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ORIGINAL PAPER

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Risk factors for overweight and obesity in pre-school children

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ABSTRACT

Introduction. Obesity in children and adolescents is a growing problem in the 21st century. The epidemic of chronic non-communicable diseases resulting from obesity is currently one of the biggest problems of modern medicine. Excessive body weight is the result of a long-lasting imbalance between the amount of energy supplied and its expenditure. Energy regulation of the body is subject to both genetic and environmental factors. Among other things, due to this, the problem of excessive body weight is most severe in societies with a high degree of socio-economic development. The aim of this paper is to determine the influence of selected environmental and social factors on the occurrence of overweight and obesity in pre-school children.

Material and methods. The study included pre-school children from south-eastern Poland. After obtaining the consent from parents, 200 children (87 boys, 113 girls) aged 3 to 6 years were examined. A questionnaire used for the research was derived from the program: European Pilot Study Evaluating the Influence of Local Promotional Activities on Prevention of Obesity in Pre-school Children. In the subjects, body weight was measured on an electronic scale three times and the body height was measured three times using a stadiometer. Obesity was determined according to the criteria developed by the International Obesity Task Force (IOTF).

Results. The prevalence of overweight and obesity among children amounted to 6.4% in 3-year-olds, 11.3% in 4-year-olds, 17.7% in 5-year-olds, and 20.7% in 6-year-olds. A factor significantly increasing the risk of obesity among the examined girls and boys was the mother's BMI index.

Conclusion. Although knowledge about the factors that promote overweight and obesity is common, it is still a common health problem. Particular attention should be paid to the prevention of obesity in children of parents with a BMI above 30 kg m^2. Early maternal education can change the lifestyle of the whole family.

Keywords. child obesity, inheritance of obesity, causes of obesity

Introduction

Child obesity is a progressive global problem. In 1975, the average BMI of children aged 5 - 19 years was 17.2 kg/m^2. The increase in BMI by 2016 was, on average, 0.32 kg/m^2 per decade for girls and 0.40 kg/m^2 for boys. The smallest increase in BMI was found in south Asia and East Africa. The largest occurred in Polynesia and Micronesia.[1] There were 42 million children with ex-

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cessive body mass worldwide in 2013, of which 31 million reside in highly developed countries.[2] In the US, the number of obese children has tripled in the last 30 years. Up to 9% of children are obese in pre-school age. In addition, it has been observed that nutrient deficiencies such as iron and zinc accompany increasing body weight.[3,4]

Obesity of children has serious health consequences. Adipose tissue induces inflammation resulting in hypertension, dyslipidemia, heart disease, asthma, sleep apnea, and inflammation of the joints. Other diseases associated with obesity include insulin resistance, type 2 diabetes, non-alcoholic fatty liver and orthopedic problems resulting from excessive joint load.[4,5] Shashaj et al. studied 219 pre-school children with excessive body mass in 2014. Twenty-four per cent were diagnosed with dyslipidemia, 31% with hepatic steatosis, and 35% with insulin resistance. Changes in health appear soon after weight gain.[6] Research by Freedman et al. showed that obese children are more likely to become obese adults. Excessive body weight in childhood affects the state of health in later years. Obese children are four times more likely to develop type 2 diabetes, as adults, even if they normalize body weight in adulthood. Inflammation induced in childhood by adipose tissue results in increased CRP in adulthood and greater chances of developing metabolic diseases.[8,9] In addition, children with excessive body mass are characterized by low self-esteem and low self-confidence, which may result in poor academic performance. Behaviors discriminating against obese children have already been diagnosed in two-year olds.[10,11]

The causes of obesity can be different: socio-economic, environmental, genetic or behavioral. The excess energy consumed with food, exceeding the energy expenditure of the body should be considered the direct cause of fat accumulation. Consuming highly processed, high-energy food such as sweets, salty snacks or fast-food as well as spending free time in front of a TV or computer screen are the causes of the accumulation of excess fat tissue.[13] Another cause of obesity is the genetic factor. Heredity of body mass and genetic susceptibility to the environmental impact is significant. Marques-Lopes et al. determined that the influence of genes on the mechanisms of energy accumulation in the form of adipose tissue amounts to 40%.[14] The researchers are focusing on variants of obesity-promoting genes, e.g. (FTOrs9939609), melanocortin-4 (MC4R) and FLJ35779 (rs2112347) receptors more and more frequently. Along with the development of genetics, obesity risk diagnostics may take place in the early years of life.[13]

Dysfunction of the body's energy homeostasis may also result from medical reasons such as:

– disorders within the central nervous system (CNS): hypothalamus diseases, tumors and CNS inflammatory conditions, intracranial hypertension, neurological disorders, Froehlich syndrome, Blount syndrome,
– endocrinopathies: hypothyroidism, Cushing's syndrome, growth hormone deficiency, hyperinsulinism, polycystic ovarian syndrome, alleged hypoparathyroidism, hypogonadotropin hypogonadism, status post ovarectomy,
– genetical syndromes: Turner, Klinefelter, Willi-Prader syndromes, Dercun's disease, Lawrence-Moon--Biedl disease,
– drug-induced obesity, such as: neuroleptics, antidepressants, phenothiazine derivatives, antiepileptic drugs, steroids and glucocorticoids.[15]

Children with excessive amounts of adipose tissue become adults susceptible to civilization diseases to a greater extent than those with normal body mass. Therefore, prophylactic measures such as nutritional education should be introduced both at parents during pre-conception and later as well as in pre-school children.

Aim of the study

The aim of this study was to determine the impact of selected environmental and social factors on the prevalence of overweight and obesity in pre-school children.

Material and methods

The study covered children from kindergarten, in which we obtained the consent of the director of the facility and parents who were also asked to complete the questionnaire. The survey was taken from the program: European Pilot Study Evaluating the Impact of Local Promotional Activities on Prevention of Obesity in Preschool Children. The consent to use the survey was received from the coordinator of the above-mentioned program. In total, the study covered 200 children, including 87 boys and 113 girls, aged from 3 to 6 years. In children, the body weight was measured on an electronic scale three times and the height was measured using a stadiometer. Preschoolers were weighed and measured without shoes, in underwear. The BMI was calculated from the obtained measurements. Obesity was determined based on criteria developed by the International Obesity Task Force (IOTF).[16] The parents' body mass index was determined on the basis of their body weight and height obtained from the measurement on the weight and using the stadiometer. The calculated BMI was classified according to the WHO criteria.[17]

In order to examine the influence of risk factors on the occurrence of obesity, odds ratio (OR) for each factor was calculated assuming the significance of differences at the level of $p < 0.05$. The statistical analysis was carried out using the R software (version 2.13.1) and an

Excel spreadsheet. The differences were tested using the chi-square test (for qualitative variables), the student's t-test for quantitative variables and the Wilcoxon test for non-parametric variables. Using the ×2 test, the differences in the risk of obesity were verified in the group of children subjected to the given factor in relation to the group of children without the obesity.

Results

Table 1 presents the general characteristics of the examined children. The boys had a slightly higher average body weight (19.40 kg) than girls (19.05 kg). The mean BMI values for boys were 15.99 kg/m^2 and for girls 15.32 kg/m^2.

When analyzing Cole's coefficient, no obesity was found in 3-year-old preschoolers (Table 2). Among boys at this age, overweight accounted for 6.5%, while for girls 6.2%. In the study group, 4-year-old girls did not experience obesity, while in boys it was 11.4%. Overweight at this age occurred in 2.5% of boys and 7.4% of girls. In the group of 5-year-old girls obesity most often occurred at the level of 12.5%, while in boys it was 3.3%. In 6-year-olds, obesity occurred only in boys and accounted for 10%. Overweight in 6-year-old boys was at 15%, and in girls - 11.1%. Analyzing the average body weight values in children, it was found that overweight and obesity was more common in boys than in girls. Among the male subjects, overweight constituted 9.5%, while in the female subjects it was 6%. Obesity in boys was found in 6% and in girls in 4.7%. The body mass deficit in girls was found in 22.6%, and in boys slightly less, 22.4%.

Analyzing the body mass (p = 0.037) and the mothers' BMI (p = 0.012) and the child's BMI (p = 0.001) and body mass (p = 0.001), significant relationships were found (Table 3). The mean body mass of mothers with normal BMI index was on average 61.54 kg, and in children with overweight and obesity 65.81 kg. The mean BMI values in mothers of children who had normal body mass amounted to 22.31 kg/m^2, while in mothers of children with overweight and obesity 24.09 kg/m^2.

According to the data from table 4, the following factors were statistically significant among boys: birth weight of a child p = 0.0026, maternal BMI p = 0.0489, birth body length p = 0.016. Mean maternal BMI values of boys with normal body mass amounted to 22.39 kg/

Table 1. General characterictics of the studied children

Parameter	Total		Boys		Girls		P
	N	X± SD	n	X± SD	N	X± SD	
Age (years)	200	4.37 ± 1.00	116	4.34 ± 1.05	84	4.40 ± 0.92	0.6329
Child's body mass [kg]	200	19.25 ± 3.59	116	19.40 ± 3.59	84	19.05 ± 3.59	0.4987
Child's body height [m]	200	1.11 ± 0.09	116	1.10 ± 0.09	84	1.11 ± 0.08	0.3474
Child's BMI [kg/m^2]	200	15.71 ± 2.09	116	15.99 ± 2.20	84	15.32 ± 1.87	0.0247
Centile according to Cole*	200	3.84 ± 0.99	116	3.84 ± 1.04	84	3.82 ± 0.91	0.8000

N – number of subject studies; (X ± SD) – arithmetic mean ± standard deviation, p < 0.05 differences statistically significant.
* 1,2,3 underweight, 4 normal, 5 overweight, 6 obesity.

Table 2. The incidence of body mass disorders in the studied children according to Cole

	Boys				p	Girls				p	Total		p
											B*	G*	
Age (years)	3	4	5	6		3	4	5	6		4.34 ± 1.05	4.40 ± 0.92	
Underweight (%)	25.8	17.1	30	15	0.392	12.5	22.2	21.9	44.4	0.542	22.4	22.6	0.17
Normal (%)	67.7	68.6	50	60		81.2	70.4	62.5	44.4		62.1	66.7	
Overweight (%)	6.5	2.5	16.7	15		6.2	7.4	3.1	11.1		9.5	6	
Obesity (%)	0	11.4	3.3	10		0	0	12.5	0		6	4.7	

*B – Boys, G – Girls, p < 0.05 differences statistically significant.

Table 3. Selected parameters affecting the prevalence of overweight and obesity in preschoolers

Parameter	Children with normal body mass		Children with overweight and obesity		p
	N	X±SD	N	X±SD	
Child's sex: 1-boy, 2-girl	128	1.44 ± 0.50	27	1.33 ± 0.48	0.324
Mother's age [yrs]	128	32.96 ± 4.19	27	35.19 ± 5.68	0.128
Father's age [yrs]	128	35.13 ± 4.93	27	36.74 ± 5.35	0.136
Mother's height [cm]	128	166.02 ± 4.94	27	165.30 ± 6.36	0.566
Mother's body mass [kg]	128	61.54 ± 8.15	27	65.81 ± 9.51	0.037
Mother's BMI [kg/m²]	128	22.31 ± 2.70	27	24.09 ± 3.31	0.012
Father's height [cm]	128	179.04 ± 6.31	27	178.22 ± 5.73	0.312
Father's body mass [kg]	128	86.09 ± 12.30	27	85.59 ± 9.01	0.895
Father's BMI [kg/m²]	128	26.82 ± 3.26	27	26.95 ± 2.68	0.977
Mother's gestational weight gain	123	14.06 ± 4.40	23	15.83 ± 5.81	0.208
When was the child born: 1-preterm, 2-on time, 3-postterm	128	2.02 ± 0.51	27	2.19 ± 0.40	0.120
Child's body mass at birth [g]	126	3401.83 ± 499.51	25	3592.40 ± 428.40	0.087
Child's body length at birth [cm]	126	54.79 ± 3.02	25	56.32 ± 2.73	0.010
Was the child breast fed: 1-no, 2-don't know, 3-yes	128	2.88 ± 0.49	27	2.93 ± 0.38	0.603
How long was the child breast fed: 1-less than 1 month, 2-up to 3 months, 3- up to 6 months, 4-up to 9 months, 5-more than 1 year	119	3.08 ± 1.20	26	3.04 ± 1.08	0.753
Child's body mass [kg]	128	18.97 ± 2.70	27	23.74 ± 4.87	<0.001
Child's body height [cm]	128	1.10 ± 0.08	27	1.10 ± 0.10	0.782
Child's BMI [kg/m²]	128	15.71 ± 0.98	27	19.50 ± 1.97	<0.001
Mother's BMI: 1-underweight,2-normal, 3-overweight, 4-obesity	128	2.13 ± 0.40	27	2.30 ± 0.54	0.106
Father's BMI: 1-underweight,2-normal, 3-overweight, 4-obesity	128	2.82 ± 0.67	27	2.81 ± 0.68	0.967
Child's body mass: 1- below 2500g,2-2500g to 3500g, 3-3500g to 4000g 4- over 4000g	128	2.50 ± 0.80	25	2.84 ± 0.75	0.042
Centile according to Cole: 1,2,3 underweight, 4 normal, 5 overweight, 6 obesity.	128	4.00 ± 0.00	27	5.41 ± 0.50	<0.001

N – number of subjects; X ± SD – arithmetic mean ± standard deviation; p < 0.05 differences statistically significant

m², and mothers of boys with overweight and obesity 23.74 kg/m². Body weight of boys with normal BMI was 19.33 kg, while boys with overweight and obesity 23.17 kg. Birth length was statistically significant in boys. Boys with normal body mass at birth were 54.73 cm and with an excess of 56.69 cm. Among girls, the maternal body weight p = 0.0237 and mother's BMI p = 0.0171 turned out to be a statistically significant parameter. Mothers of overweight and obese girls increased their body weight during pregnancy to an average of 18.00 kg, while mothers of girls with normal body mass, took on average 13.54 kg. The mean BMI values of girls' mothers with excessive body weight were 24.81 kg/m², while those with normal body weights - 22.21 kg.

The quotient of obesity risk in children for overweight and obese mothers, and normal weight mothers is OR = 21.64 (Table 5).

The relationship between environmental and nutritional factors influencing the incidence of overweight and obesity in preschoolers was also checked (Table 6). Not all of the analyzed parameters were statistically significant. A whole group of children ate meals prepared in kindergarten at the same frequency, regardless of whether they were breakfasts, dinners or afternoon teas. Both parents of overweight or obese children and parents of children with normal body mass allow their offspring to consume fast-food dishes less than once a month. The question concerned both replacing main

Table 4. Selected anthropometric parameters affecting the occurrence of overweight and obesity in boys and girls

Parameter	Boys with normal body weight		Boys with overweight and obesity		p	Girls with normal body weight		Girls with overweight and obesity		p
	N	X ± SD	N	X ± SD		N	X ± SD	N	X ± SD	
Mother's body mass [kg]	72	61.44 ± 8.28	18	64.28 ± 7.86	**0.187**	56	61.66 ± 8.06	9	68.89 ± 12.12	**0.0237**
Mother's BMI [kg/m²]	72	22.39 ± 2.80	18	23.74 ± 2.39	**0.0489**	56	22.21 ± 2.59	9	24.81 ± 4.74	**0.0171**
Father's body mass [kg]	72	86.01 ± 12.45	18	86.56 ± 8.12	**0.8234**	56	86.18 ± 12.20	9	83.67 ± 10.84	**0.5633**
Father's BMI [kg/m²]	72	26.73 ± 3.19	18	27.10 ± 2.74	**0.6274**	56	26.93 ± 3.37	9	26.66 ± 2.70	**0.8194**
Estimated gestational weight gain [kg]	71	14.44 ± 4.40	15	14.67 ± 5.69	**0.8844**	52	13.54 ± 4.39	8	18.00 ± 5.76	**0.0129**
Body mass at birth [g]	71	3452.54 ± 537.94	16	3662.50 ± 439.48	**0.1104**	55	3336.36 ± 441.25	9	3467.78 ± 401.43	**0.4055**
Body length at birth [cm]	71	54.73 ± 3.08	16	56.69 ± 2.65	**0.016**	55	54.87 ± 2.96	9	55.67 ± 2.92	**0.4572**
Do you think your child is*	72	3.47 ± 0.95	18	3.78 ± 0.88	**0.2047**	56	3.54 ± 0.87	9	4.44 ± 0.73	**0.0044**
Which figure does your child have	72	2.19 ± 0.99	18	2.67 ± 1.37	**0.1835**	56	1.96 ± 0.97	9	2.89 ± 1.45	**0.0165**
Child's age [yrs]	72	4.25 ± 1.06	18	4.78 ± 1.00	**0.0586**	56	4.27 ± 0.90	9	4.67 ± 0.87	**0.2216**
Child's body mass [kg]	72	19.33 ± 2.78	18	23.17 ± 4.54	**0.0026**	56	18.51 ± 2.53	9	24.89 ± 5.58	**<0.0001**
Child's height [m]	72	1.10 ± 0.08	18	1.09 ± 0.11	**0.7457**	56	1.10 ± 0.07	9	1.12 ± 0.07	**0.3344**
Child's BMI [kg/m²]	72	16.04 ± 0.95	18	19.54 ± 2.05	**<0.0001**	56	15.28 ± 0.84	9	19.44 ± 1.91	**<0.0001**
Mother's BMI **	72	2.17 ± 0.41	18	2.22 ± 0.43	**0.6211**	56	2.07 ± 0.37	9	2.44 ± 0.73	**0.0544**
Father's BMI **	72	2.75 ± 0.67	18	2.83 ± 0.71	**0.6526**	56	2.91 ± 0.67	9	2.78 ± 0.67	**0.5819**

