European average (15.51 vs. 11.25 – data from Eurostat in 2014).[4] In 2016 there were 9,861 suicide attempts and 5,405 people took their own lives (data from Polish Central Statistical Office).[5] The problem concerns young people- most suicide attempts in 2016 were taken by people aged 30-49.[5] Intoxication is a rare tool for attempting suicide-it takes 11th place of types of the ways of suicides.[6] Rarely poisoning, but often with severe patient status, is intoxication with hypoglycemic drugs, including insulin. Since 2013, there have been 22 patients documented with that type of poisoning in the Department of Toxicology and Cardiology in Lublin. Patients with diabetes (type 1 and also type 2) have about twice a higher risk of depression as the general population.[7] There are publications which show the relation between diabetes and depression.[8,9,10] For example, a meta-analysis from 2001 showed that in patients with diabetes, depression co-occurred with a frequency of 28.5% throughout life.[11] The higher rate of depression in diabetic patients may be due to an increased occurrence of depression in diabetic patients or an increased incidence of diabetes in patients with depression. There are data suggesting that this relationship is indeed two-way.[12] Therefore, patients with diabetes are at higher risk for suicide.[13] However, in diabetic patients who have access to insulin, it has been shown that only less than 5% of suicide attempts were made with insulin.[14]

Case reports

First case report: 22-year-old patient was hospitalized in the Department of Toxicology and Cardiology due to suicide attempt in the way of insulin poisoning. He was found unconscious in his room with 10 empty vials for insulin with a farewell letter. Unfortunately, the time that had passed since the poisoning was unknown. From the medical history, it is known that the patient was not treated for diabetes. The first glucose measurement showed that the level was indeterminable. Patient was treated with intensive pharmacotherapy, including concentrated glucose solutions and glucagon and glycaemia was 3 mg/dl. The patient had respiratory failure and could not breathe himself, therefore he was intubated. During intensive treatment we could observe large fluctuations in glucose concentration (from 3 mg/dl up to 400 mg/dl). Due to the features of brain edema and its consequences, the patient was consulted neurologically. Although the cerebral edema receded, hypoxia-ischemic lesions were visualized within the caudate and lenticular nuclei. In the MRI, the features of the broad white leiomyosis of the periventricular white matter, cortical and subcortical atrophy of the brain, corresponding to severe changes in the nature of hypoglycemic brain damage in the chronic phase were visualized. He required gastric consultation due to bleeding from the gastrointestinal tract. In the first days of hospitalization, the patient also had arrhythmia and high blood pressure. After the hospitalization which lasted 5 months, the patient was discharged from the hospital in a stable state, he was conscious, with preserved circadian rhythm, but with no verbal contact, with quadriplegic paralysis and he was fed by gastrostomy. Patient was referred for further care and convalescence.

Second case report: Second case of patient was a 41-year-old woman with depression in medical history, who was hospitalized two times in the Department of Toxicology and Cardiology due to suicide attempts by insulin poisoning. During the first hospitalization, she was treated due to intoxication with an analog of long-acting insulin 900 IU and 60 tablets of glimepiride (3 mg). During admission to the hospital she was unconscious, with glycaemia 25 mg/dl. Two years later, the patient was admitted to the Department of Toxicology and Cardiology because of another suicide attempt by injection of 1800 IU of long-acting insulin. She was in a medium-heavy state, conscious. She admitted that she took insulin to draw attention to herself. Interestingly, the patient was not treated for diabetes and her medicines belonged to her husband. The patient required intensive pharmacotherapy, including a specific antidote – glucagon, concentrated glucose solutions and steroid therapy. In each case, severe recurrent hypoglycemia was observed up to 25 mg% until the fifth day of hospitalization. Despite recurrent hypoglycemia, the patient's condition improved due to effective medical intervention and there was no development of any serious complications. Probably the most important thing was that the medical intervention was applied on the same day. In response to interviews, the patient was referred for further psychiatric treatment after each hospitalization.

Discussion

Diabetes is a civilization disease and many people are treated with hypoglycemic drugs. Intentional poisonings are usually associated with the intake of much

higher doses of insulin than during accidental poisoning and therefore they are linked with a worse prognosis also due to lack of seeking medical help.[15] The patients who are exposed to long-lasting and severe hypoglycemia are also linked with worse prognosis, due to the risk of neuroglycopenia.[16] Another risk factor is the implementation of treatment above six hours after poisoning, because it is known as appropriate time to respond to insulin poisoning.[15] In studies of insulin-induced hypoglycemia in monkeys, 5–6 hours of blood glucose concentrations of less than 1.1 mmol/l (20 mg/dl) were associated with neurological damage and it caused brain death.[17] Some researchers also believe that the duration of hypoglycemia depends more on the dose of insulin that has been taken and period of action of insulin is not so much important, because even short-acting insulin can work extremely long.[18,19] Therefore, patients who have received a high dose of insulin are in the group of high risk. We can see in the case of 41-year old patient who recovered completely after two intoxications because of treatment which was used in the suitable time. The 22-year old patient did not get medical care at the right time and treatment was started too late and it caused many complications after a toxic dose of insulin. It should be remembered that there are always exceptions in medicine and, for example Thewjitcharoen et al. presented in 2008 a clinical case describing an 80-year-old non-diabetic patient who survived with any complications a suicide attempt by giving himself 16,000 U of insulin (10,000 U of Humulin R and 6,000 U of Humulin N) probably due to consumption of many chocolate bars and high-carbohydrate drinks prior to the attempt.[20] However, it should be remembered that these are only exceptions and, in general, insulin poisoning can have a severe course and irreversible effect.