N – number of subjects; (X± SD) – arithmetic mean ± standard deviation; p < 0.05 differences statistically significant
* 1 very slim, 2 slim, 3 slightly slim, 4 normal body weight, 5 slight overweight, 6 overweight, 7 obese, **1 underweight, 2 normal, 3 overweight, 4 obese

Table 5. Odds ratio for selected biological risk factors for obesity in girls

Parameter	OR	95% CI	p
A mother with overweight and obesity / mother with normal weight	21.64	1.49 – 1271.77	0.0100
Body mass at birth > 4000 g ≥ 2500 g i < 3500 g	6.82	0.076- 603.87	0.2619

OR – odds ratio; CL – confidence interval; p – statistical significance level; p < 0.05-differences statistically significant

meals with fast food and eating them as an additional meal. As the least liked product, both groups of children enumerated legumes, while their favorite food products included sweets in the first place. Parents of both groups of children determined that children eat meals watching TV on average twice a week. The time of watching television is about 1 hour a day, while on Saturdays and Sundays it is extended to 2 hours a day. The amount of time devoted to watching TV did not affect the weight of children. Similarly, the amount of body fat was not affected by the time spent on computer games. On weekends children spent a little more time on computer games than on weekdays. While on working days, parents often declared that the child plays less than an hour or not at all, during the weekend this time extended to 1 hour. Obese children were more likely to eat fish than normal body mass children, but less often legumes, milk, cheese and fruit. Children with normal

Table 6. Environmental and nutritional parameters influencing the incidence of overweight and obesity in preschoolers

Parametrs	Children with normal body mass		Children with overweight and obesity		p
	N	X±SD	N	X±SD	
How often does a child eat breakfast in the kindergarten: 1 – never,2 – 1 once a week, 3 – several times a week, 4 – every day	127	3.89 ± 0.44	27	3.85 ± 0.60	0.9592
How often does a child eat dinner in the kindergarten: 1 – never,2 – 1 once a week, 3 – several times a week, 4 – every day	128	3.89 ± 0.49	27	3.89 ± 0.42	0.8289
How often does a child eat evening tea in the kindergarten: 11 – never,2 – 1 once a week, 3 – several times a week, 4 – every day	127	2.76 ± 1.42	27	2.93 ± 1.41	0.5696
How often do you take your child to have fast-food for dinner or supper: 1 – never, 2 – once a month, 3 – several times a month, 4 – 1 to 2 times a week, 5 – 3 or more times a week	128	1.25 ± 0.47	27	1.52 ± 0.80	0.0932
How often do you take your child to have fast-food for a snack between meals: 1 – never, 2 – once a month, 3 – several times a month, 4 – 1 to 2 times a week, 5 – 3 or more times a week	128	1.64 ± 0.51	27	1.85 ± 0.72	0.1887
Child likes eating the best: sweets	128	0.37 ± 0.48	27	0.30 ± 0.47	0.4856
Child dislikes: legumes	128	1.00 ± 0.00	27	1.04 ± 0.19	0.0295
How often does your child eat watching TV/playing games: 1 – never/rarely, 2 – a few times a week, 3– once a week, 4 – a few times a day	128	1.95 ± 0.98	27	2.00 ± 1.11	0.9581
How often does your child watch TV a day on week days: 1– never, 2 – less than 1h a day, 3 – 1h to 2 h a day, 4 – 2 to 3h a day, 5 – more than 3h	128	2.76 ± 0.71	27	2.59 ± 0.75	0.2863
How often does your child watch TV a day at the weekend: 1– never, 2 – less than 1h a day, 3 – 1h to 2 h a day, 4 – 2 to 3h a day, 5 – more than 3h	128	3.48 ± 0.83	27	3.41 ± 0.84	0.6373
How often does your child play computer games a day: 1– never, 2 – less than 1h a day, 3 – 1h to 2 h a day, 4 – 2 to 3h a day, 5 – more than 3h	128	1.43 ± 0.62	27	1.59 ± 0.64	0.1560
How often does your child play computer games a day at the weekend: 1– never, 2 – less than 1h a day, 3 – 1h to 2 h a day, 4 – 2 to 3h a day, 5 – more than 3h	128	1.77 ± 0.85	27	2.15 ± 0.95	0.0373
Child likes eating the best: sweets	128	1.03 ± 0.17	11	1.18 ± 0.40	0.0188
Child dislikes: legumes	128	1.00 ± 0.00	11	1.09 ± 0.30	0.0006
Child likes eating the best: milk and cheese	128	1.29 ± 0.46	11	1.00 ± 0.00	0.0381
Child dislikes: fruit	128	1.04 ± 0.19	11	1.18 ± 0.40	0.0384
Child attends sport classes: No. of days a week	35	1.66 ± 0.97	4	1.00 ± 0.00	0.0003

N – number of subjects; (X ± SD) – arithmetic mean ± standard deviation; $p < 0.05$ differences statistically significant

body mass more often participated in sports activities than children with obesity. In children with excessive adipose tissue in the week, it was only one day and in children with normal amount of adipose tissue on average 1.6 days.

Discussion

With age, the likelihood of overweight or obesity increases.[6] In studies by Shashaj et al., overweight in children aged 2 years was 7% and in children aged 5 it was 16.9%. Obesity at the age of 2 years is 1.1% and by the age of 5 was already 2.9%. A similar tendency was observed by Majcher A. et al.[18] examining adolescents with obesity and their previous developmental history. At the age of 2, 53% of them were overweight or obese, at the age of 4 this rose to 71.6%, while at the age of 6, to as much as 85.4%. A similar tendency is presented by a study of population in kindergartens in south-eastern Poland. Overweight children at the age of 3 accounted for 6.35% of the total, and at the age of 6, 13.05%. Obe-

sity in the group of 3 year-olds did not occur, and in 6-year-old children it was 5%.[6] This dependence can be explained by a longer time of exposure to environmental factors such as excessive energy consumption from food and too little physical activity.[15] For this reason, it is necessary to monitor the child's body weight at various stages of development and to introduce nutritional education from the earliest years. Prevention of obesity in children cannot focus only on children, but should cover the whole family. Children usually eat the foods offered them by caregivers, which means that exclusive education of children is less effective.[19]

It can be suggested that parents are responsible for the weight of their children also before they are born. Animal studies showed that the BMI of their descendants was much higher when mothers were fed unhealthy, high-calorie foods during pregnancy.[20] For this reason, mothers from south-eastern Poland, who had gestational weight gain on average 18 kg gave birth to children who later developed excessive body mass, while those who increased body weight by 13.54 kg gave birth to children with normal body mass at a later stage of development. Improper nutrition of women during pregnancy resulted in excessive weight gain during this period, which influenced the development of obesity in their offspring. It has also been proven that the choice of food for pregnant women influences the nutritional preferences of their children after birth. Mennella A. et al.[21] observed in pregnant women the following relationship: children of women who during the pregnancy drank large amounts of carrot juice, at the first administration of carrots were less grimacing compared to children whose mothers did not drink carrot juice. The breastmilk is another factor responsible for the predispositions of a baby to become overweight at an early stage of their lives. Numerous studies show that naturally-fed children are less likely to be obese.[22] However, this correlation was not found in this study. Equally often children fed with natural milk and artificial milk had excessive or normal BMI. Statistically significant was the relationship between the body mass of mothers and their children. In the study group from the south-east of Poland it was found that mothers of children with excessive body mass have a higher BMI than mothers of children with normal body mass. The question whether the dependence of the excessive weight of the parents and their descendants results from the inheritance of the tendency to gain weight or eating habits remains an issue. We can look for genetic predispositions to obesity, however, Lobstein et al. claims that there is no consensus on the extent of genes and the environment in the formation of obesity. Genetic factors indicate population studies, which show that obese parents more often than slim parents have obese children. If both parents are obese, 2/3 of their children will be obese, if one of the parents is obese, nearly half of their children will be fat. Genetic predisposition leads to excessive accumulation of adipose tissue when specific environmental conditions occur.[23] On the other hand, Mazur et al. suggests that the increasing prevalence of obesity in children and adults in recent years should be explained not by genetic changes but by environmental factors. In his research, the author states that obesity in fathers significantly increases the risk of obesity in offspring, but to a lesser extent than maternal obesity.[24] It can be assumed that the unhealthy eating habits of mothers resulted in improper feeding of the children, which is reflected in the weight of the body. Myles et al. in their review showed that in 19 out of 22 studies, heredity of eating behaviors has been proven[25] Studies reveal that it is mainly mothers who are responsible for the selection of foods in the home diet. Obese mothers are mainly focused on the family, have excessive control over the children and are overprotective towards them, overfeeding them.[26]

This paper confirms the greater impact of maternal obesity than father's obesity on the increased amount of adipose tissue in a child. This trend is also confirmed by the study of Portela et al., who claims that maternal obesity is the most frequent risk factor for the formation of excessive body mass in children aged 6 years.[27] The risk of obesity in a child in correlation with obesity and overweight of the mother was higher in girls than in boys. A statistically significant relationship was found between maternal obesity and daughter obesity. The odds ratio for this factor was OR = 21.64. Perez-Pastor et al.,[28] estimate that the obese mother is ten times more likely to have an obese daughter than a mother with a normal body weight. It is noted that the overweight of the mother affects the weight of the daughter, not the son. However, the an overweight father affects the weight of the son, not the daughter. Studies[28] conducted on 226 families show that in women with obesity up to 41% of daughters aged 8 years showed body mass that was too high. However, in women with normal body mass, only 4% of obese daughters were present. Among boys, these proportions are smaller; obese fathers have about 18% of sons with excessive body mass, while in fathers with normal BMI, sons with overweight and obesity are only 3%. In both cases, the researchers did not detect the relationship between mother-son and father-daughter. They argue that the inheritance of obesity from mother to daughter and from father to son has nothing to do with genes. The reason is watching the behaviors that later cause one to become overweight. The sons imitate the behavior pattern of the father and the daughter copy their mother.[28] Parents' perceived feeding habits are transferred to the way children are fed. However, not all dietary choices in this study have been reflected in excessive body weight in children. In the study, obese children were eating fish more often than

children of normal weight. Fish, although considered healthy, are often eaten by children in the form of fried fillets or fish fingers, which reduces their nutritional value and exceeds the number of calories accepted. Children with excess body weight were less likely to eat legumes. It is believed that legumes are beneficial in the prevention of obesity due to the high content of prebiotic compounds that is regulating the intestinal microflora, which disorders are an important risk factor for the development of excessive body mass.[29] Legumes are a valuable source of vitamins and minerals responsible for the expression of genes that regulate the lipogenesis process. They reduce insulin resistance and normalize lipid metabolism.[30] Another important relationship is the fact that children with excessive body weight consumed fruit less often. According to Harton et al.[31], pre-school children eat fruit or sweets most often in the form of snacks. Fruits are a better choice because they contain fewer calories and have a higher nutritional value. Other studies also confirm that sweets are a frequent choice of preschool children as snacks.[32] Replacing sweet snacks with fruits can significantly affect the child's body weight. Another important aspect is the reluctant consumption of dairy products by overweight and obese children. Numerous studies confirm the beneficial properties of dairy products in the prevention of overweight. Weijing et al.[33] assessed that the risk of obesity decreased by 16% when consuming 200g of milk per day. There are several potential mechanisms underlying the impact of dairy products on the risk of obesity. A high-energy diet reduces energy storage through mechanisms associated with calcium levels in adipocytes, increases thermogenesis and reduces appetite.[33]

Proper nutrition of children is important due to their rapid developmental pace, which carries a high demand for nutrients. In addition to providing essential nutrients in the development of a child, physical activity is also important. It is an important factor affecting the body weight in children. Children from the south-east of Poland, with a correct body weight, were much more likely to participate in sports activities than children with overweight or obesity. It is recommended for children to devote about 60 minutes a day to physical activity. Only 1/3 of school children spend their free time actively. However, every seventh child does not take part in any physical activity other than that provided at school.[34] According to Kwiecień et al.[35], in Europe it was observed that with age, children spend less and less time on extracurricular physical activity and show less willingness to participate in physical education classes. Time spent passively is often devoted to watching TV and simultaneously advertising of food products. Brzozowski et al.[36] determined that pre-school children who often watch television and advertisements of food products later reach for it much more willingly. Limiting the amount of advertising for unhealthy food should be implemented to a significant extent. As many as 47.4% of children reach for "junk" food. According to Lioutades et al.[37], advertisements operate on four levels. First, they motivate children to buy unhealthy products, changing their expectations in relation to the type of food. Secondly, they present the product in a good light by tuning the child to it positively emotionally. In the third aspect, the funny dimension of advertising creates a pleasant mood related to the advertised food. The last dimension is the lack of objective assessment of the child in relation to the advertisement and the product itself.

Conclusion

Overweight and obesity occurred in 13.5% of all preschoolers surveyed. With age, the number of children with overweight and obesity increases. The prevalence of excessive body weight among preschoolers was 6.4% in 3-year-olds, 11.3% in 4-year-olds, 17.7% in 5-year-olds, and 20.7% in 6-year-olds. Among boys, obesity and overweight occurred more often than among girls. In the female children, excessive body mass was at the level of: 6.2% in 3-year-olds, 7.4% in 4-year-olds, 15.6% in 5-year-olds, 11.1% in 6-year-olds. In male children, excessive body weight occurred successively: 6.5% in 3-year-olds, 13.9% in 4-year-olds, 20% in 5-year-olds, and 25% in 6-year-olds. A factor significantly increasing the risk of obesity among the examined children was the maternal BMI index. This is closely related to the heredity of eating habits passed on to the young generation by mothers. Nutrition education should concern entire families, in particular mothers and children from the earliest years.

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ORIGINAL PAPER

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Sweat lead and copper concentrations during exercise training

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ABSTRACT

Introduction. Skin is the largest organ of the human body. It plays an important role in protection against harmful substances found in the surrounding environment and takes part in the elimination of heavy metals from the body by sweating. The aim of the study was to evaluate the changes in the concentration of lead and copper in the sweat collected on the first and the fourteenth day of endurance training.

Materials and methods. The research included 43 patients undergoing a supervised, two-week endurance training on a cycle ergometer and cross-trainer. The lead and copper contents were presented in relation to the sodium content as an indicator of the amount of excreted sweat.

Results. The lead concentration in relation to the sodium content in the samples of sweat taken with the use of swabs is statistically significantly higher on day 1 (*Me* = 1.64-E-4) than the 14th day (*Me* = 0.37-E-4) $p = 0.027$. In the sweat samples collected with a plaster, the lead concentration on day 14 of rehabilitation (*Me* = 0.08-E-4) is statistically significantly lower than before the beginning of the training cycle (*Me* = 1.19-E-4) $p = 0.044$. The concentration of copper in sweat samples collected with swabs and patches on day 1 of the rehabilitation cycle does not significantly differ from the content of samples collected on day 14.

Conclusions. Endurance training with submaximal heart rate results in reduced excretion of lead in the sweat and does not significantly affect the level of copper. Further research into the impact of physical effort on the excretion of metals from the body can help explain the results

Keywords. copper, lead, physical effort

Introduction

Skin is the largest organ of the human body. It plays an important role in protection against harmful substances found in the surrounding environment and takes part in the elimination of heavy metals from the body by sweating. Determining the concentration of biomarkers in sweat can be helpful in diagnosing certain diseases as well as in detecting traces of drugs and narcotics.[1] Lead is a toxic metal, especially for the nervous system.[2,3] Previous studies have reported excretion of lead with

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sweat while in the sauna, which indicates that increased sweating under the influence of a thermal stimulus contributes to detoxification.[4] Lead is excreted to a greater extent in a bound form than in the form of ions. Haber et al. found a decrease in the content of lead in the blood of people threatened with exposure to this element in the workplace during endurance training. The authors suggest that sweating is the main route for lead elimination.[5] Percutaneous absorption of lead causes the element to appear in sweat and saliva, but not in blood and urine. Lead absorbed through the skin is quickly eliminated from this location, so it is not as dangerous as, for example, taken orally.[6] Copper is an important building block of many protein enzymes. It is also a component of cytochrome C oxidase, which participates in the transformations associated with cellular respiration. Homeostasis disorders of copper ions occur in Alzheimer's disease and Wilson's disease.[7–9]

Objective of the paper

The aim of the study was to assess the changes in the concentrations of lead and copper in the human sweat collected on the first and fourteenth day of endurance training.

Material and methods

Characteristics of the studied group

The research included 43 patients undergoing supervised endurance training on cycle ergometer and cross-trainer in the conditions of a Rehabilitation Center. Patients reported to the Center because of lower back pain of low or moderate intensity. They were asked not to take painkillers throughout the training. Persons with diagnosed rheumatological or endocrine diseases, pregnancy, fever, diabetes or cardiovascular diseases, chronic neurological disorders and liver diseases were excluded. Participants were informed about the course of the research process and expressed their written consent to participate in the study in accordance with the protocol approved by the local bioethics commission. The population surveyed was dominated by women, which accounted for almost three quarters of the whole sample (72.4%). The youngest patient was 21 years old, the oldest was 68, and the average age of the respondents was nearly 48 years (M = 47.62; SD= 15.91). The height of patients in the study ranged from 158 to 187 cm, while on average it was close to 170 cm (M = 169.62; SD= 8.42). Patients weight was from 47 to 92 kg, and their average body weight is over 70 kg (M = 70.14; SD = 11.07).

The body mass index (BMI) of the participants ranged from 17.26 to 29.74 and was 24.36 on average (SD= 3.29). Almost half of the people (48.3%) were overweight, almost 45.0% had normal body weight (44.8%), while 6.9% of patients were underweight.

Twenty-nine participants completed the whole training cycle. It included exercises on a cycle ergometer and cross-trainer, five days a week for two weeks. In the first week, the training lasted 16 minutes, in the second 20 minutes. The intensity of the training was submaximal, calculated on the basis of the age of the subjects and was 85% of the maximum heart rate obtained from the formula 208-0.7× age (in years).[10]

Sweat analysis

Sweat samples were collected using two methods. The first method was to use a PharmChem patch applied between the shoulder blades, the second sample was taken using a cotton swab immersed in a drop of sweat from the forehead area. Samples were collected at 1 and 14 days of training.

The concentration of Pb and Cu (and Na) in the collected sweat was determined in samples diluted 50 times with deionized water (0.1 ml sweat sample + 4.9 ml deionized water).

Determinations were made with a ThermoScientific model XSERIES2 mass spectrometer, with a collision-collision reaction chamber, with ionisation in inductively coupled plasma (*ICP-MS)*.

The analytical process used reagents of purity for trace analysis and deionized water purified with the Millipore Simplicity 185 UV apparatus. The InorganicVenturesANALITYK-122 pattern was used for calibration of the spectrometer. For the correctness of the calibration curves and for quality control of the performed analyzes, the following certified reference materials were used: EnviroMat ES-L-2 and EnviroMat ES-H-2.

The concentrations of lead and copper expressed in ppb (parts per billion) are presented with reference to the sodium content as an indicator of the amount of sweat produced. The median amount of sodium in the samples taken on the first day with a patch was 183.8 ppm (parts per million). The median amount of sodium in the samples taken on day 14 was 214.7 ppm. These values did not differ with statistical significance ($p> 0.05$).

Statistical tools used

Statistical analyzes were performed using the IBM SPSS 21 statistical suite. The characteristics of the test sample were based on the calculation of the distribution of percentages of the occurrence of qualitative variables, and mean value, standard deviation, and the minimum and maximum for quantitative parameters. The shapes of the distribution of the analyzed data were verified using the Shapiro-Wilk test. The analysis of intra-group differences in the field of variables whose distributions departed from the Gaussian curve was carried out using the Wilcoxon non-parametric signed-rank test, and the results were further refined by means of effect size mea-

sures, which were calculated using a *r* Cohen two-level rank correlation coefficient for matching pairs. The values of individual parameters, due to their lack of conformity of their distribution with the normal distribution, were interpreted on the basis of the median, which is a congruent estimator of the expected value in the population without any asymptomatic load. The values of the quarter interval as well as the supplementary - mean and standard deviation are also given.

The work assumes the limit level for false positive error of 0.05.

Results

The results of the comparison of the lead concentration in relation to the amount of sodium in the sweat samples collected from the patients with the PharmChem patch applied between the shoulder blades and the cotton swabs dipped in a sweat spot from the forehead on 1st and 14th day of training are shown in Table 1.

A graphical illustration of the obtained research results is shown in Chart 1.

The median concentration of lead in relation to the amount of sodium in sweat samples taken from patients with a PharmChem patch on day 1 of the rehabilitation cycle is 1.19-E-4, and in samples taken on the 14th day of exercise it was 0.08-E-4.

The calculations demonstrate that lead concentration in comparison to the amount of sodium in the sweat samples of the subjects taken with the PharmChem patch on the 14th day of training (*Me* = 0.08-E-4) is significantly statistically lower than before the start of the rehabilitation cycle (*Me* = 1.19-E-4) $Z = 2.01$, $p = 0.044$. The recorded size of the effect informs about the occurrence of average correlation between the considered dimensions of $r_c = 0.46$.

The median lead concentration in relation to the amount of sodium in sweat samples taken from patients with swabs immersed in a sweat drop from the forehead area on day 1 is 1.64-E-4, while in samples taken on day 14 of exercises it was 0.37-E-4.

Based on the statistical analyzes carried out, it was found that the lead concentration in relation to the

Table 1. Comparison of the lead concentration in relation to the amount of sodium in the sweat samples collected from the patients in the training cycle

Method	Measured value								Intra-group comparisons		
	On the 1st day of exercise				On the 14th day of the exercise						
	Me	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	*Z*	*P*	r_c
patch	**1.19E-4**	13.24E-4	1.73-E-4	3.64-E-4	**0.08E-4**	0.29-E-4	0.099-E-4	0.08-E-4	2.01	0.044	0.46
swab	**1.64-E-4**	24.87-E-4	5.22-E-4	8.74-E-4	**0.37-E-4**	4.16-E-4	1.10-E-4	1.42-E-4	2.22	0.027	0.57

Me - median; *Q* – quartile deviation; *M* – mean; *SD* – standard deviation; *Z* – Z test; *P* – p-value; r_c – Cohen's correlation coefficient; E-4 – the exponential form e.g: 1.19-E-4=1.19×10^{-4}

Source: own research results.

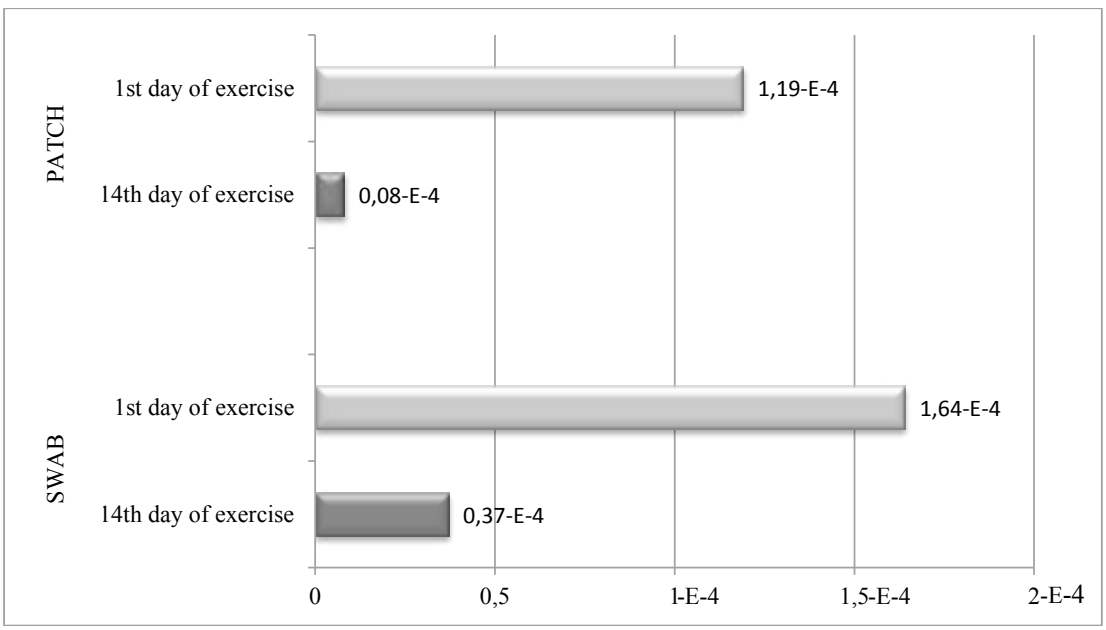

Figure 1. Comparison of the lead concentration in relation to the amount of sodium in the sweat samples collected from the patients in the training cycle

Source: own research results

amount of sodium in the sweat samples of the subjects taken with swabs dipped in a sweat drop from the forehead area, it is statistically higher on day 1 (*Me* = 1.64-E-4) than the 14th day (Me = 0.37-E-4) *FROM* = 2.22, $p = 0.027$, and the obtained effect size indicates a strong correlation between the considered dimensions, $r_c = 0.57$.

The results of the comparison of the copper concentration in relation to the amount of sodium in the sweat samples collected from the patients with the PharmChem patches applied between the shoulder blades and swabs dipped in a sweat spot from the forehead on 1 and 14 days are shown in Table 2.

A graphical illustration of the obtained research results is shown in Figure 2.

The median concentration of copper in relation to the amount of sodium in sweat samples taken from patients with a PharmChem patch on day 1 of the rehabilitation cycle is 6.98-E-4, and in samples taken on the 14th day of exercise it was 8.39-E-4.

The calculations demonstrate that the concentration of copper in relation to the amount of sodium in the samples of sweat tested with the PharmChem patch in 1st (*Me* = 6.98 -E-4) and in 14th (*Me* = 8.39-E-4) day of rehabilitation is comparable, $Z = 0.77$, $p = 0.445$. The median concentration of copper in relation to the amount of sodium in sweat samples collected from patients using cotton swabs dipped in the forehead sweat on day 1 of the rehabilitation cycle is 95.65-E-4, while in samples taken on the 14th day of exercise it was 80.57-E-4.

On the basis of statistical analyzes it was found that the concentration of copper in sweat samples taken with the help of swabs dipped in a sweat drop from the forehead area on day 1 of the rehabilitation cycle (*Me* = 95.65-E-4) does not differ significantly from the content of samples collected on day 14 (*Me* = 80.57-E-4) $Z = 0.93$, $p = 0.352$.

Discussion

As the amount of sweat collected by means of a patch or swab is not known, the amount of lead and copper in

Table 2. Comparison of the copper concentration with respect to the amount of sodium in the sweat samples taken from the patients in the training cycle

Method	Measured value								Comparisions Intra-group	
	On the 1st day of exercise				On the 14th day of the exercise					
	Me	*Q*	*M*	*SD*	***Me***	*Q*	*M*	*SD*	*Z*	*P*
patch	**6.98-E-4**	742.80-E-4	95.62-E-4	206.74-E-4	**8.39-E-4**	93.99-E-4	17.74-E-4	24.51-E-4	0.77	0.445
swab	**95.65-E-4**	2281.62-E-4	339.31-E-4	734.00-E-4	**80.57-E-4**	869.17-E-4	197.05-E-4	280.74-E-4	0.93	0.352

Me – median; *Q* – quartile deviation; *M* – mean; *SD* – standard deviation; *Z* – Z test; *P* – *p*-value; r_c – Cohen's correlation coefficient; E-4 – the exponential form e.g: 1.19-E-4=1.19×10^{-4}

Source: own research results.

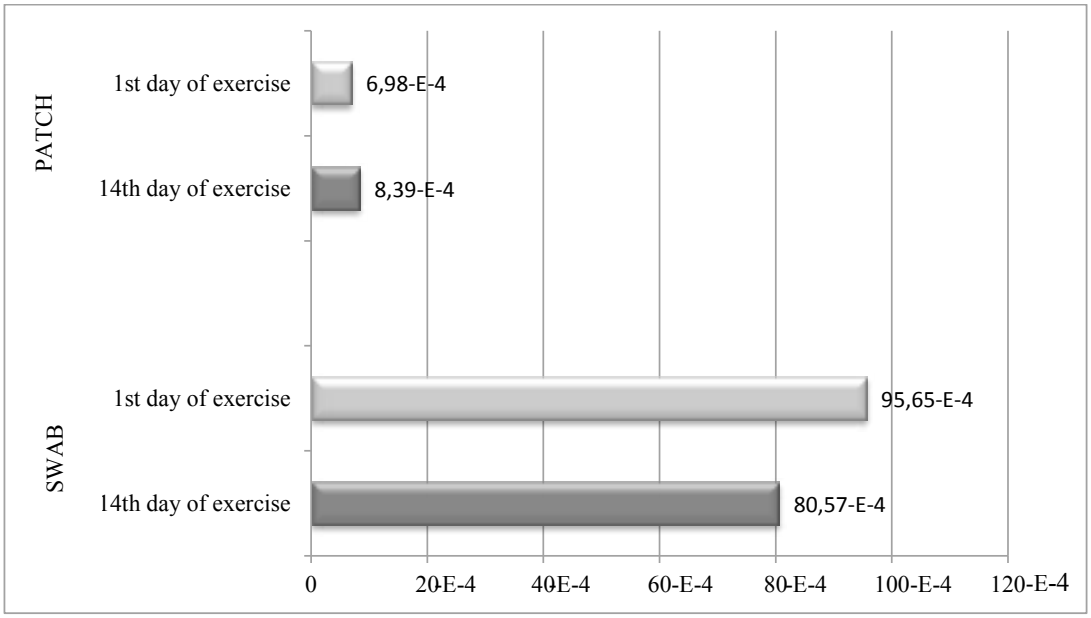

Figure 2. Comparison of the copper concentration with respect to the amount of sodium in the sweat samples taken from the patients in the training cycle

Source: own research results.

the samples was compared to the amount of sodium. As a result of the training, the average amount of sodium in the sweat samples did not change. Based on this observation and literature data, it was considered that sodium ions may be an internal marker of the amount of sweat collected. In addition, the concentration of physiological ions such as sodium ions does not change in the test subjects as the intensity of perspiration increases.[11,12]

Based on the results obtained, it can be concluded that after a fourteen-day training the lead concentration in the sweat samples tested decreases significantly. The lead content on day 14 was lower than on the first day in samples taken with both a patch and a cotton swab. Increased sweating caused by physical effort or environmental temperature causes excretion of toxic substances along with sweat.[4] Earlier works relate mainly to the elimination of elements after their administration or exposure related to work.

In studies carried out by Omokhodion and Crockford no increase in excretion of lead from sweat while driving on an ergometer in a hot environment was observed in people who were previously administered lead chloride orally. An increase in its blood and urine levels has been observed.[13] In case of workers exposed to lead, higher lead concentrations were observed in both sweat, and blood and urine, than in the control group.[14] Obtained discrepancies may result from the lead absorption path. The concentration of lead in the sweat can not be directly related to the content of lead in the blood, as well as the same assessment of lead content in the sweat can be disturbed due to contamination within the epidermis.[15] Exercises may, however, be a preventive tool against oxidative stress and inflammatory processes induced by exposure to lead, thereby counteracting the toxic effects of lead on the body.[16]

The results presented in this paper may form confirmation of the influence of endurance training on excretion of lead as an element (in free and bound form) in the sweat as a result of increased lead movement from tissue stores. However, the lack of a comparative blood analysis makes it impossible to unequivocally confirm changes in lead serum levels. Thus, the assessment of the significance of these reports in the context of monitoring the level of lead and its elimination from the body is not unambiguous.

The second objective of the present study was to examine the effect of training on the copper concentration in human sweat. Based on the results, it was found that the differences in copper content in the sweat samples tested on the first and fourteenth day of training were not statistically significant.

In studies on the concentration of copper and lead obtained by iontophoresis, lower concentrations of these elements were found in women. Sweating induced by a stay in sauna resulted in a similar excretion of copper by both sexes.[17] In other studies on the composition of sweat, in which the method applied was a *total body washdown technique*, sweating was considered the main way to eliminate zinc and copper from the body. On the other hand, the concentration of lead was comparable to its urine content.[18] Campbell et al. found increased urinary excretion of copper as a result of aerobic physical training.[19] Perhaps physical exercise is a factor regulating the level of copper in the body, but according to our research, probably not through the path of sweating. It should be noted that the metal concentration varies depending on where the sweat sample is taken.[20,21] In this work, the results of samples obtained using two methods, and from two body regions, indicate that the differences in the composition of sweat have a similar tendency, but the total amount of elements in relation to sodium differed in both methods of collection, especially when it comes to the copper.

Conclusions

Endurance training with submaximal heart rate results in reduction of lead excretion in the sweat and does not significantly affect the level of copper excretion. Further research into the impact of physical effort on excretion of lead and copper can help explain the results.

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ORIGINAL PAPER

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Statodynamic characteristics of the spine in a sitting position

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ABSTRACT

Introduction. Due to the unprecedented development of media and information technology, modern lifestyles have been changing from active to passive (sedentary). A sitting position dominates today both in the professional and the non-professional sphere of people's life. It seems that a human does not realize what is the position of individual segments of his body, especially the torso while sitting. The torso, as the segment with the highest mass, is the source of the highest mechanical loads acting on the spine. Hence, in the habitual sitting posture, the optimal spine position has been lost.

Objective. The aim of this study is to analyze statodynamic parameters of the spine in a sitting position and answer the question which of them determine the habitual sitting posture.

Material and methods. The study included 372 people declaring themselves as healthy. The research program consisted of statodynamic parameters of the spine in a standing position and in 6 sitting positions: sitting position freely, favourite sitting position, sitting position with a crossed leg over the right and left thigh, and sitting position with a feet resting on the left or right knee.