Summary

Diabetes is a civilization disease (422 million people have diabetes). Most of them are treated with hypoglycaemic drugs. Consequently the number of poisonings is increasing in both directions: accidental and suicide attempts. Insulin poisoning can be severe, because of neuroglycopenia, but due to effective treatment at the right time, patients can recover completely.

Conclusion

In conclusion, insulin is a toxic agent that people may have access to at work, such as medical professionals, that may be used it for attempting suicide. Other people using this as a suicide measure are family members of people with type 2 diabetes and diabetics who suffers from depression. Depression sufferers should be covered by treatment and psychiatric care to prevent such incidents.

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CASUISTIC PAPER

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Combined aplasia of frontal and shenoid sinuses with hypoplasia of the maxillary sinus

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ABSTRACT

Introduction. Combined aplasia of multiple sinuses is extremely rare. Agenesis of the paranasal sinuses is an uncommon clinical condition that appears mainly in the frontal (12%) and maxillary (5-6%) sinuses.

Case report. In this paper, we present the case of a 74-year-old woman with combined frontal and shenoid sinus aplasia accompanied by unilateral maxillary sinus hypoplasia. The findings were confirmed by a computed tomography scan of paranasal sinuses. The reason for admission was persistent headache, numbness of the left cheek and left alveolar process, and occasional nasal blockage.

Discussion. The uniqueness of our case is that the patient is an elderly female with combined aplasia of the frontal and sphenoid sinus with hypoplastic maxillary sinuses, whereas previously reported cases were found in children and in young adults.

Summary and conclusions. These anomalies can be misdiagnosed as chronic sinusitis or neoplasm. All potential sinus anomalies will have clinical implications and will hinder conventional and functional endoscopic sinus surgery.

Keywords. frontal sinus aplasia, maxillary sinus hypoplasia, paranasal sinus anomalies, paranasal sinus aplasia

Introduction

The paranasal sinuses are air-filled spaces located within the bones of the face and skull. They are thought to contribute to voice resonance, humidifying and warming inhaled air, increasing olfactory membrane area, absorbing shock to the face and head, providing thermal insulation for the brain, contributing to facial growth, representing vestigial structures, and to lighten the skull and facial bones.[1,2] The process through which the paranasal sinuses develop begins prenatally. They vary in terms of the development period and the level of pneumatisation. They can manifest different anomalies, for instance proper sinus development can be disturbed by many harmful factors and is associated with pneumatisation. Fractures, tumors, mucoceles, primary ciliary dyskinesia, infections, and some syndromes may have adverse effects on paranasal sinus development.[2,3] Various other clinical syndromes are found to be associated with agenesis of paranasal sinuses, such as Down's syndrome, cystic fibrosis, craniosynostosis and osteodysplasia.[2] Combined aplasia of multiple sinuses is extremely rare, as is hypoplasia of other sinuses. Agenesis

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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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of the paranasal sinuses is an uncommon clinical condition that appears mainly in the frontal (12%) and maxillary (5-6%) sinuses.[2] These anomalies can be asymptomatic or misdiagnosed as chronic sinusitis or neoplasm. All potential sinus anomalies will have clinical implications and will hinder conventional and functional endoscopic sinus surgery.[4]

In this paper, we present the case of a 74-year-old woman with combined frontal and shenoid sinus aplasia accompanied by unilateral maxillary sinus hypoplasia. The findings were confirmed on a non-contrast computed tomography scan of paranasal sinuses.

Case report

A 74-year-old woman was admitted to the Department of Laryngology of the District Hospital in Skarzysko-Kamienna. The reason for admission was persistent headache, numbness of the left cheek and left alveolar process, and occasional nasal blockage. Her complaints persisted throughout the day, and were aggravated in the early morning and during cold weather. She had already undergone medical treatment on several previous occasions, but with only temporary relief. There was no family history of similar complaints. No past history of any nasal surgery, facial trauma or any systemic disease involving the skeletal system was found, and haematological and other laboratory findings were normal. She was referred to a dentist to exclude all possible dental and oral abnormalities. A dental examination at the Department of Oral Surgery Poznan University of Medical Sciences revealed no dental and oral abnormalities and diseases. The ENR examination revealed no other clinical abnormalities. Computed tomography (CT) detected unilateral aplasia of the right frontal and right shenoid sinuses accompanied by unilateral hyperplasia of the left maxillary sinus (Fig. 1, Fig. 2, Fig. 3).

Additionally, CT revealed the sclerotic structure of the mastoid process of the right temporal bone and the