Results. The conducted research has shown that setting the spine in a habitual sitting posture is determined only by a change in the statodynamic parameters in the sagittal plane and generally does not depend on the range of motion in other planes.

Conclusions. Habitual sitting postures are determined by the size of angles of the *thoracocervical* and thoracolumbar transitions as well as the size of the amplitude of the pelvic movements. The research has indicated worrying trends to misuse of kinematic redundancy in the spine while sitting in the sagittal plane.

Keywords. maladaptive postural behaviour, statodynamic parameters, sitting position

Introduction

Due to the unprecedented development of media and information technology, a modern lifestyle has been changing from active to passive (sedentary). A sitting position dominates today both in a professional sphere and in non-professional parts of life.[1,2,3,4,5] It is also visible in our country due to the fact that about 40% of Poles work in a sitting position and over 60% spend their free time in a passive way. [6] Therefore, we can see that mental activity and intellectual effort replace the physical activity and physical effort that shape human health to a large extent. It is obvious that sitting is an essential and

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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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natural element of a human life from an early age. However, a permanent sitting with elimination of all types of physical activity impairs the adaptive mechanisms of a human body. This is particularly visible in an organ of movement and concerns the main axis of the body, i.e. the spine. The human body, as a self-regulating system with a multi-element structure, reaches various compromises associated with minimization of functional costs while sitting. We can notice this phenomenon especially in the sagittal plane in which the asymmetric mass distribution of the upper body is balanced. It seems that a human does not realize the positioning of individual segments of his body while sitting, especially when we consider the torso. The torso, as the segment with the largest mass, is the source of the greatest mechanical loads, i.e. forces and their moments acting on the spine. It is worth mentioning that the most important element that arranges the human body in a sitting posture is the position of the head and the need to meet visual requirements. We cannot disregard the significance of postural behaviour and space geometry in the process of adapting the human body to the sitting position. That is why, in a habitual sitting posture the maladaptive torso position and the optimal spine position are lost in the hypothetical neutral zone of Panjabi[7]. It leads to postural disorders and in time to overload and pain changes of the spine, which constitute a significant medical disorder. At the same time, overload and pain problems due to their prevalence constitute a significant socio-economic problem.[8,9]

Objective of the work

The aim of this study is to analyze the statodynamic parameters of the spine in a sitting posture and answer the question which of them determine the habitual sitting posture.

Material and test method

372 people, who declare healthy and professionally active lifestyles, have been examined. Participation in the research was voluntary. The inclusion criteria were a lack of diseases of the locomotor system and surgical procedures and spinal injuries. The studied group included 212 women and 160 men aged 20-50 years ($\bar{x}$ = 36,55), with people under 40 years of age, accounting for 60.22% of respondents, i.e. 224 people. The average body weight of the subjects was 71.51 kg and the average height was 169.63 cm, while the average BMI was 24.76 and WHR 0.83. The research program consisted of statodynamic measurements of the spine in a standing position and in 6 sitting positions: sitting position freely, favorite sitting position, sitting position with a crossed leg over the right and left thigh, and sitting position with a foot resting on the left or right knee. These were the measurements taken:

- angles of particular transitions of the spine in the sagittal plane measured with the V-Rippstein plurimeter with an accuracy of ± 1 °. The plurimeter was placed at the base of the sacrum and the segment L_4-L_5 in the measurement of the lumbosacral transition. The angle of the thoracolumbar segment was measured at the level of the Th_{12}-L_1 segment. The *thoracocervical* transition was measured by placing the base of the plurimeter at the level of the Th_1-C_7 segment, whereas the craniocervical angle was measured at the base of the cranium (O_{cc}-C_1 segment)
- angular size of curvatures of the spine, which were calculated by summing up the angles of the vertebral column transitions in the sagittal plane. The angle of lumbar lordosis was formed by the sum of the lumbosacral and thoracolumbar angles, the angle of thoracic kyphosis gave the sum of the thoracolumbar and *thoracocervical* angles, whereas and the cervical lordosis angle was formed by the sum of the *thoracocervical* and craniocervical angles
- pelvic flexion and extension are also measured by a plurimeter placed at the height of the sacral segments
- ranges of three-dimensional motion of the spine in the cervical and lumbar segments are determined by the use of the Zebris CMS 10 set based on signals received from ultrasonic sensors with an accuracy of 0.1 ° fixed at the occipital protuberance and segment C_7-Th_1 and at the base of the sacrum and vertebra Th_{12}
- projection lengths of the spine in the habitual posture and during auto-elongation were measured with the anthropometer with an accuracy of ± 1 mm. On their basis, the kyphotisation indicators have been calculated on the basis of the formula (authorship of researchers)

$$K = \frac{w - z}{w} \times 100\%$$

where: w = the projection length of the spine in auto-elongation

z = the projection length of the spine in the habitual posture.

All the research procedures were carried out with the approval of the University Bioethics Committee for Scientific Research and according to the Declaration of Helsinki 1978, amended in 1983.

The obtained results were used for statistical analysis, in which the descriptive statistics were first used in order to present the study group and determine its statistical features. Then, a test statistic was applied, suitable for the objectives set for this research. This was

the analysis of variance for repeated measurements of the projection lengths of the spine and factor analysis, which allowed to assess the sitting posture and identify indicators determining the orthogonal space and describing the phenomenon of a human kyphotisation. Further, factor analysis of other statodynamic parameters was performed in order to determine their impact on spinal setting (kyphotisation) at various positions. Statistical significance was set at $p < 0.05$.

Results

A kyphotisation indicator, which describes a certain situation important for clinical diagnosis consists of seven components, that is one indicator in a standing position and six indicators in sitting positions: sitting position freely, sitting position with a crossed leg over the left or right thigh, sitting position with a foot resting on the left or right knee and favourite sitting position. It turned out that all components of the kyphotisation indicators are variables with factor loading values > 0.7. Among them, three most representative indicators were selected by factor analysis, which do not correlate with each other and describe the phenomenon of spinal kyphotisation. They are: a kyphotisation indicator in a sitting position with a foot resting on the knee, an indicator in a favourite sitting position and a kyphotisation indicator in a standing position. Then, the remaining statodynamic parameters of the spine were subjected to factor analysis in order to determine their effect on the components of the kyphotisation indicator. As a result of this analysis, variables with absolute values of factor loadings > 0.7 were derived. Among them there were nine independent variables with the highest values of factor loadings, which describe the statodynamic variability of the spine in 83.2%. Then, in the course of multiple regression by means of backstep analysis, the effect of selected statodynamic parameters on the kyphotisation of the subjects, based on the previously determined components of kyphotisation, was investigated. In the final step, three variables have been left that have a significant (directly proportional) effect on the kyphotisation in a standing position. They are: angle of *thoracocervical* transitions in a sitting position with a leg on the thigh (THC), pelvis flexion in a sitting position with a crossed leg over the thigh (ZM) and pelvic extension in a sitting position with a foot resting on the knee (WM) (Fig. 1). In other words, the indicator of kyphotisation in a standing position depends on the mentioned predictors, i.e. angle of *thoracocervical* segment and pelvis rotation in the sagittal plane. A similar analysis of multiple regression was carried out for the dependent variable of the kyphotisation indicator in the sitting position with a foot resting on the opposite knee, directly proportional by the variables: angle of thoracolumbar transition in the sitting position with a foot resting on the knee (LTH), angle of *thoracocervical* transition in the sitting position with a leg crossed over the thigh (THC) and pelvis flexion in a sitting position with the leg on the thigh (ZM) (Fig. 2). They favour the spine kyphotisation in this sitting position, i.e. the higher they are, the greater chance of kyphotisation in the sitting position. On the other hand, as for kyphotisation in a favourite

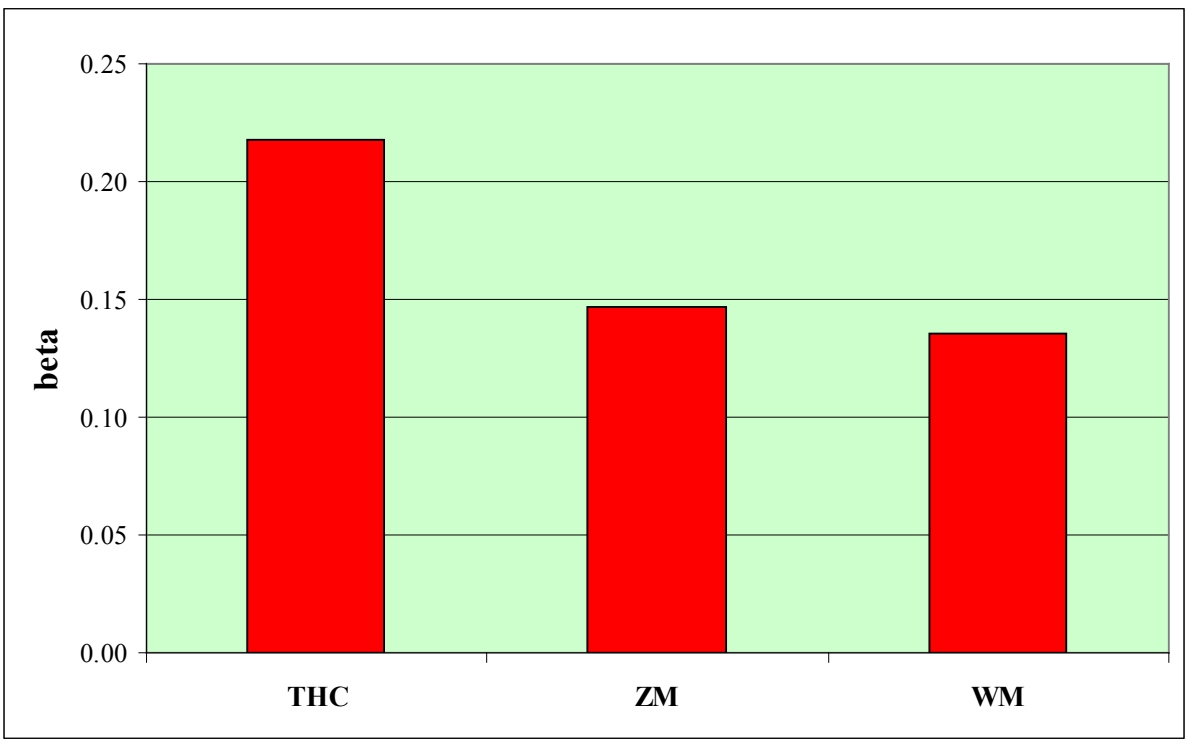

Figure 1. The influence of statodynamic parameters on kyphotisation in a standing position

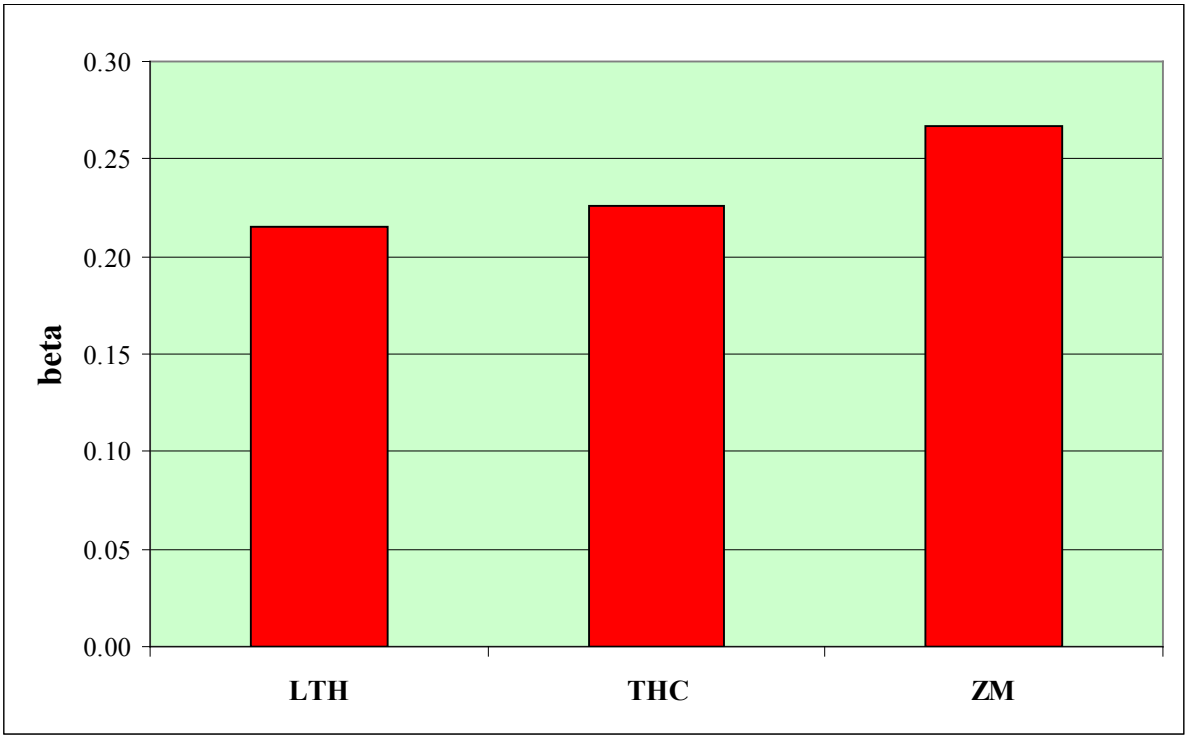

Figure 2. Influence of statodynamic parameters on kyphotisation in a sitting position with a foot resting on the knee

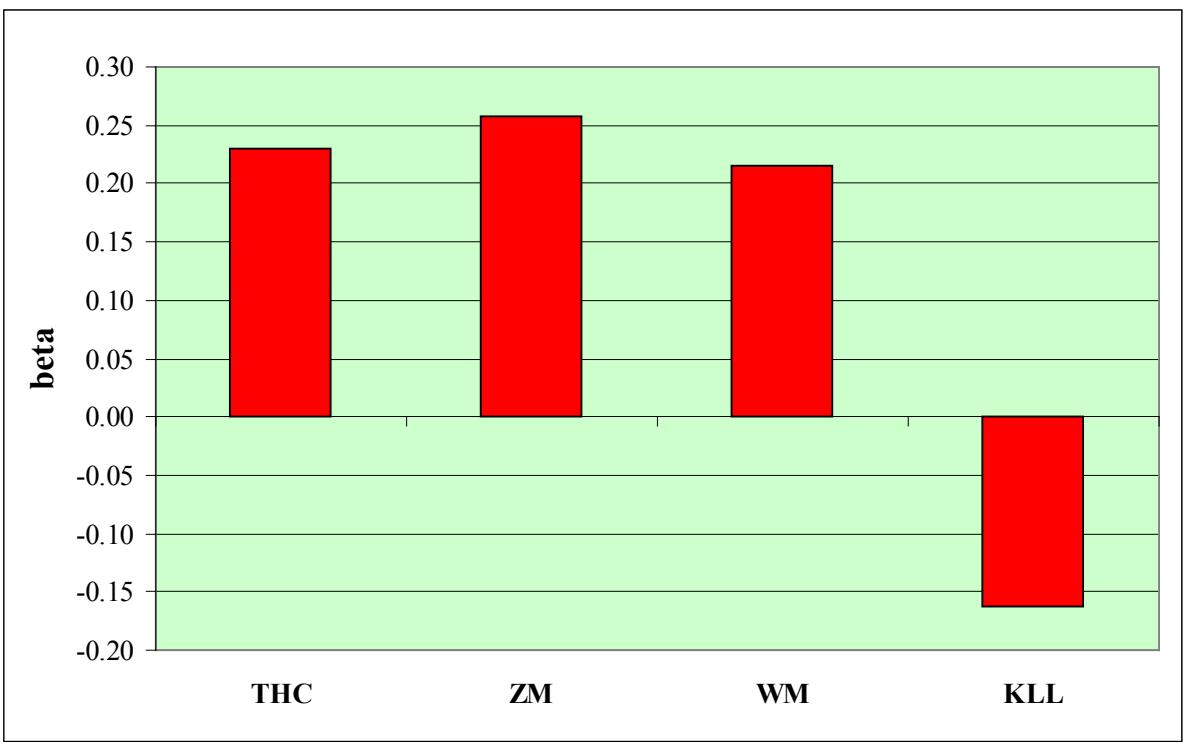

Figure 3. Influence of statodynamic parameters on kyphotisation in a favourite sitting position

sitting position, the regression analysis revealed a significant directly proportional influence of independent variables: angle of the *thoracocervical* transition in a sitting position with a crossed leg over the thigh (THC), pelvic flexion in the sitting position with a crossed leg over the thigh (ZM), pelvic extension in the sitting position with a foot resting on the knee (WM) and inversely proportional dependence on the variable of angle of lumbar lordosis in a sitting position with a foot resting on the knee (KLL), therefore the kyphotisation in a favourite sitting position is determined by the size of inclination of the *thoracocervical* transition and pelvic

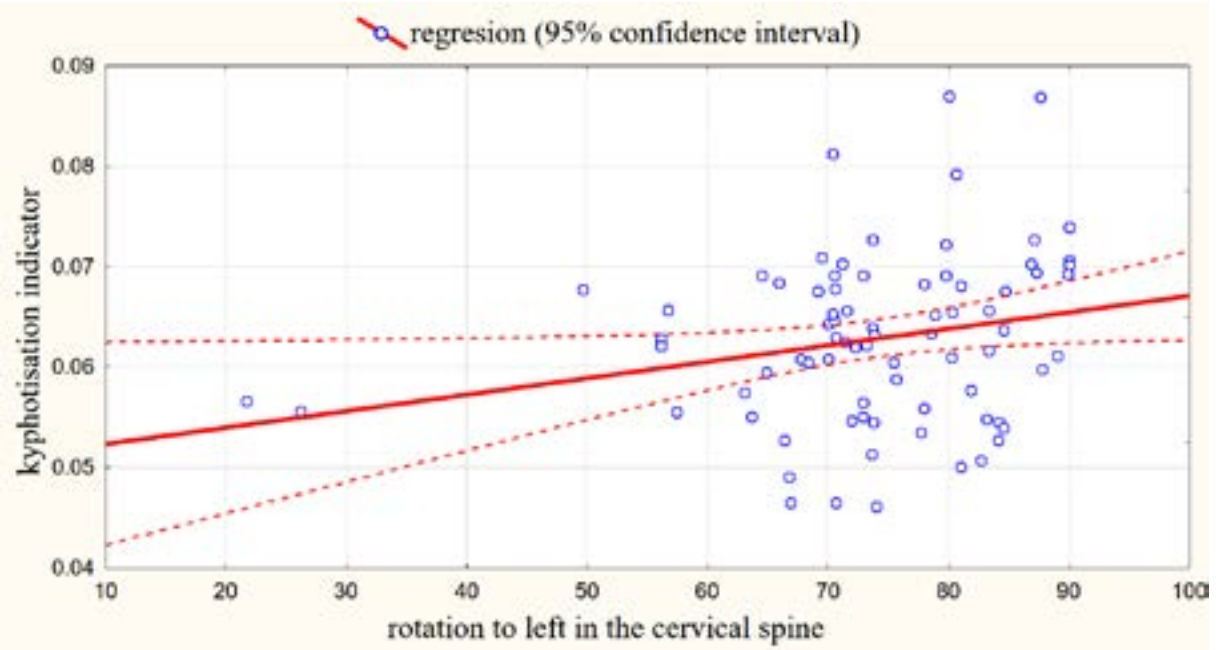

Figure 4. Effect of rotation to left in the cervical spine on kyphotisation in a sitting position with a foot resting on the knee

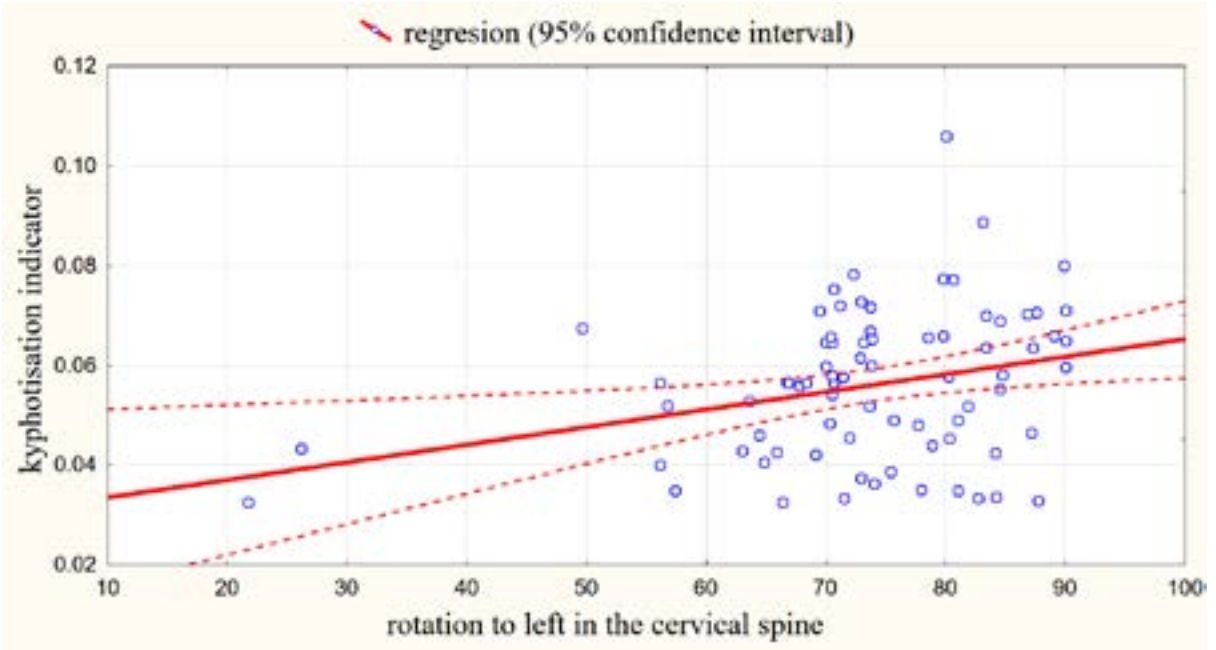

Figure 5. Effect of rotation to left in the cervical spine on kyphotisation in a favourite position

flexion and extension, while an increase in the angle of lumbar lordosis causes a decrease in the indicator of kyphotisation (Fig. 3). Factor analysis also included the ranges of motion in the cervical and lumbar sections of the spine to determine their impact on the components of the kyphotisation indicator. Seven factors with the highest factor loadings for the regression analysis were selected. Backstep analysis for the *kyphotisation indicator* in the standing position did not show the influence of any independent variable, i.e. the kyphotisation in this position does not depend on the range of cervical and lumbar spine motion. Only as for the *kyphotisation indicator* in the position with a foot resting on the knee of the opposite leg and the position of favourite sitting, rotation to left of the cervical spine has a positive effect, i.e. the higher rotation to left, the greater tendency to kyphotisation (Fig. 4 and 5).

Discussion

The sitting posture is shaped by sensorimotor education and belongs to an individual repertoire of postural and movement patterns of a person. The morphological and

functional base for the sitting posture is the bone-joint system and muscular-ligament system together with the controlling nervous system, whereas the functional balance between these three systems allows correct stabilization of the spine according to Panjabi.[7] Anatomical-kinematic links between individual segments of the human body while sitting display enormous complexity. The problem of stabilization of the human body, especially the torso, was solved in the phylogenetic process by s-shaped setting of the spinal column in the sagittal plane with greater muscle strength of the extensors in relation to the flexors so that they can effectively counteract the rotational force of gravity and by appropriate pelvic tilt, which results from the balance of muscle strength. However, due to lack of physical activity and muscular effort, deactivation of the locomotor system and functional disorders of the spine stabilizing systems take place, thus the sitting posture. It has been indicated in the research. It turned out that most respondents do not cope with the spatial arrangement of the body in the sitting posture and they automatically take (from the three described types of sitting) a frontal sitting position with the *kyphotic* spinal position. According to Snijders, a frontal sitting position is the most beneficial in the sense of functional expenditure, while a back sitting position is very demanding in terms of energy usage, and an intermediate sitting with the centre of gravity over the ischial tuberosities is unstable.[10] While sitting, quick fatigue occurs as well as failure of muscle stabilizers and shift of stabilization towards fascial-ligamentous structures susceptible to nociception. This leads to the loss of optimal (sigmoidal) spinal position and changes in its statodynamic parameters. This is done through the click-clap phenomenon with counter-nutation of the sacrum and a posterior pelvic tilt and a simultaneous compensatory change in the spinal position. It is obvious that these maladaptive changes have hidden costs in the form of increased static loading and mechanical stress of the intervertebral discs, which may lead to discomfort of sitting. Therefore, researched persons take 2 types of habitual sitting. The first of them (with a higher rate of *kyphotisation)* can be defined as a forced sitting, whereas the second one (with a lower rate of kyphotisation) may be named an antalgic sitting. Probably because of discomfort, the respondents are subconsciously looking for the optimal sitting posture.[11] The *kyphotic* spinal position in both types of sitting is most influenced by angles of the *thoracocervical* and thoracolumbar transitions as well as the rotation of the pelvis controlling the angle of lumbar lordosis. At the position of the spine in a sitting posture with a foot resting on the knee (forced sitting), the angles of *thoracocervical* and thoracolumbar transitions have the biggest influence, as well as a posterior pelvic tilt, while the spine position in a favourite sitting (antalgic sitting) is influenced by the angle of *thoracocervical* transitions and pelvic rotation. At the same time, it turns out that this postural habit is transferred by subjects from a favourite sitting position to a "standing" manner, because *kyphotisation* in a standing position is also determined by the angle of *thoracocervical* transition and pelvic rotation. On the other hand, the angle of lumbar lordosis and in a favourite sitting position remains inversely related to the *kyphotic* spinal position, which corresponds to the research of O'Sullivan and Callaghan. According to them the change in the pelvic-lumbar complex may lead to a change in the motor control of the torso muscles, i.e. the response of the torso muscles remains under the influence of the angle of lumbar lordosis.[12,13] The higher the value of lumbar lordosis is in a favourite sitting position, the smaller the spinal *kyphotisation* and the smaller stoop of the torso. Whereas, the smaller the angle of lumbar lordosis, the greater the spinal *kyphotisation* and the stoop of the torso. This is particularly evident in a sitting position with a foot resting on the knee, because the straightening of the lumbar lordosis results not only in increased *kyphotisation*, but also in the change of the angle size of the thoracolumbar transition. The setting of the spine, and therefore its kyphotisation indicator in both sitting postures also depend on back extensor muscles, which has a so-called weak point around the third thoracic vertebra, predestining to the phenomenon of flexion-relaxation in the course of sitting, i.e. myoelectric silence, which according to Callaghan already appears at the small angle of the lumbar spine flexion in the sagittal plane.[13] This is probably due to the small mass of the local "muscular" area around the third thoracic vertebra and the upper body stabilization, and especially the upper limb girdle joined only functionally to the trunk with the fascial-ligamentous system, which increases the bending moment of the thoracic spine in the upper section and thus increases the angle of *thoracocervical* transition in both types of sitting. In other words, the change in the size of the angle of the *thoracocervical* transition determines directly proportional *kyphotisation* of the spine in both types of habitual sitting. In turn, the pelvic rotation (flexion and extension movement) in the position of a favourite sitting position corresponds to the research conducted by Vergara et al., who consider pelvic postural changes during sitting (called macro movement) as a good indicator of discomfort in the back and especially of the lumbar spine.[14] Moreover, Callaghan and Mc Gill in their studies also observe a dynamic strategy of sitting associated with frequent changes in the pelvis and loins, which is explained by the mechanism of fatigue of muscle stabilizers.[15] Therefore, considering the conducted research it can be concluded that the habitual sitting posture is instinctively modified due to discomfort or back pain. Thus, the second sitting posture is not acci-

dental, it only gives the possibility of migrating the loads between the muscular-ligamentous structures stabilizing the spine and allows for hydration of the intervertebral discs. The conducted analysis shows that subjects during the sitting dangerously use kinematic redundancy of the spine (61 °) and pelvis (15 °) by pelvic maladaptation and compensatory changes in the position of the spine in the sagittal plane. This results in a change in statodynamic conditions and at the same time it is a manifestation of pathological postural motility. Kinematic redundancy of the motion system is beneficial only in the case of pathology, because it gives the opportunity to adjust the lack or functional deficiency by controlled compensation during the rehabilitation treatment. Compensation is reserved for use in medical conditions as so-called "rehabilitation potential". Thus compensatory mechanisms should not appear in healthy people in the course of sitting as adaptation strategies resulting from poor physical efficiency and low "threshold" of peripheral fatigue, which is accompanied by central fatigue associated with the demand for relief and static position change. The studies also show that setting the spine in a habitual sitting posture is determined only by a change in the statodynamic parameters in the sagittal plane and does not depend essentially on the range of motion in the other planes. Only rotation to left in the cervical segment has a positive effect on the *kyphotic* spinal position while sitting, which is probably a kind of functional habit associated with the adjustment of the head to organize a work station, for example a computer monitor. The cervical segment rotation is a very beneficial form of adaptive activity from the point of view of energy costs while working in a sitting posture, due to the fact that it does not move the centre of gravity of the head in relation to its fulcrum. Thus, we can see that the surveyed persons use incorrectly the kinematic redundancy of their motor system while sitting by creating maladaptive postural-motor patterns, which will cause in time degenerative overload changes in the spine. To sum up, it should be stated that a modern "Homo sedentarius" should use various preventive and therapeutic strategies that will help maintain optimal spine stabilization while sitting. For this purpose, the most often used exercises are strengthening the muscles of the pelvis and loins complex, which, improving motor control, contribute to the reduction of spinal overload and pain problems. However, according to some authors, they are insufficient and sometimes even unnecessary.[16,17,18,19] It seems that people with a sedentary lifestyle should reach primarily for cognitive therapies, and among them especially for educational programs such as "explain pain" or strategies for counteracting spinal overload and pain problems.[20,21,22]

Conclusions

1. Habitual sitting postures are determined by the size of angles of the *thoracocervical* and thoracolumbar transitions as well as the size of the amplitude of the pelvic movements.
2. The research has indicated worrying trends to misuse of kinematic redundancy in the spine while sitting in the sagittal plane.

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REVIEW PAPER

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The significance of NGAL and KIM-1 proteins for diagnosis of acute kidney injury (AKI) in clinical practice

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ABSTRACT

Introduction. Despite advances in medical care AKI (*acute kidney injury*) is associated with high morbidity and mortality. The lack of adequate early renal injury biomarkers is often a problem for an early AKI diagnosis.

In recent years, numerous scientific studies have been carried out which reveal new urine and serum markers to assess the period of the kidney injury before revealing its late clinical effects.

In most clinical settings, AKI is due to acute renal tubular necrosis which results in protein accumulation in urine. Determination of the concentrations of proteins such as NGAL-(*neutrophil gelatinase-associated lipocalin*) and KIM-1 (*kidney injury molecule-1*) are of great significance in the diagnosis of AKI.

Aim. The purpose of the study was to review the literature about significance of NGAL and KIM-1 proteins for diagnosis of acute kidney injury (AKI) in clinical practice.

Materials and method. Analysis of Polish and foreign literature.

Keywords. NGAL proteins, KIM-1 proteins, acute kidney injury (AKI)

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) proteins have recently been used as biomarkers for renal injury. Their biological function is not fully understood. Both in a healthy kidney and in the urine, the level of these proteins is almost undetectable. As a result of renal ischaemia or the potentially nephrotoxic substances action, their expression and synthesis are intensified. Expression of NGAL like KIM-1 proteins are stimulated in damaged epithelial nephrons cells in the distal tubule and the S3 segment of the proximal tubule respectively.

Therefore, these are early, non-invasive markers of kidney injury detected especially in urine prior to the

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development of a full-blown AKI.-

Biochemical description of the NGAL protein

Neutrophil gelatinase-associated lipocalin also known as lipocalin-2, siderocalin, uterocalin and 24p3, is a 25 kDa protein belonging to the lipocalin superfamily.[1,2] The lipocalin protein family is a large group of small extracellular proteins.[3] Lipokalin can have different functions. They participate in the processes of regulating cell aging, differentiation and modeling of the immune response. In the human body, NGAL also influences the growth, development and differentiation of various cells.[4] A similar secondary and tertiary structure as well as the presence of ligand binding sites is a common feature of lipocalin. NGAL may exist in the form of a monomer, a dimer or in combination with other molecules, e.g. metalloproteinase-9 (*matrix metalloproteinase-9;* MMP-9), also called gelatinase B or collagenase type IV.[5]

Neutrophil gelatinase-associated lipocalin are endogenous and ubiquitous biomarker molecules that are secreted by various types of human tissues, including the gastrointestinal tract, respiratory tract and kidneys. In the kidneys, NGAL is secreted into the urine by the thick ascending limb of loop of Henle and collecting ducts of the kidney, with synthesis in the distal nephron.[6,7] Due to its small molecular size, the NGAL protein is freely filtered and can be easily detected in the urine. Neutrophil gelatinase-associated lipocalin in urine (uNGAL) appears in a very short time and its increased concentration is a result of acute kidney injury, diabetic nephropathy, nephritic syndrome, tubulointerstitial nephritis, and IgA nephropathy.[8,9,10]

Biochemical description of the KIM-1 protein

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein with an ectodomain containing both an Ig-like and a mucin domain. It was discovered in renal tubular epithelial cells in a screen for molecules involved in the pathogenesis of AKI.[11]

The glycoprotein is located primarily on the apical surface of the proximal tubule of the nephron in the outer core layer. With the participation of metalloproteinases, the extracellular domain is cleaved and excreted into the urine. That ectodomain is a quantitative marker of kidney damage. The soluble KIM-1 has a molecular weight of around 90 kDa.[12,13]

During normal kidney function, KIM-1 is almost undetectable in the urine. Renal ischaemia, and also damage to various nephrotoxic factors induces its expression and synthesis. Therefore, the molecule is a quantitative biomarker of kidney injury.14 Clinical studies show that KIM-1 is upregulated in tubules of patients with focal segmental glomerulosclerosis, IgA nephropathy, or membranoproliferative glomerulonephritis and that it is associated with tubular injury and interstitial fibrosis. These results have led to the suggestion that KIM-1 may be a promising, non-invasive, easily detected in urine biomarker of chronic tubulointerstitial damage.15,16

The role of NGAL and KIM-1 proteins in AKI

current clinical diagnosis allows for detection of new biomarkers of renal tubular damage such as NGAL, KIM-1, interleukin 18 (IL-18), liver-type fatty-acid-binding protein (L-FABP), insulin-like-growth-factor-binding protein 7 (IGFBP7), and tissue inhibitor of metalloproteinase 2 (TIMP-2). Marking them by clinicians enables diagnosis of acute renal injury in the preclinical phase of the disease.[17]

Due to some renal complications, e.g. due to cardio-renal syndrome, attempts have been made to adapt several well-characterized markers for early diagnosis of kidney damage in patients treated for cardiovascular diseases. Among the large group of known markers, NGAL has the highest diagnostic significance due to the very fast response time to tubular lesions. This is particularly important because AKI complications during cardiac surgery are a cause of prolonged hospitalization and increased mortality.[18,19]

Neutrophil gelatinase-associated lipocalin seems to be a good biomarker of injury, due to the fact that the increase in the concentration of this protein both in plasma and in urine is observed already 2 hours after the kidney damaging factor action that significantly precedes serum creatinine elevation that occurs around 24-48 hours after kidney injury.