Fig. 1. Aplasia of the right frontal sinus

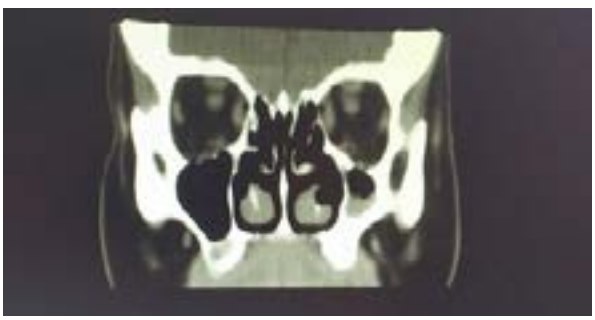

Fig. 2. Hypoplasia of the left maxillary sinus

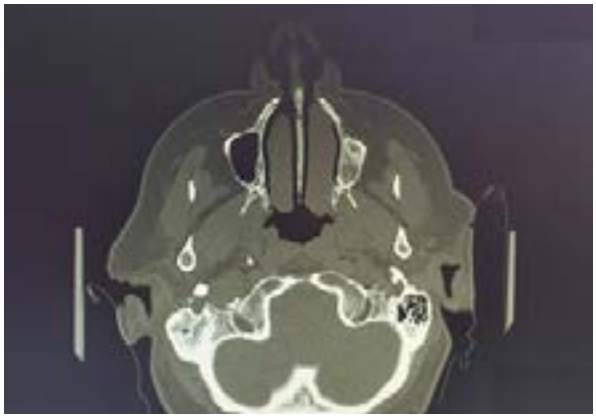

Fig. 3. Hypoplasia of the left maxillary sinus, sclerotic structure of the mastoid process of the right temporal bone

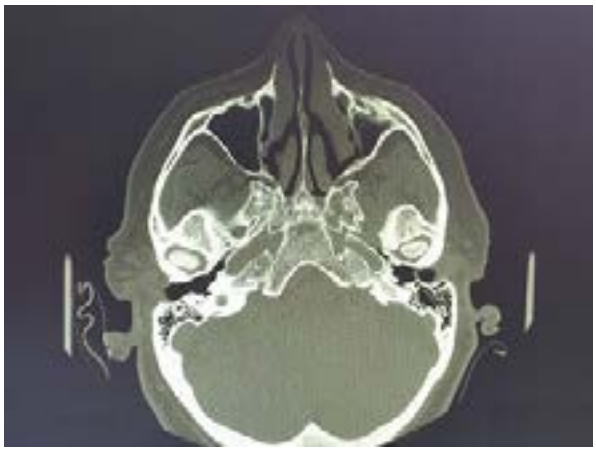

Fig. 4. Aplasia of the right shenoid sinus and the hypoplasia of the left maxillary sinus

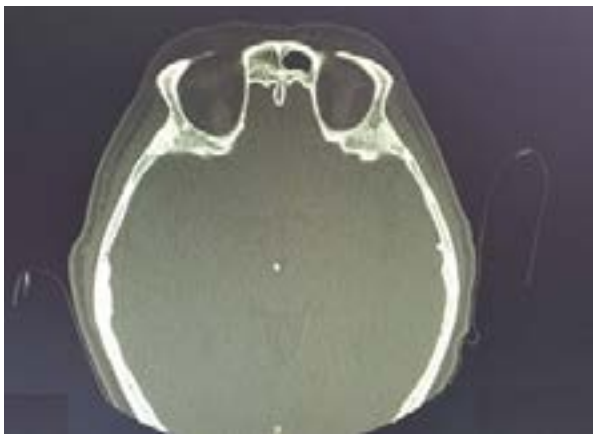

Fig. 5. Aplasia of the right frontal sinus

thickness of the inner lamina of the frontal bone, shenoid and parietal bones (Fig. 4, Fig. 5).

Discussion

Isolated sinus aplasia or hypoplasia is detected quite often and can result from congenital or acquired post-traumatic or post-infectious abnormalities. However, combined or bilateral sinus aplasia or hypoplasia are extremely rare. Agenesis of the frontal sinus is the most common and accounts for 12% of cases. In some populations, it appears at a higher proportion. Furthermore, the configuration and development of frontal sinus and its possible anomalies within each population also depends upon the constitutional (age, gender, hormones and craniofacial configuration) and environmental factors (climatic conditions and local inflammation).[5,6] A bilateral absence and unilateral absence of the frontal sinuses was found in 3.8% and 4.8% of cases, respectively.[7] According to Ozcan et al., combined aplasia of both frontal and maxillary sinuses is the most common radiological and clinical pattern and can have clinical implications for sinus surgery.[8] The findings of aplasia/hypoplasia of the frontal and or sphenoidal sinuses may be part of the spectrum of primary ciliary dyskinesia and this finding should prompt exclusion of this condition.[9] The uniqueness of our case is that the patient is an elderly female with combined aplasia of the frontal and sphenoid sinus with hypoplastic maxillary sinuses, whereas previously reported cases were found in children and in young adults. The sinus anomalies were asymptomatic for many years and were not properly diagnosed. Frontal sinus aplasia is found more often in young women than men.[10] It is not known whether there is a female predilection for frontal and shenoid sinus aplasia. The frontal sinus is absent at birth and develops after the age of 2 years. The frontal sinuses arise from one of several outgrowths that originate in the region of the frontal recess of the nose, and their site of origin can be identified on the mucosa as early as 3 to 4 months in utero. Less commonly, the frontal sinus develops from the anterior ethmoid cells of the infundibulum. Its development is quite variable but the final adult proportions are reached only after puberty. Because the left and right frontal sinuses develop independently, a significant asymmetry between these sinuses can arise in the same individual.[3] This independent development results in more common unilateral aplasia or hypoplasia. The shape, dimensions and limits of the frontal recess are determined by its surrounding structures, and a frontal sinus cannot exist without a recess. When a frontal sinus is agenetic, the contralateral sinus may expand and cross the midline toward the agenetic side, which mimics the presence of bilateral frontal sinuses.[3] Replacing the agenetic frontal sinus with the contralateral sinus ensures the proper drainage system and an asymptomatic presentation of this anomaly. Furthermore, it is not known if the craniofacial changes and asymmetries are determined by real bone asymmetry, or if they appeared as a compensatory mechanism.[11,12] The sphenoid sinus reaches its maximum size by the late teenage years, but shows variation in pneumatisation. Previous case reports have shown that agenesis of the sphenoid sinus occurs in 1–1.5 % of the population.[13] One of the most common possible results of shenoid sinus aplasia or agenesia is chronic headache. On the other hand, shenoid sinus agenesia does not result in facial asymmetry. The maxillary sinus is the first sinus to develop and is usually found to be less pneumatized in the early years of life. Hypoplasia of the maxillary sinus is quite uncommon and often misdiagnosed as chronic sinusitis or neoplasm and is seen unilaterally in 7 % and bilaterally in 2 % of adults.[2] It has been reported in 1.73% to 10.4% of patients with sinus symptoms.[11] However, it is sometimes asymptomatic and is diagnosed using radiological evaluation. Maxillary sinus hypoplasia (MSH) is classified into three types. Type 1 MSH shows mild maxillary sinus hypoplasia, type 2 shows significant sinus hypoplasia with a narrowed infundibular passage and hypoplastic or absent uncinate process, and type 3 is cleft-like maxillary sinus hypoplasia with an absent uncinate process.[14-16] Possible aplasia or hypoplasia maxillary sinus can due to facial changes, especially in the infraorbital area and in dental arch development.[17] Findings such as uncinate process abnormality, orbital enlargement, sphenomaxillary plate, canine fossa elevation, infraorbital fissure enlargement, thickening of the sinus wall and mucosal pathologies can be seen together with maxillary sinus anomalies.[18]

Summary and Conclusions

In conclusion, the low percentage of the frontal sinus agenesis must be taken into consideration during the pre-surgical planning related to the sinus.[19] Therefore, analysing DVT images of the frontal sinus is a useful tool to identify its size and configuration and to minimize the risk factors associated with surgical procedures.[20] Chronic headache, sinusitis and asymmetric craniofacial changes can be associated with paranasal sinus anomalies, but these abnormalities can be asymptomatic for many years.

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CASUISTIC PAPER

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Investigation of focal necrotizing pneumonia after diesel fuel ingestion

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ABSTRACT

Introduction. Diesel oil is a mixture of hydrocarbons. These compounds are widely used in everyday life. Oral exposures are most often accidental and affect mainly children, but they also happen in adults. Oral ingestion may lead to aspiration of pulmonary alveoli which may cause necrotizing pneumonia.

Aim. The aim of the study is to assess te severity of diesel oil intoxication on an example of a presented case.

Methods. The analysis of the clinical patient history and review of available literature.

Results. A 27 year old patient was admitted to the toxicology department due to accidental diesel poisoning. Patient drank a small amount diesel oil, then suffered nausea and vomiting, which resulted in aspiration of diesel to respiratory system. During hospitalization focal necrotizing pneumonia was diagnosed. Patient was treated with intensive specific pharmacotherapy. On the 11th day of stay, the patient was discharged with recommendation of control in the pulmonological and toxicological clinic and chest x-ray examination in order to diagnose the suspicious oval change discovered in the right lobe during hospitalization.

Conclusion. First toxicity symptoms are non-specific, so well collected anamnesis is crucial. Complications of hydrocarbon ingestion can be a threat to patient's life. Due to rarity of the problem, there are no clearly defined treatment guidelines.

Keywords. diesel fuel, intoxication, necrotizing pneumonia

Introduction

Diesel oil is a product of distillation of crude oil mainly consisting of a mixture of aliphatic hydrocarbons with a C9-C24 chain length.[1,2] These compounds are used in everyday life by almost every person. Frequent contact with diesel fuel is a potential source of exposure and poisoning. Oral poisoning with diesel is most often accidental and mainly affects children under 5 years. The frequency of this poisoning is about 1/3 of cases in the USA.[3-5] Among adults, oral diesel intoxication is most

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common in developing countries.[6] Oral poisoning with hydrocarbons may cause pneumonia, which in rare cases can even lead to death (less than 1% of cases).[7] It is rare to drink over 10 ml of hydrocarbons at once because of their unpleasant taste. However, pneumonia may be caused be the aspiration of small dose such as 2 ml to the bronchial tree.[7] The group most exposed to such cases are fire swallowers and workers of petrochemical industry. A common practice used mainly in developing countries is siphoning fuel from the vehicle's tank, which aspirated into oral cavity may contribute to poisoning.[8] Derivatives of crude oil may occur to injury of respiratory, digestive and cardiovascular systems or renal failure associated with multiorgan failure or rhabdomyolysis.[9]

Case report

A 27 year old patient was admitted to the hospital emergency department and then to the toxicology department due to accidental diesel poisoning. During anamnesis, the patient admitted to drinking small amount of diesel. He could not determine the exact volume of consumed toxic. In the pre-hospital conditions, patient suffered nausea and vomiting, which resulted in aspiration of diesel to respiratory system. On the day of admission to the toxicology department, patient was in middle condition. He was conscious, in verbal logical contact, cardiovascular and respiratory efficient. He reported stabbing chest pain in the heart area and increased dyspnea at rest. In the physical examination doctors detected weakened alveolar murmur and characteristic smell of diesel noticeable from the mouth. Laboratory tests showed: leucocytosis- 16400/ul; CRP- 22 mg/l, pCO_2- 53.1 mmHg, pO_2- 25.8 mmHg; saturation- 46.2%. In a performed x-ray of the chest, we observed massive densities on the right side near the heart and a small amount of liquid in the right diaphragm-rib angle (figure 1). To verify the examination computed tomography (CT) was performed. In the CT we observed: in segments 7-10 above diaphragm and in heart area merging densities with hypodens areas, in segments 8-10 parenchymal lesions (ground glass opacity) corresponding to symptoms of necrotizing pneumonia (figure 2).