A large number of experimental studies confirm the usefulness of NGAL-1 gene in the diagnosis of AKI. In the ischemic and toxic AKI model, it was shown that the NGAL-1 gene was more expressed, and the protein encoded by it was one of the biomarkers of kidney damage synthesized in the largest amounts.[20,21] The usefulness of NGAL as "renal troponin" has also been confirmed in clinical trials.[22] Patients hospitalized in the intensive care unit with symptoms of acute renal failure were characterized by a significant increase in NGAL concentration, both in urine and plasma.[23]

It was noted that in adult patients who underwent cardiac surgery who developed post-operative AKI diagnosed on the third day after surgery evidenced by a 50% increase in serum creatinine in relation to its baseline, plasma NGAL concentration increased only 1-3 hours after surgery.[24] Similarly, it was observed among children operated on due to congenital heart disease who also developed AKI as a result of disorders of renal blood supply due to extracorporeal circulation.[25]

In a cohort of high-risk adult patients undergoing cardiac surgery, there was an increase in postoperative AKI and 1-year mortality in patients with higher preop-

erative serum NGAL. Those patients with serum NGAL above 220 ng/L had an estimated twofold increase risk of cardiovascular and all-cause mortality at 1 year following cardiac surgery.[26] In other studies of patients with acute nephropathy caused by radiographic contrast agents, urea and serum NGAL elevation were also observed.[27]

A meta-analysis summarizing the usefulness of the determination of NGAL in patients at risk of AKI after cardiac surgery during treatment in intensive care units after administration of contrast agents, clearly confirmed the reliability of the determination of NGAL concentration using standardized reagent kits. Based on a thorough review of the test results, covering over 2,500 patients, the predictive value of NGAL concentration was found to be higher than 150 ng/ml. Attention was also drawn to the higher predictive value of NGAL concentration in AKI in children.[28]

The high clinical utility of the marker NGAL can be demonstrated by the synthesis of lipocalin-2 and its use in relieving the symptoms of ischemic acute myocardial injury. In an established murine model of renal ischemia-reperfusion injury, intravenous NGAL administered before, during, or after ischemia resulted in marked amelioration of the morphologic and functional consequences, as evidenced by a significant decrease in histopathologic damage to tubules and in serum creatinine measurements. Neutrophil gelatinase-associated lipocalin-treated animals also displayed a reduction in the number of apoptotic tubule cells and an increase in proliferating proximal tubule cells after ischemic injury. The results indicate that NGAL may represent a novel therapeutic intervention in ischemic acute renal failure, based at least in part on its ability to tilt the balance of tubule cell fate toward survival.[29]

The therapeutic success of surgical treatment is largely associated with the lack of serious postoperative complications. Such postoperative complications include AKI, in which, depending on the type of surgery, mortality affects from 7-60% of patients.[30-32] Acute kidney injury markedly increases peri-operative mortality risk. However, despite the development of less invasive techniques, cardiac surgery remains the first option in many conditions such as severe coronary artery disease, valve diseases and complex interventions. Therefore, there is interest among cardiologists and cardiosurgeons in research on new markers of kidney damage. Numerous studies in the field of cardiology include issues of elevated serum NGAL levels, which are a consequence of cardiac surgery using extracorporeal circulation[18], as well as coronary bypass and coronary revascularization.[19]. The results of the study clearly show that the increase in lipocaline precedes the increase of serum creatinine concentration by 24-48 hours, which gives the opportunity to use serum and urinary NGAL determinations in monitoring kidney damage of patients after these procedures and rapid implementation of measures to protect the kidneys from progressive damage.[19]

Malyszko et al.[33] also see the usefulness of the determination of serum NGAL concentration in the diagnosis of kidney damage in the course of hypertension. Based on the concentration of NGAL, groups of patients at risk of early deterioration of renal function in the course of hypertensive nephropathy may be identified and appropriate treatment may be implemented.

A higher concentration of NGAL was found in the urine in patients with left ventricular hypertrophy in the course of primary hypertension[34], with atherosclerosis.[35] The prognostic significance of the NGAL concentration assessment in the urine and serum in renal syndrome[36] and in the assessment of acute renal failure in patients with acute congestive heart failure was also demonstrated.[37]

Studies show a positive correlation of higher levels of lipocalin-2 with a worse prognosis in patients with serum NGAL levels above 140 ng/ml at admission where seven times more often the deterioration of kidney function develops until failure. During long-term follow-up, shorter survival periods of patients with acute congestive heart failure have been demonstrated with initially higher levels of NGAL (above 215 ng/ml).[37,38]

Determination of NGAL levels in humans is used primarily in the assessment of AKI, but also in the diagnosis of the progression of chronic kidney diseases, e.g. obstructive nephropathy[39], IgA nephropathy[40] or progression of kidney damage in systemic diseases such as diabetes[41,42], arterial hypertension[43] or lupus erythematosus.[44] A positive effect of the determination of NGAL concentration for the evaluation of nephrotoxicity of contrast agents used in radiological diagnostics[45] and the nephrotoxicity of some drugs, e.g. cisplatin, was also demonstrated.[46]

The potential role of KIM-1 as a biomarker in various pathologies of the kidneys is still intensively studied, and in many analyses it has been shown that KIM-1 is a sensitive and specific marker of proximal tubular damage.[47] Any increase in KIM-1 in the urine indicates kidney damage, because the protein is not out of the kidney in an amount that can change its level in the urine. Both experimental and clinical studies indicate that KIM-1 is a biomarker of tubular lesions already appearing, similarly to NGAL-1, on the first hours after toxic or ischemic damage to the nephrons tubules.

Determination of this protein as a laboratory AKI index is also important in the assessment of the severity of this disease. Liangos et al.[48] embraced the study of 201 hospitalized AKI patients and found that those with the highest urinary KIM-1 level had a statistically 3-fold higher dialysis implementation frequency. A sim-

ilar relationship was found in a subsequent study evaluating the need renal replacement therapy and mortality among AKI development patients.[49]

Clearly increased KIM-1 concentrations were also observed in patients with acute tubular damage that developed due to previous cardiac surgery or with symptoms of acute renal failure in the course of sepsis.[50,51] In addition, it was observed that the risk of rejection of allogenic kidney transplant increases with elevating urinary KIM-1 concentration.[52,53] Increased KIM-1 secretion into the urine was also revealed in patients with clear cell renal carcinoma.[54]

Clinical usefulness of KIM-1 protein is not limited to the diagnosis of acute renal injury or failure. There are studies showing KIM-1 utility in diagnosing and monitoring of chronic kidney disease (CKD). Analysis of changes in the concentration of this marker in the urine may indicate the transition of AKI into the CKD.[55] Patients with chronic heart failure and cardiorenal syndrome were characterized by a higher concentration of KIM-1 in urine in relation to healthy people.[56]

This protein proves to be useful for identifying groups of patients with AKI or those at risk of its occurrence, especially those undergoing cardiac surgery. Early diagnosis and appropriate treatment may help reduce the number of patients requiring renal replacement therapy.

Conclusions

Biochemical diagnostics of AKI mainly based on classical laboratory parameters such serum creatinine or urea is still insufficient. There is a need to extend diagnostics with new protein biomarkers discussed briefly in this work. The use of NGAL and KIM-1 in combination with other biomarkers, i.e. cystatin C, nαKlotho protein or IL-18, allows an earlier and more reliable diagnosis of AKI in the preclinical phase of disease. Such knowledge will enable the rapid implementation of appropriate and effective therapeutic procedures.

There is hope that the determination of the protein biomarkers in the urine and plasma will become a routine diagnostic procedure in patients with AKI risk factors in clinical settings.

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REVIEW PAPER

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Plants – a source of therapeutic material?

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ABSTRACTS

Introduction. The use of plants with therapeutic or medicinal properties is as ancient as human civilization and for many years prior to the 20th century, plants and animal products were the main source of therapeutic medicinal drugs.

Aim. The discovery of new plant-derived drugs continues to be an active field of research in medical science today. The aim of this article is to describe several main classes of natural products currently under investigation.

Material and methods. Analysis of literature.

Keywords. plants, natural products, drug, therapeutic material

Introduction

The isolation and evaluation of natural products from plants have made an enormous impact on the discovery of new drugs and has led to advances in medical science.[1,2] Many natural plant substances are used for general health maintenance or for serious conditions such as cancer, asthma, AIDS, multiple sclerosis, and arthritis.[3-5]

Plant Anticancer Agents

Plants are an excellent source of anticancer agents.[6] Each year, more evidence for their use against tumors in traditional systems of medicine is reported in the literature. Several main types of natural products derived from plants that are especially of interest in anticancer research are listed below:

- The alkaloids [6-14]
- The terpenes[15-20]
- The lignans [21-23]
- The macrolides [24-25]

Historical evidence for the use of plants used as anticancer agents is well known. Over the past 40 years, major chemotherapy agents derived from this source have been introduced to the pharmacy market. Several examples of anticancer substances and their plant source are

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as follows: alkaloids from Vinca species (vinblastine, vincristine, vindesine, vinorelbine), camptothecin derived cytotoxics (topotecan and irinotecan) and more recently homoharringtonine from Cephalotaxus trees, lignans based on the podophyllotoxin class (etoposide and teniposide), terpenes like betulin and taxanes (paclitaxel and taxotere and ingenol mebutate, and the macrolide maytansine from Gymnosporia spp. Plants are a rich sources of cytotoxic agents and given the likelihood of a vast number of active substances which are still unknown, their continued discovery and investigation are warranted pursuits in medicinal chemistry. In addition, advances in genome biology and target selection will ensure that plants will continue to provide new anticancer drugs.

The alkaloids

Alkaloids are an immensely important group of naturally occurring bioactive compounds and contain one or more amine functional groups.[6-8] In addition, they are easily extracted and purified with simple partition and precipitation methods and they were isolated in high purity very early on in the history of drug discovery.[9-12] This group includes some compounds with neutral and even weakly acidic properties. Molecules of alkaloids, depending on the pH, mimic the chemistry of endogenous amines acting as physiological compounds. Many alkaloids are endowed with an exquisite selectivity for their targets, allowing them to exert their effects at very low concentrations. Ironically, many alakaloids were also used as poisons in ancient times.

Important anticancer alkaloids have been derived from Vinca species.[12-16] Bioassays performed on Madagascar perivinkle (Catharanthus roseus, Vinca rosea) led to the discovery of the active alkaloidal compounds vincristine and vinblastine. Both, vincristine and vinblastine are dimeric indole alkaloids and their laboratory synthesis is difficult and expensive. The yield of alkaloids from Vinca rosea is low and equals 0.0002% for the alkaloid vincristine only. Vincristine, is also known as leurocristine and is marketed under the trade name Oncovin®. Vinblastine is marketed under the trade name Velbe® and is used to treat acute Hodgkin's disease. These alkaloids cause M phase specific cell cycle arrest by disrupting microtubule assembly and proper formation of the mitotic spindle and the kinetochore, each of which are necessary for the separation of chromosomes during the anaphase of mitosis. The alkaloid Vindesine (a synthetic derivative of vinblastine) is used to treat leukemia and lung cancers. Vinorelbine (marketed as Navelbine®) is used as a treatment for non-small cell lung cancer and breast cancer in combinations with cisplatin.[16]

The terpenes

Terpenes are class of organic compounds, produced by a variety of plants. They alter anticancer therapy; however there are still difficulties isolation and characterizations. Terpenes do not precipitate or form crystals easily. Terpenes are the essential oils of many types of medicinal plants and flowers.[17-20] They are complex mixtures with very low polarity making their separation difficult. Commonly known medicinal plants which possess terpenes are Taxus brevifolia (the source of Taxol) and Euphorbia (the source of the diterpene known as ingenol mebuatate). Betulic acid is a well-known topoisomerase inhibitor with antiretroviral and antimalarial properties extracted from the bark of White birch (Betal alba).

The lignans

Lignans are wide variety of polyphenols present in more than 55 plant families. Due to numerous arrangements of these polymerized polyphenols, they provide a wide array of chemical functionality. Lignans consists of two phenylpropane units linked by a C-C bond between the central atoms of the respective side chains.[21-23] Lignans have been implicated in reduction in the occurrence of certain types of estrogen-related tumors. They found in sesame seeds, black tea, soy milk and coffee, garlic, asparagus and carrots, lentils and beans.

The macrolides

Macrolides are microbial secondary metabolites. Those compounds have antibiotic and cytotoxic properties.[24-25] Today, they are recognized to be plant-derived natural products with anticancer properties synthesized by endophytic microorganisms.

Microbial Anticancer Agents [24]

- The anthracyclines
- The bleomycines
- The actinomycins

Marine anticancer natural products

The ocean covers 70% of the Earth's surface and is a vast reserve for the discovery of new natural products drugs. This huge environment is home to a fantastic range of diverse organisms and of the 28 major animal classes, 26 exist in aquatic regions and eight are exclusively aquatic. The discipline of marine natural product chemistry is comparatively young compared to phytochemistry with relatively small numbers of natural products having been reported.[26] At present, Ecteinascidin-743 is a marine natural product that is used as an anticancer drug clinically for soft-tissue sarcoma branded under the name Yondelis®. Ecteinascidin-743 is also under clinical trial for breast and prostate cancer.[27-28]

Further strategy

There are a number of potential strategies that can be applied to attempts to discover new natural product cytotoxic agents, such as: to obtain biomass in previously

unexplored environments and the collection of marine organisms. Many marine natural products have shown antimicrobial and anticancer activity but are not yet approved for clinical use.[29]

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Plant medicinal products and drug interactions

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ABSTRACT

Introduction. Some herbal medicinal products may be beneficial in certain respects but many can be dangerous for patients taking doctor-prescribed medications. Plant medicinal products are often taken with conventional drugs by patients. Interactions are possible between herbal medicinal products and conventional medications that can lead to toxicity due to increased drug plasma levels or drug treatment failure.

Aim. The aim of the study was to review the study of plant medicinal products and drug interactions.

Materials and method. Analysis of literature.