During 11 days of hospitalization patient required intensive pharmacotherapy. We applied combination of antibiotics (clindamycin and ceftriaxone), steroid therapy and normalized electrolyte balance. Due to increased dyspnea, patient temporally required passive oxygen therapy. As a result of performed therapy, patient's condition improved, disappearance of reported complaints and regression of changes in imaging examinations were observed. Control x-ray of chest showed reduction of parenchymal lesions and rounded cyst of about 4cm. In case of improvement of clinical condition, patient was signed out with recommendation of control in the pulmonological and toxicological clinic and chest x-ray examination after 10 days in order to diagnose the suspicious oval cyst in the right lobe.

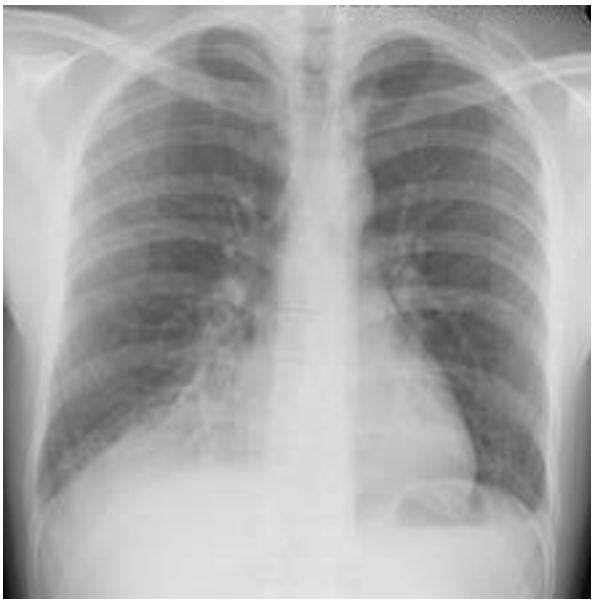

Fig. 1. Posteroanterior chest radiograph demonstrating alveolar infiltrates, more in the right lower zone

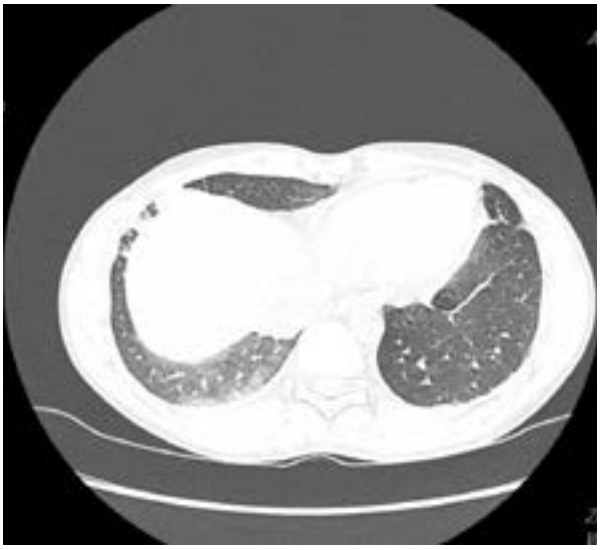

Fig. 2. Computed tomography scan demonstrating right lower lobe consolidation

Discussion

Aliphatic hydrocarbons are well absorbed through the gastrointestinal tract, skin and lungs. Absorption depends on its chemical structure. The longer is carbon chain, the lower is absorption of hydrocarbons.[9] Toxic effects of this substances also depends on their physical properties: viscosity, volatility, solubility and surface tension.[9] The main factor contributing to their aspiration is low viscosity, while the lower the viscosity, the higher risk of aspiration and its penetration to bron-

chial tree.[10] Aspirated oily hydrocarbons do not stimulate cough reflex, but they reach the pulmonary alveoli causing chemical damage to capillaries, interalveolar septum and epithelium. This results in local edema of alveoli and disruption of surfactant secretion.[11] Surfactant, as a compound reducing surface tension, does not fulfil its function. Pulmonary alveoli collapse. This causes disproportions of alveoli ventilation and secondary leads to hypoxia.[12] During the inflammatory reaction, macrophages are activated and inflammatory cytokines are released. This process contribute to the bronchial tree contraction and dysfunction of cilia located in airways.[13,14]

General symptoms of poisoning appear a few hours after aspiration (coughing, dyspnea, choking) and increase from 2 to 8 days.[12] Delayed symptoms result from the lipophilicity of hydrocarbons. These compounds easily penetrate into the fat tissue, where they are accumulated and released over time.[9] Additionally this ability simplify their passage through the cerebral and cellular barriers.[1] Complications of intoxication of diesel oil which worsen patient's prognosis are pleuritis, emphysema, chemical pneumonia, or bacterial infection.[12] A small group of patients may develop cysts, pulmonary abscess, pleural effusion or pulmonary fibrosis.[15] In the case of lung aspiration, the first radiological changes may occur within 2-6 hours after aspiration. In presented case we observed massive inflammatory changes in the right lobe with clinical symptoms of respiratory tract damage. In the control x-ray of chest oval shading (diameter about 4cm) was observed. The image of this change correlated with the picture of post inflammatory cyst of cavity. Mechanism of creating of cavity of cyst after hydrocarbons aspiration is unclear. It is believed that thickened inflammatory wall of alveoli may contribute to accumulation and entrapment of air on the principles of valve mechanism. This may cause creation of pneumatocele. Such phenomenon can also depend on mean time from aspiration to applied treatment, as well as the type and amount of aspirated hydrocarbons.[16]

Due to small number of reports in the literature concerning pneumonia caused by the aspiration of oil derivatives, there are no clearly defined rules of treatment.[17] The main treatment used is symptomatic, aimed at supporting respiratory functions and preventing complications. After hydrocarbon ingestion, giving medical charcoal or gastric emptying is not recommended due to increasing risk of aspiration.[20] When respiratory failure occurs, patients often require respiratory therapy or extracorporeal membrane oxygenation (ECMO) techniques. One of the indications fur using ECMO is severe respiratory failure due to chemical pneumonia.[18] The applied steroid therapy contributes to the reduction of inflammatory response and prevention of pulmonary fibrosis.[9]

Summary

The presented issue of chemical pneumonia does not exhaust the broad symptomatology and numbers of complications associated with crude oil derivatives poisoning. Acute and especially chronic exposure to aliphatic hydrocarbons may lead to multi organ dysfunction, severe condition and death.

Conclusions

- Crude oil derivatives poisoning is extremely dangerous, because even a slight aspiration of these compounds may result in the development of serve complications which may be a serious threat to patient's life.
- First symptoms of intoxication are non-specific. Therefore, it seems crucial to have a properly collected anamnesis and performed fast treatment in order to reduce the risk of developing complications.
- Due to rarity of the problem, there are no clearly defined treatment guidelines, what often complicates and lengthens the healing process of patient.

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CASUISTIC PAPER

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Hyponatremia secondary to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) during the course of lung cancer. A case report

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ABSTRACT

Introduction. Hyponatremia is a frequently observed electrolyte disorder among patients with cancer. In 1957, Schwartz et al. reported the first case of a patient with hyponatremia due to SIADH, secondary to lung cancer. From that moment on, there has been data published that indicates patients with SIADH are less responsive to chemotherapy, have greater predisposition to central nervous system metastases and are often characterized by an advanced stage of cancer during time of diagnosis. Hyponatremia has many possible causes, and the differential diagnosis can pose a challenge.

Aim. The aim of the study was to consider the occurrence of secondary hyponatremia in the course of cancer and the significance of this disorder in the prognosis of the disease.

Methods. An analysis of the clinical history of the patient and a review of available literature.

Results. A 66-year old patient with hyponatremia was admitted to the Department of Endocrinology, and lung cancer was determined as the cause of the aforementioned electrolyte disorder.

Conclusion. SIADH secondary to cancers should be included in a differential diagnosis of every case of hyponatremia of undetermined etiology.

Keywords. hyponatremia, SIADH, lung cancer

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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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Introduction