Keywords. drug interactions, natural products, drug, therapeutic material

Introduction

Synthetic drug interactions with plant-based medicinals can take place in several phases: at the stage of absorption of drugs from the gastrointestinal tract (pharmacokinetic phase), during drug metabolism by cytochrome P-450 enzymes (pharmacokinetic phase).[1-6] Conventional drug and plant medicinals may also exhibit additive and hyperadditional synergism during the pharmacodynamic phase.[7-10] Complications arise if an interaction lessens the intended effect or amplifies an adverse side effect.[11-14]

Disorders in the absorption stage of synthetic drugs are the main cause of therapy failure. Among the plant medicines contributing to this type of interaction of note are flax seeds (Lini semen) and the seeds of the plantago (Plantaginis ovatae semen). The absorption of synthetic drugs from the gastrointestinal tract is also influenced by their mobility through the digestive tract. Herbal laxatives containing extracts of aloe vera, rooting root, buckthorn bark, or senna leaves intensify intestinal passage, leading to a reduced absorption of conventional synthetic drugs.[15-20]

Interactions at the metabolic stage result from the inhibition or activation of cytochrome P-450 enzymes. Clinical St. John's wort (Hyperici herba) is an import-

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ant therapeutic resource that is known to disturb the metabolism of a variety of drugs. The active substances contained in St. John's wort extract include hyperoside, hypericin, and pseudohipericin induce the activity of P-450 type CYP3A4, CYP2C9 and CYP2C19 isoenzymes. As a result of the combined use of drugs that are substrates of these isoenzymes and St John's wort, there is a reduction in the therapeutic concentration of synthetic drugs in the blood. The most dangerous interactions with St John's wort include anticoagulants, immunosuppressants, antivirals (including those used in HIV therapy), non-steroidal anti-inflammatory drugs and statins. Flavonolignans, phytosterols and flavonoids are raw plant-based materials that also cause an increase in CYP3A4 activity. Decreasing the metabolism of drugs broken down by this route increases the bioavailability of these drugs and the result is a significantly elevated blood concentration of drugs that are substrates of CYP3A4. These medicines include erythromycin, diazepam, alprazolam, verapamil, loratadine, hydrocortisone, sertraline, dextromethorphan, indinavir, caffeine, and paracetamol.[21-26]

By definition, the traditional herbal medicinal products are those herbal medicinal products that have been used for at least 30 years, including at least 15 years within the EU and are intended to be used without the supervision of a medical practitioner and are not administered by injection. [26-30] Some examples of herbals used in traditional herbal medicinal products are: Calendula officinalis L.; Echinacea purpurea L., Moench; Eleutherococcus senticosus (Rupr. et Maxim.) Maxi; Foeniculum vulgare Miller subsp. vulgare var. vulgare; Foeniculum vulgare Miller subsp. vulgare var. dulce (Miller) Thellung; Hamamelis virginiana L; Mentha x piperita L. and Pimpinella anisum L.[26-30]

It is possible for herb-drug interactions to enhance treatment by improving drug bioavailability but harmful herb-drug interactions are understandably a higher priority in terms of public health. An herbal product will be considered a medicinal product when presented as having properties for treating or preventing disease in human beings or where it has a pharmacological, immunological or metabolic action. [31-33]

It is known that the anticoagulant warfarin is the most common drug involved in interactions with the herb St John wort. The concomitant use of St John's wort (*Hypercium perforaum*) with immunosuppressives (e.g. ciclosporin), antiretrovirals (e.g. indinavir), cardiac (e.g. digoxin) or antineoplastic (e.g. irinotecan) drugs may result in reduced plasma concentration of the drug and reduced efficacy by various mechanisms. Ginko biloba has been reported to cause spontaneous bleeding and may produce an additive effect with anticoagulants and antiplatelet agents. Herbal medicine may also interact with each other and many herb combinations. Herbal products may also be taken to enhance the effect of conventional drugs and reduce side effects showing the potential for beneficial herb-drug interaction. [34-36]

Pharmacodynamic interactions involve:
- receptor binding
- post-receptor effects
- systematic or organ effects
- chemical interactions

Interactions can sometimes be predicted on the basis of their pharmacology and chemistry. Pharmacokinetic interactions occur when drug Absorption Distribution Metabolism or Execretion (ADME) processes are altered by another drug and these are the most common types of mechanism (Figure 1).

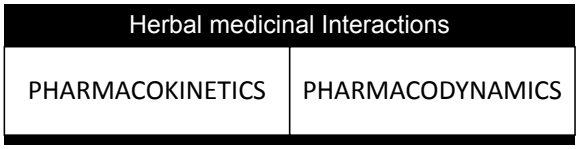

Figure 1. The main interaction classifications of herbal drugs

Table 1 illustrates the interactions activity between some drugs and herbal medicinal products.

Table 1. Some examples of documented interactions of herbs that may be taken in self- medication

Herbal Drug	Prescribed Drug	Interactions
Cranberry[5]	Warfarin	Increased plasma level of warfarin
Ginkgo biloba[8,9]	Tolbutamide	Decreased tolbutamide blood concentrations
Ginseng[10]	Phenelzine	Sleeplessness
Hibiscus[13]	Paracetamol	Changes in paracetamol pharmacokinetics
St John wort [15,16]	Alprazolam, amitriptyline, bupropion, imatinib	Decreased blood concentrations

Glycoprotein is a multidrug resistance protein that transport many important drugs. Glycoprotein is regulates drug bioavailability. In the intestinal epithelium, it pumps drugs back into the lumen; in the liver it excretes them into bile ducts; in the kidney it excretes them into the urine; and in the blood brain barrier it pumps them back into the capillaries.[27-30]

Conclusion

Herbal medicines are widely used by older adults and children and both groups have different rates of me-

tabolism. Older patients in addition to having a generally slower metabolism may take herbal medicines for degenerative conditions and are also more likely to be taking multiple medications. The use of herbal and nutritional supplements is increasing and the practice of integrated medicine is becoming more acceptable. This mean that the pharmacist and other health professionals may be asked by patients about the advantages (if any) of talking certain herbal medicines.

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Fundamentals of the use of *Berberis* as a medicinal plant

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ABSTRACT

Introduction. The daily use of medicinal plants has increased in recent years. The study of drugs of natural origin as an academic discipline and its applications in healthcare has changed remarkably but still focus on the quality of products and the development of new medicines.

Aim. This study covers all fundamental aspects of pharmacognosy as well as topics relating to the therapeutic use of plant drugs known as phythotherapy. The purpose of the study was to review the literature about the use of *Berberis.*

Materials and method. We reviewed the literature regarding the use of *Berberis* published between 1933 and 2018. We found more than 500 articles studying the properties of *Berberis* for digestive disorders, antibacterial, antidiabetic, hypotensive effects, anti-inflammatory effects, cholesterol regulation, cardiovascular disease, hyperlipidemia, cerebral ischemia trauma, mental disease, Alzheimer disease and osteoporosis. Our review includes recent studies regarding chemical composition and medicinal outcomes of *Berberis.*

Keywords. medicinal plants, phythotherapy, *Berberis*

Introduction

Plants have been the basic medical systems for thousands of years, particularly in China and India. Studies dealing with medicinal and other useful plants and their bioactive compounds have used many concepts and methodologies.

In the January 2018, The National Center for Biotechnology Information (NCBI) PubMed Data Base showed that the total number of publications regarding *Berberis* and its use is 500. The healing properties of *Berberis* have been known and appreciated for thousands of years. *Berberis* occurs in central and southern Europe, northwest Africa and western Asia. The genus *Berberis* includes about 500 species worldwide such as *Berberis vulgaris, Berberis aristate, Berberis darwinii*, *Berberis*

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dictyophylla, *Berberis julianae*, *Berberis thunbergii* and *Berberis verruculosa*.[1-3]

Figure 1. The basic applications and properties of *Berberis*. The percent of publications pertaining to individual Berberis properties are as follows:
– anti-inflammatory effects 20%,[7-9]
– antibacterial 16%,[10,11]
– cholesterol regulation 12%,[12,13]
– digestive disorders 12%,[14]
– antidiabetic 9%,[2]
– cardiovascular disease 9%,[15]
– anticancer 8%,[16,17]
– mental disease 4%,[18]
– Alzheimer disease 4%,[18]
– osteoporosis 3%,[18]
– hypotensive properties 2%,[19]
– cerebral ischemia trauma 1%,[15]

In natural medicine, *Berberis* leaves are also used being harvested in May or June and dried in natural drying rooms. The *Berberis* fruit which is harvested in August or September is also used for medicinal purposes. They are dried in heated ovens, initially at a temperature of about 30 degrees Celsius, and then further dried at 50-60 degrees Celsius. *Berberis* fruits are very rich in vitamin C; therefore they have a vitaminizing effect. *Berberis* species are rich in polyphenolic constituents such as anthocyanin and have shown significant free radical-scavenging activity.[4] *Berberis* species are rich in polyphenolic constituents such as anthocyanin and have shown significant free radical-scavenging activity showed that leaves and fruits of *Berberis crataegina* contain predominant phenolic compounds which includes rutin and chlorogenic acid.[4,5] A new a porphine base named O-methylcorydine-N-oxide together with berberine, palmatine, jatrorrhizine and oxyacanthine have been isolated from *Berberis chitria*.[6] Berberine has antimicrobial and ameocidical properties and is used either in the form of the pure compound or as a component of plant extract. Berberine is known to be a substrate of p-glycoprotein and to affect expression of cytochrome P (CYP) 450 enzymes, 3A4 and others.

Berberis leaves are used mainly for colds and for general strengthening of the body; in addition, the *Berberis* fruits have antipyretic effects. In folk medicine, *Berberis* has been used among others, for liver disease and other digestive ailments, e.g. digestive disorders or lack of appetite. *Berberis* has a diuretic effect, therefore it is recommended to use it during any problems with the urinary tract. The properties and basic applications of *Berberis* species are presented in Figure 1.

Chemical composition of *Berberis*

The main ingredient of *Berberis* are berberine and berbamine.[20] The chemical composition of *Berberis* include alkaloids, tannins and phenolic compounds. The triterpene lupeol, separated from fruits, and oleanolic acid, isolated from ethanolic extracts. Sterols stigmasterol, obtained from hexane extracts, and stigmasterol glucoside from ethyl acetate extracts. Alkaloids include berberamine and palmatine.[20] Other important alkaloids are oxyberberine, columbamine, isocorydine, lambertine, magniflorine and bisbenzlisoquinolines such as oxycanthine were reported to have been extracted from *Berberis* plants.[20] Leaves, bark and roots contain isoquinoline-derived alkaloids such as berberine, jatririzine, palmatine, and magnoflorin. Berberine is the main alkaloid, its content in leaves and root barberry is estimated at 1.5-2%. In addition, the leaves, bark and *Berberis* roots contain tannins, resin and wax. The fruits of *Berberis* contain pectin and organic acids, among others ascorbic (vitamin C, about 1.5%), whereas apples contain about 6% and lemons from 2 to 4%.[21] In addition, the fruit contains sugars (glucose, fructose) and a set of bioactive components that support the action of ascorbic acid. A dozen polyphenolic compounds (anthocyanins and flavonoids, including rutoside) and other plant dyes, i.e. beta-carotene and provitamin A that support visual processes and are helpful in dermatological diseases are also found. The red color of berries is provided by anthocyanin compounds and lutein, which, along with vitamin A, support the functioning of the eye.[21]

Below is a graph (Figure 3) showing an increase in the number of *Berberis* publications from 1933 to present.

Berberis has yellow flowers that bloom in May and June. In early autumn, it yields fruit as hard, oblong red fruits that appear on the twigs. The fruit has a sour and tart flavor. *Berberis* covers about 500 species, the most popular being grown, for example, in north-eastern re-

Figure. 2. Dry fruits of *Berberis*

gions of Iran. The cultivation of *Berberis* in South Khorasana dates back two hundred years. Mokhber-Dezfuli et al. in their work described the phytochemical and pharmacological activity of various species.[22] Whereas Srivastava et al. summarized the taxonomic, ethno-botanical, pharmacognostic, photochemical and pharmacological properties of many *Berberis* species.[23] The research team of Bhardwaj & Kaushik stated that due to the fact that when collecting plants, the whole plant is dug together with roots it is necessary to look at how root properties compare to leaves and fruit as the main compounds exhibiting beneficial properties exist in different plant parts.[24] Therapeutic uses have a long tradition in Asia in Chinese medicine and Ayurvedic medicine, where the bark and root of *Berberis* has been used for several thousand years. The main goal is to support the functioning of the intestines and liver as well as alleviate skin problems, cleanse the body and strengthen immunity. In natural medicine, barberry is widely used. It has a high content of vitamin C, so it is used for colds and fever as well as prophylactic. In addition, it positively affects our nervous system, used in neuroses and problems with insomnia. In addition, it has a beneficial effect on the level of concentration and a positive mood. In addition, it is used in people who are overweight, due to the fact that fruit tea has a positive effect on metabolism, additionally has a diuretic and slightly laxative effect. *Berberis* extracts show positive effects in applications for diarrhea, intestinal cramps and other gastrointestinal bowel disorders.[25] Another property of *Berberis* is antibacterial activity. Bark and root infusion is used for bacterial infections, due to their high content of berberine which has antibacterial properties. Bakht et al., in studies from 2017, evaluated the antibacterial activity of *Berberis* extract against gram-positive, gram-negative bacteria and fungi. Different fractions showed different degrees of antimicrobial activity.[26] A decoction of *Berberis* leaves has a positive effect on rheumatic pains and neuralgia. Thanks to its bactericidal properties, barberry has also found its use in cosmet-

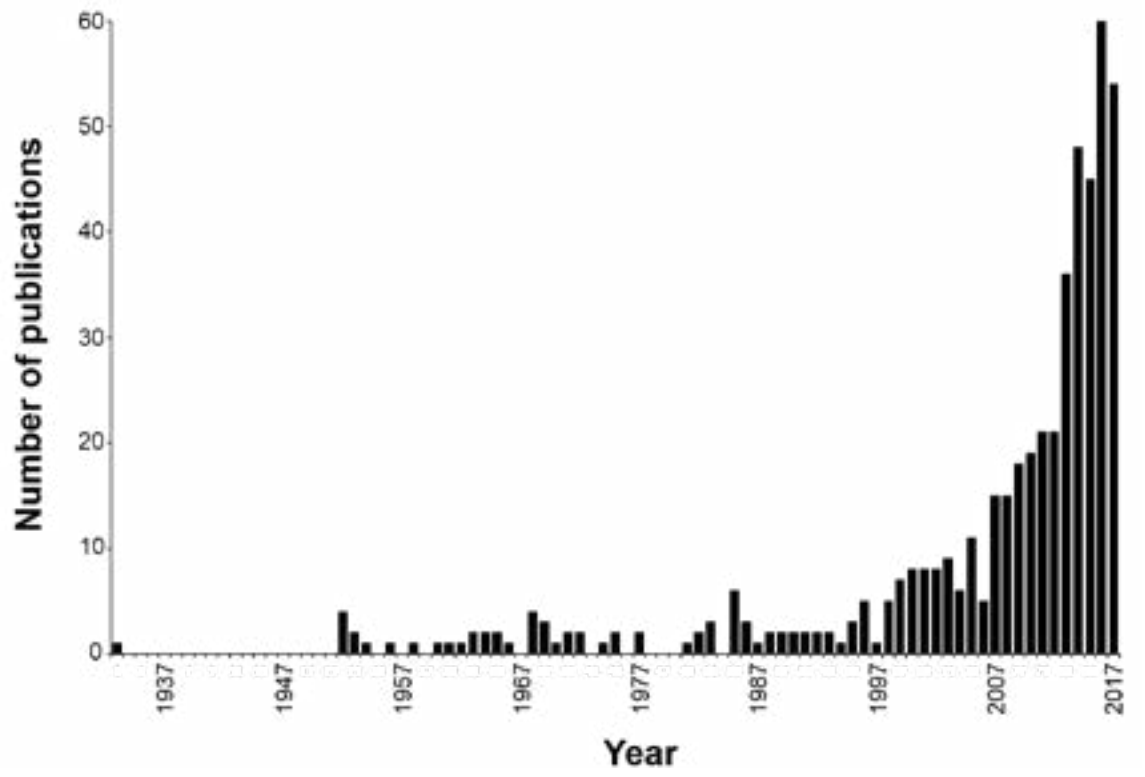

Figure. 3. Number of publications on *Berberis* collected from the Library of National Center for Biotechnology Information (NCBI) PubMed Data Base over the years starting from 1933

ics in the treatment of acne, and due to the high content of vitamin C, flavonoids and flavones, it is often used in preparations used for skin discoloration. Imenshahidi & Hosseinzadeh in 2016 presented in their work, the latest information on the pharmacological action of berberine used, inter alia, for the treatment of tumors, diabetes, cardiovascular disease, hyperlipidemia, inflammation, bacterial and viral infections, ischemic injuries, mental illness, Alzheimer's disease, and osteoporosis.[27] The Rahimi-Madiseh group assessed the possibility of using barberry fruits in the development of new medicines.[28] In the article by Imanshahidi & Hosseinzadeh the authors looked at traditional applications and pharmacological action of the active ingredient *Berberis vulgaris*.[29] *Berberis* has played an important role in the herbal treatment for over 2500 years. Arayne et al. presented barberry as a therapeutic agent used in homeopathic treatment of kidney pain and kidney stone removal.[30] In addition, these healing properties are appreciated in traditional Iranian medicine. Rahimi-Madiseh et al., based on a review of the literature, showed that *Berberis* contains a large number of positive substances that include ascorbic acid, vitamin K, several triterpenoids, more than 10 phenolic compounds, and more than 30 alkaloids. Due to these phytochemicals, its anti-cancer, anti-inflammatory, antioxidant, antidiabetic, antibacterial, analgesic and antinociceptive as well as hepatoprotective properties can be attributed.[28] The most important active ingredient contained in the bark and root of *Berberis* is berberine. In addition, isoquinoline alkaloids are include berbamin, palmatine and magnoflorine. Together, these substances are widely used in traditional medicine. They positively affect the metabolism and the entire digestive system. They especially support the metabolism of glucose and cholesterol and the proper functioning of the liver and gall bladder. *Berberis* leaf infusion is used for digestive disorders to relieve stomach aches, nausea and overeating. In addition, it can be helpful in stopping bile production and restoring normal function.[31] The use of the *Berberis integerrima* extract may also have a positive effect on insulin sensitivity. Fallah and their research group tested the effect of the extract on sensitivity in insulin-resistant rats with a high fructose diet. The results of the study revealed that *Berberis integerrima* may be a candidate for protecting against type II diabetes/insulin resistance through direct insulin-like effects and an increase in adiponectin levels.[32] In addition to insulin sensitizing, berberine has a cholesterol-lowering effect. Guarino et al. in their studies checked the effect of berberine and silymatin on abdominal fat in patients with overweight or obesity with concurrent type II diabetes. Based on the analysis of over 100 volunteers, a clinically significant effect was demonstrated in obese people with T2DM and metabolic syndrome.[33] In addition, berberine has a positive effect on the nervous system, showing sedative properties as it has been shown to minimize problems with insomnia and aid in lowering blood pressure. Fatehi et al. in their studies investigated the effect of *Berberis* extract on blood pressure in rats and the effect on potassium currents recorded from cells in the crescent and cerebellum which re-connect with the rat's brain. It has been noticed that a dose of extract of 0.05-1 mg/100 g of rat body weight exerts a positive effect. A significant reduction in mean blood pressure and heart rate was noted.[34] Thanks to this research, it can be concluded that barberry extract has a beneficial effect on the cardiovascular and nervous systems. The berberine present in the roots and bark is anti-inflammatory. *Berberis* berries, due to the content of pectins, also have anti-inflammatory effects. Both the roots and stem bark of *Berberis orthobotrys* have long been traditionally used to treat joint pain. The anti-arthritic potential was assessed *in vitro* using protein denaturation (bovine serum albumin and ovalbumin) and membrane stabilization methods at 12.5-800 μg/ml and *in vivo* using Freund's turpentine oil, formaldehyde and complete adjuvant models at the age of 50 at doses of 100 and 150 mg/kg. In conclusion, these results confirm traditional use of *Berberis* as a potent anti-arthritis agent will potential for the treatment of rheumatoid arthritis.[35] Sengupta et al. in their studies evaluated the antitumor potential of methanol extracts from the *Berberis aristata* root and Azadirachta indica seeds prepared by various extraction techniques in human osteosarcoma cells (HOS). *Berberis aristata* was found to be active against sensitive and drug-resistant HOS cells depending on the method of extraction.[36] Extracts from barberry are also used in cosmetics and for the skin in the form of ointments. Nimisha et al. in their studies investigated the effect of using a transferosomal gel with barberry extract on psoriasis. The gel contained *Berberis aristata* extracts (roots, ethanolic 70%). It has been demonstrated that the gel has anti-inflammatory and adjunctive activity against psoriasis.[8] Antioxidant activities of the ethanolic extracts of roots, twigs and leaves of common barberry *Berberis vulgaris* and *Berberis croatica Horvat* were studied.[37] The antimicrobial activity of hydroalcoholic extracts of four *Berberis* species *viz. Berberis aristata*, *Berberis asiatica*, *Berberis chitria* and *Berberis lycium* were tested against eleven bacterial and eight fungal strains. *Berberis aristata* root extract gave low MICs values against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus flavus* while stem extracts gave low MICs values against *Berberis cereus* and *Streptococcus pneumoniae.*