Hyponatremia is defined as a serum sodium level <135 mEq/l.[1] Hyponatremia is due to impaired renal function and failure to secrete and retain water. Antidiuretic hormone (ADH) affects localized V2 receptors at the basolateral aspect of the collecting duct cells and leads to increased aquaporin expression on the luminal aspect of the collecting duct cells which increases water absorption and diminishes thirst. Normally, thirst and secretion of an antidiuretic hormone depend on plasma osmolality. Hyponatremia occurs if there is persistent ADH stimulation resulting in increased water retention and plasma dilution.[2] Symptoms of hyponatremia depend on the severity of plasma sodium deficiency and the time period in which hyponatremia has established.[2] Acute hyponatremia has a duration of less than 48 hours. In patients with acute hyponatremia, neurological symptoms are observed as a result of the water moving to the brain tissue, according to the concentration gradient, and resulting brain edema.[2] Seizures, speech impairment and even coma or death can also be observed. If hyponatremia persists over 48 hours, it is recognized as chronic. It occurs much more often than acute hyponatremia. The sodium concentration is typically above 120 mEq/L. Chronic hyponatremia is often asymptomatic, since there is enough time for an osmolyte shift (including sodium, potassium and chloride) from the brain cells to the cerebrospinal fluid, which prevents cerebral edema.[3,4] Nonetheless chronic hyponatremia can cause symptoms such as nausea, vomiting and neurological symptoms including fatigue, headaches, confusion, epileptic seizures, especially in the case of sudden serum sodium level decrease. Coma is also probable[5] and there may be subtle neurological abnormalities. Elderly people may experience frequent falls or gait disturbances.[6] Hyponatremia is also observed in the following endocrine disorders: adrenocortical insufficiency and hypothyroidism.[7] It occurs in patients with a decrease in blood volume - for example due to hemorrhage or in chronic diseases characterized by edema, such as in liver cirrhosis or heart failure.[2] The syndrome characterized by excessive secretion of antidiuretic hormone, and is known as a syndrome of inappropriate antidiuretic hormone secretion (SIADH) which was initially described by Leaf and Mamby.[8] SIADH is characterized by hyponatremia, inadequately elevated urine osmolality, significant urine sodium excretion and decline of serum osmolality usually in an euvolemic patient. Peripheral edema is usually absent. The diagnosis requires exclusion of diuretic treatment, and normal cardiac, renal, adrenal, hepatic and thyroid function.[9] Hyponatremia accompanies approximately 30% of hospitalizations and SIADH is the most common cause of hyponatremia.[9,10] SIADH can be caused by various diseases, such as central nervous system disturbances, malignancies like lung tumors, especially small cell carcinoma which produce ADH ectopically, cancers of the pancreas, duodenum, head and neck.[9] Many drugs used in the cancer treatment can also cause SIADH.[9] However, not only drugs used to treat cancer can cause hyponatremia, it is very often caused by thiazides and antidepressants.[11] Pulmonary diseases such as pneumonia or bronchial asthma, atelectasis, acute respiratory failure and pneumothorax can cause SIADH.[9] It can also be observed in tuberculosis.[12] Surgical procedures, such as abdominal and chest surgeries, can cause excessive secretion of ADH, probably by the mechanism of pain receptors stimulation.[9] Also, neurosurgical interventions, especially those performed in the area of the pituitary gland, may result in the development of SIADH syndrome.[9] It was also described in the process of two genetic syndromes: nephrogenic syndrome and hypothalamic syndrome. Nephrogenic syndrome is caused by a gain-of-function mutation in the gene for V2 receptor, which is located on the X chromosome.[13] Hypothalamic syndrome is caused by a mutation in the transient receptor potential vanilloid type 4 (*TRPV4*), which encodes the central osmolality sensing mechanism.[14] Both HIV and AIDS are associated with excessive secretion of the antidiuretic hormone and SIADH syndrome due to existing adrenocortical insufficiency, opportunistic infections and cancer associated with HIV infection.[9] There are studies indicating the importance of hyponatremia as a HIV-disease severity index.[15] Hyponatremia often occurs in older patients with diabetes and during the course of many infections.[11] It is important also to mention idiopathic SIADH, the causes of which remain unknown.[9] As mentioned above, there is a relationship between lung cancer and the occurrence of SIADH due to ectopic ADH secretion. Hyponatremia is a frequent electrolyte disorder among patients hospitalized for cancer diagnosis or treatment.[16] Depending on the type of tumor and the clinical condition of the patient, the incidence of hyponatremia varies.[17] The frequency of hyponatremia is estimated between less than 1% to more than 40% due to reports from general hospitals.[18] Large group of patients have showed that SIADH occurs in in 15% of cases of small-cell lung cancer.[19] 2% -4% refer to patients with non-small cell lung cancer.[20,21] A common cause is abnormal, ectopic release of antidiuretic hormone (SIADH), independent of tonicity maintained by non-osmotic factors.[20] In 1957, Schwartz et al. described the first case of a patient with hyponatremia due to SIADH, secondary to lung cancer.[22] Chute et al. stated that the patient with SIADH is less responsive to chemotherapy, has greater predisposition to central nervous system metastases and is often characterized by advanced stage of cancer at the time of diagnosis.[23] To illustrate this data, we would like to present a case of a patient with hyponatremia, in whom lung cancer was

detected in the course of the differential diagnosis of the etiology of low serum sodium level.

A case report

66 year old patient was admitted to the Endocrinology Department due to electrolyte disorders in the form of chronic hyponatremia of unknown etiology. Patient's medical history included post-operative hypothyroidism, hypertension, ischemic heart disease and primary Sjögren's syndrome. She was admitted to the Department of Endocrinology for further evaluation of the causes of hyponatremia, after previous hospitalization in the Department of Gastroenterology, in the time of which the thyroid function disorders and adrenal insufficiency were ruled out as potential causes of electrolyte disorder. During fifteen days of hospitalization, a number of laboratory tests were carried out. It was found that hyponatremia still persisted at a moderate level; hypothyroidism (due to inadequate substitution dose of L-thyroxine) and adrenocortical insufficiency were again excluded. The results of the study also showed normal diuresis, decreased plasma osmolality, normal urine osmolality, sodium excretion in the daily urine collection higher than 30mmol/l, and decreased urea and uric acid levels.

This clinical and laboratory presentation is very characteristic of SIADH syndrome. As mentioned above, SIADH often has a paraneoplastic etiology, therefore it was decided to extend diagnostics procedures accordingly. In course of the previous hospital stay, the patient had a chest x-ray, which did not show any pathology. At the time of hospitalization in the Clinic of Endrocrinology, a CT scan of the neck, chest and abdomen were performed. The thyroid ultrasound visualized a bundle of cervical lymph nodes with central vascularization on the right side, however CT did not confirm the presence of enlarged lymph nodes in the neck. The CT scan however, did show mediastinal lymph node infiltrates with present necrosis and associated mass in the upper lobe of the right lung, as well as involvement of supraclavicular and subclavian lymph nodes. The CT of the abdomen also showed the bilateral presence of focal lesions of the adrenal glands of benign phenotype, with high lipid content, suggestive of adenomas. Hormonal evaluation was performed that revealed no pituitary-adrenal axis disturbances. Due to the the cause of observed SIADH syndrome the Patinet was reffered to the Pulmonology Department for a more extensive evaluation (to determine the type of neoplasm and staging). In process of further patient hospitalization, endobronchial ultrasound (EBUS), the material for histopathological examination was collected. The histopathological evaluation of the material collected from the outbreak and the lymph nodes found in the imaging studies revealed small-cell lung cancer. After the diagnosis, appropriate treatment was initiated.

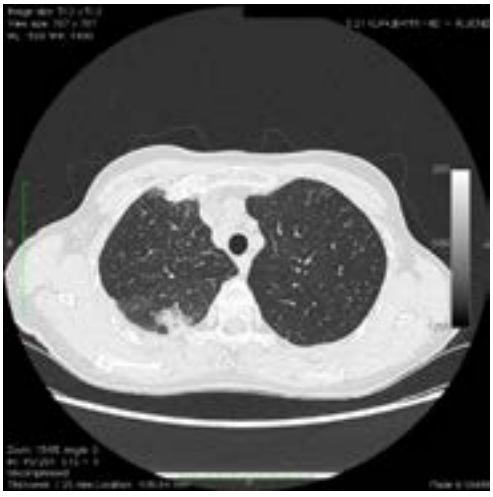

Fig. 1. Computed tomography (CT) of the patient's chest.

Discussion

Hyponatremia is the most frequent electrolyte disorder in oncological patients.[24,25] Such a condition can be the result of tumor antidiuretic hormone (ADH) production. Also treatment with such agents as vincristine, vinblastine and cyclophosphamide used in the treatment of lung cancer can also be the cause of hyponatremia.[26] Cyclophosphamide enhances the action of ADH at the renal tubule level. Stimulation of ADH secretion can also be stimulated by phenothiazines used as antiemetics medicaments, antidepressants- such as tricyclic drugs and selective serotonin reuptake inhibitors (SSRI), and

Table 1. The results of basic tests

WBC	RBC	HCT	PLT	pH	pO_2
$3.99 \times 10^3/\mu L$	$3.8 \times 10^6/\mu L$	33.1%	$305 \times 10^3/\mu L$	7.41	90.6 mmHg

pCO_2	CRP	Cholesterol	Creatinine	TSH	Cortizol
36.4 mmHg	0.964 mg/L	199 mg/dL	0.5 mg/dL	2.118 mIU/L	192.40 µg/24h

Table 2. Laboratory results of tests performed to diagnose SIADH

Sodium concentration in serum	The value of sodium in the daily collection of urine	Serum osmolality	Urine osmolality
128 mmol/L	72 mmol/L	254 mOsm/kg H_2O	375 mOsm/kg H_2O

opioid analgesics.[26] Nausea and vomiting after chemotherapy can also result in hyponatremia. Congestive heart failure seems to be associated with hypovolemic hyponatremia, which complicates the treatment of oncological patients using anthracyclines.[27] This electrolyte disorder can also be observed in patients suffering from paraneoplastic syndrome, like nephrotic syndrome or renal minimal change disease.[28] Hyponatremia may also be a final result of cancer metastases to the central nervous system or adrenal cortex, leading to adrenal insufficiency. Hyponatremia, as mentioned above, can serve as a marker for the disease severity. Hyponatremia has been shown to be an independent prognostic marker in oncological patients.[29,30] Among patients with lung cancer, hyponatremia is a negative prognostic factor at the time of hospital stay and in patients with erlotinib-based treatment regiments.[31,32] It should be taken into account that the prognostic value of hyponatremia may vary depending on such factors as: the type of tumor, the severity of the disease and the initial treatment of hyponatremia. The worst prognosis concerns the following cancers: small cell lung cancer (SCLC), mesothelioma, gastrointestinal cancer, renal cell carcinoma and lymphoma.[33,34,35,36,37] Rapid correction of serum sodium correlates with longer overall survival and improvement of clinical condition.[38] Petereit et al. conducted a study in 10 patients diagnosed with SCLC and SIADH. Patients were selected based on the histologically confirmed diagnosis of SCLC and the clinical picture of neurocognitive deficit induced by SIADH- associated hyponatremia. All patient data were monitored for clinical improvement based on ECOG (fitness scale according to the Eastern Cooperative Oncology Group) status, time of chemotherapy initiation and sodium levels correction. The treatment was conducted according to the diagnostic and therapeutic algorithm. It led to effective correction of both clinical problems and sodium level in peripheral blood plasma. Patients started chemotherapy treatment at the same time. Then were treated with tolvaptan which led to performance status improvement based on the ECOG score. All patients benefited from the effective treatment of SIADH, avoiding long-term hospitalization. It has been shown that the serum sodium normalization failure after the chemotherapy initiation is a negative prognostic factor.[39] Hyponatremia seems to be in negative correlation with the treatment efficacy.[40] The management of any cancer patient should always take into account an evaluation of sodium level.

Summary

The possibility of SIADH as a paraneoplastic syndrome should be considered in patients with hyponatremia of undetermined etiology. In the case of abnormal sodium concentrations, extensive differential diagnosis should be performed. SIADH can be caused by ectopic neoplastic ADH production, be connected to ADH secretion stimulation or even the result of AVP (the antidiuretic hormone arginine vasopressin) due to anticancer therapy or palliative treatment. Studies have shown that hyponatremia has a prognostic value in groups of patients diagnosed with lung cancer. It is crucial to gradually and effectively correct the hyponatremia, keeping in mind that the process should be spread out over time.

Conclusion

SIADH secondary to different types of malignant neoplasm should be included in a differential diagnosis of every case of hyponatremia of undetermined etiology. Imaging studies and tumor markers evaluation are an important part of diagnostic procedures. Correction of the serum sodium level improves the patient condition.

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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.
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