Conclusion

Berberis has pro-health effects. All components of barberry plants, apart from their flowers, are a raw material for obtaining biologically active substances with pro-

health properties. The abundance of medicinal compounds with barberry medicinal properties can be used to treat many diseases, especially those associated with inflammatory processes, and microbial etiology. Due to the fact that the substances contained in barberry are deposited in our body, it should be used very carefully, in addition, the therapy should not be prolonged.

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REVIEW PAPER

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Anticancer properties of *Viburnum*

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ABSTRACT

Aim. The aim of this paper is to provide an overview of the anticancer properties of different species of *Viburnum*.

Materials and methods. Forty nine papers that discuss the medicinal history and current research of *Viburnum species* as phytotherapeutic agent were used for this discussion.

Literature analysis. The results of scientific research conducted in vitro indicate that the compounds present in the extracts of Viburnum significantly affect the development of cancer cells such as leukemia, cervical cancer, breast, colon, lung, skin and stomach. This indicates that they may be used as a therapeutic agent to support oncological therapies.

Keywords. antitumor activity, *Viburnum species*, cytotoxic activity

Introduction

Viburnum (*Viburnum L.*) is a shrub currently belonging to the family *Adoxaceae* previously *Viburnaceae* or *Caprifoliaceae* and within the genus represents over 250 species around the world.[1] It is a species widely distributed in the temperate zone in central and southern Europe and North America and in the mountains of northern Africa and south-east Asia.[2-4] In Poland, the wild species found are viburnum coral (*Viburnum opulus*) (Figure 1 and 2) and viburnum hordowina (*V. lantana*).[5] It is a shrub blooming white (Figure 3) – marginal and middle pink-white flowers.

Figure 1. Fruit of viburnum coral (*Viburnum opulus)* (photo by Małgorzata Szczygieł)

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Figure 2. Shrub of viburnum coral with ripe fruits (photo by Małgorzata Szczygieł)

Figure 3. Shrub of viburnum coral with flowers (photo by Amelia Prus)

Many species of *Viburnum* are widely known and have been used for many years in folk medicine due to its valuable properties. They are also characterized by antibacterial, anti-inflammatory, sedative, hepatoprotective, antinociceptive and anti-asthmatic properties.[6-9] Numerous biologically active compounds are responsible for these properties which originate from various species of *Viburnum*.[10-16] The scientific literature presents the results of phytochemical studies confirming the presence of triterpenoid compounds [17-22], iodoids, [23-25] diterpenoid/diterpenes [26,7], vibroids, lignans [31-34], flavonoids [35-36], polyphenols [18-27], and vitamins in fruit, leaves, twigs of various species of Viburnum (table 1). These compounds are an important group among compounds derived from natural products in the prevention and treatment of tumors. Our review presents the characteristics of cytotoxic and antineoplastic extracts and compounds isolated from various species of *Viburnum*.

Waheed et al. (2013) in their study analyzed the obtained extract and fractions from the leaves of Viburnum foetens L. for interaction with human breast tumor cell lines (MDA-MB-468) and colon (Caco-2).[37] Based on the analysis of the obtained incubation results with these cell lines, they determined that the extract (organic solvents) and selected fractions inhibited cell proliferation.

Further researchers also undertook the analysis of Bibi et al. to assess Viburnum foetens cytotoxicity against breast cancer cell lines (MCF-7).[38] The phytochemical analysis of the crude extract from the leaves of Viburnum foetens showed the presence of anthraquinones, saponins, tannins, flavonoids and coumarins. On the other hand, the methanol fraction of the extract, less expensive than anthraquinones and saponins, was characterized by the highest high anti-cancer activity.

The above results suggested to researchers the need to undertake further more precise phytochemical analyzes of the obtained extracts and fractions from Viburnum in order to isolate and define specific compounds responsible for cytotoxic and anticancer properties.

This was accomplished by Fukuyma et al. by determining that the extract obtained from the species Viburnum luzonicum is a source of iridoid glycosides and aglycones (Table 1, item 1). Analysis of the results of incubation of these compounds obtained from the V. luzonicum extract with the same human epithelial tumor cell line (HeLa S3) allowed for an assessment of their cytotoxicity. It was demonstrated that glycosides **1** and **2** and agglutinates **5-9** showed moderate cytotoxicity against this line, while **3** and **4** showed no cytotoxic activity.

Researchers continued research on the isolation of further compounds from the extract from the dried leaves of Viburnum luzonicum. Spectroscopic methods confirmed the chemical structure of four iridoid aldehydes bearing (E) – or (Z) – p-coumaroyl group (**1**-**4**) (Table 1 item 2). The cytotoxicity test of these compounds also against the same HeLa S3 tumor cell line indicated that compounds **1**-**3** exhibited moderate cytotoxicity.[40]

In his research, Cheng et al. (2011)[41] isolated from an extract of Viburnum chingii leaves, oleanane triterpenoids (**1**-**2**) and vibesan diterpenoid (**3**) together with **7** other compounds (**4**-**10**) (Table 1, item 3). They then examined the cytotoxicity of these compounds to human cell lines myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), alveolar basal epithelial cells (A-549), breast (SK-BR-3) and epithelioid carcinoma (PANC- 1). The highest cytotoxicity for all cell lines was demonstrated by compound **3** and the lowest compounds **2, 4, 5, 9** and **10** with the exception of compound **4** showing no cytotoxicity to the SMMC-7721 cell line.

Li et al. (2015)determined that the branches and leaves of Viburnum odoratissimum var. odoratissimum contain diterpenoids and six known compounds with confirmed structure (Table 1, item 4).[42] Subsequently, they investigated the cytotoxicity assessment of newly

Table 1. Compounds isolated from various species *Viburnum*

Compounds	Method analysis -techniques	References
1. Viburnum		
I. iridoids glucosides: **1**. luzonoside A , **2**. luzonoside B, **3**. luzonoside C, **4**. luzonoside D, **iridoid aglycons bearing (*E*)- or (*Z*)-*p*-coumaroyl groups**: **5**. luzonoid A, **6**. luzonoid B, **7**. luzonoid C, 8. luzonoid D, **9**. luzonoid E, **10**. luzonoid F, **11**. luzonoid G	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared Spectroscopy (IR); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Fukuyama et al. (2004)[39]
2. Viburnum luzonicum		
II. aldehydes: **1**. luzonials A, **2**. luzonials B, **3**. luzonidials A, **4**. luzonidials B	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared Spectroscopy (IR); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Fukuyama et al. (2005)[40]
3. Viburnum chingii		
III. oleanane triterpenoids: **1**.1α,3β- dihydroxy-11α-methoxy-olean-12-ene, **2**.1α,2β--dihydroxy-olean-9(11),12-diene, **3**. vibsane-type diterpenoid: **IV.** vibsaol B, **5**. 2α,3β-dihydroxy-20(29)-lupene, **6**. 6α-hydroxy-3-on-20(29)-lupen-28-oic acid, **7**.3,6-dion-20(29)- lupen-28-oic acid, **8**. hederagenin acid, **9**. castanopsone, **10**. 3α,6β -dihydroxy-20(29)-lupene	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared Spectroscopy (IR); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Chen et al.(2011)[41]
Viburnum odoratissimum var. odoratissimum		
IV. diterpenoids: **1**. dehydrovibsanin G, **2**. (+) - 9′-*0*-senecioyllariciresinol, **3.** vibsanin C, **4**. vibsanin H, **5**. (8 Z) -10-epivibsanin C, **6.** (+)-9′-*0*-isovaleryllariciresinol , **7**. 9-aldehynevibsanol, **8**. vibsanol	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared Spectroscopy (IR); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Li et al. (2015)[42]
Viburnum odoratissimum		
V. vibsane-type diterpenoids: **1**. vibsanin A,	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Yu et al. (2016) [43]
Viburnum odoratissimum		
VI. diterpenoids: **1**.vibsanol C, **2**. vibsanol D, **3**. vibsanol E, **4**. vibsanol F, **5**.Vibsanol G, **6**. vibsanol H, **7**. vibsanin X.	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared (IR); Electron Impact Mass Spectrometry (EIMS); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	He et al. (2016) [44]

Viburnum mongolicium		
VII. nor-dammarane triterpenoids: **1**. 3β,12β-dihydroxy-25,26,27-trinordammara-22-en -24,20-olide , **2**. 3β,12β-dihydroxy-24α-methoxy-25,26,27-trinordammara-20,24-epoxy, **3**. 3β-*0*-acetyl-12β-hydroxy-23,24,25,26,27-hexanordammarane-20-one **4**.12β-*0*-acetyl-15α-hydroxy-17β-methoxy-3-oxo-20,21,22-23,24,25,26,27-octanordammanrane, **5**. 12β-*0*-acetyl-15α,17β-dihydroxy-3-oxo-20,21,22-23,24,25,26,27 -octanordammanrane, **6**.12β,15α-dihydroxy-3-oxo-17-en-20,21,22-23,24,25,26,27-octanordammanrane , **7**.12β-hydroxy-3-oxo-24α-methoxy- 25,26,27- trinordammara-20,24-epoxy, **8**. 3β,12β-dihydroxy-23,24,25,26,27-hexanordammarane-20-one		Wang et al. (2013) [45]
Viburnum sambucinum Reinw. ex Blume		
	Mass Spectrometry (MS) Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C).	Nguyen et al. (2017) [46]
Viburnum hainanense Merr. et Chun		
VIII. nordammarane triterpenes: **1**.12β-O-acetyl-17β-hydoxy-3,15-dioxo-20,21,22,23,24,25,26,27-octanordammanran, **2**.12β-hydoxy-17β-methoxy-3,15-dioxo-20,21,22,23,24,25,26,27-octanordammanran, **3**. 3-12β-Oacetyl-3,15-dioxo-17-en-20,21,22,23,24,25,26,27-octanordammanran, **4**.12β-hydoxy-15α-O-acetyl-3-oxo-17-en-20,21,22,23,24,25,26,27-octanordammanran, **5**. 3β-hydoxy-17-oxo-12-en-20,21,22,23,24,25,26,27-octanordammanran	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); Infrared (IR); Ultra Violet (UV); Electron Impact Mass Spectrometry (EIMS); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	Wang et al. 2016 [47]
Viburnum awabuki		
X. vibsane-type diterpenoids:, **1**.vibsanin P, **2**.vibsanin Q, **3**. vibsanin R -W **4**. vibsanin S, **5**.vibsanin T, **6**. vibsanin U, **7**. vibsanin V, **8**. vibsanin W	Nuclear Magnetic Resonance (NMR ^{1}H and ^{13}C); High Resolution Electrospray Ionization Mass Spectrometry (HR-ESI MS).	El-Gamal et al. (2004) [48]

discovered diterpenes against human tumor cell lines: myeloid leukemia (HL-60), skin (A-431), colon (HT-29), breast (T47-D) and lung (A-549). Both new diterpenoids (**1**, **2** compounds) showed inhibitory activity against the human tumor cell lines A431 and T47D. Compound 1 showed higher inhibitory activity on these two cell lines than compound 2.

In the work of Yu et al. (2016) a study of vibsanin A, a vibrate diterpenoid isolated from leaves and twigs Viburnum odoratissimum against the human myeloid leukemia cell line (HL-60) was performed (Table 1, item 5).[43] It was determined that vibsan A induces the differentiation of myeloid leukemia cells. These results indicate that vibsanine A is a powerful tool to understand the potential pathophysiological and therapeutic roles of PKC and justifies further assessment as a potential therapy for differentiating myeloid leukemia.

In subsequent studies, also the analysis of the extract of leaves and twigs Viburnum odoratissimum confirmed the new vibansan diterpenes, vibsanol C-H (**1**-**6**) and vibsanin X (**7**) (Table 1, item 6).[44] Their cytotoxicity was evaluated against human tumor cell lines. Based on the results of the tests, it was found that compound **1** showed significant cytotoxicity to all human cell lines tested: myeloid leukemia (HL-60), cancer liver (SMMC-7721), cancer lung (A-549), breast cancer (MCF-7) and cancer colon (SW-480). Compounds **4** and **5** showed only significant cytotoxicity against the SMMC-7721 cell line, while compounds **3**, **6** and **7** are not cytotoxic.

Successive researchers (Wang et al., 2013) determined that another species Viburnum mongolicium is a source of triterpenoid (Table 1. item 7).[45] Isolated compounds were incubated with 7 human tumor cell lines: lung (A-549), gastric carcinoma (BGC-823), hepatocellular carcinoma (HepG2), myeloid leukemia (HL-60), breast (MCF-7), hepatocellular carcinoma liver (SMMC-7721) and colon (W-480)) to assess their potential for cytotoxicity and antioxidant properties (free radical scavenging activity). Compounds **4**-**6** showed the highest cytotoxic activity against all tumor cell lines tested and antioxidant properties. However, compounds **2** and **7** showed lower values, while compounds **3** and **8** showed the lowest cytotoxic potential. Compound **1** was determined to have no cytotoxic activity.

Nygem et al. showed that leaf extracts from V. sambucinum Reinw. ex Blume contains, among others, dammarane type triterpenoid and other compounds (Table 1. item 8).[46] Analysis of the interaction of the isolated compounds from the extract of this kind of Viburnum relative to the following human cell lines were tested: epithelial carcinoma in the mouth (KB), hepatocellular carcinoma (HepG-2), cancer lung (LU-1) and breast cancer (MCF-7). The dominating activity revealed derivatives of octaordamarane compounds **6** and **7** in all analyzed 4 cell lines. In contrast, compound **1** was more active against LU-1, HepG2 and MCF7 cell lines than KB cell lines, and ursolic acid (**10**) only with LU-1 and MCF-7 cell lines. The compounds **5,9,13,15,16** were characterized by cytotoxic activity and the remaining compounds by non-cytotoxicity.

On the other hand, it was determined by the spectral methods that the extract from the entire plant Viburnum Hainanense Merr. et Chun are nor-dammaran triterpenes (Table 1. Item 9).[47] Isolated compounds were tested for their cytotoxic properties to human tumor cells: cervix carcinoma (Hep-2), skin squamous cell carcinoma (SCL-1), squamous cell carcinoma (CAL-27), head and neck squamous carcinoma (UMSCC-1), pharyngeal carcinoma (Detroit 562) and squamous carcinoma (TCA-83). It was determined that compounds **1**-**4** showed cytotoxicity against all tested cancer cell lines resulting, inter alia, in from the presence of the corresponding tri-terpenes of an acetylene group or a hydroxyl group in the C-12, α, β-unsaturated ketonic at C-15 position.

A vibsane -typ diterpenoids / vibsane diterpenoids (**1**-**8**) were identified in the extract fractions of leaves and twigs Viburnum avabuki K. Koch. (Table 1, item 10).[48] Their cytotoxicity was tested against the cell lines: A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia). They showed significant cytotoxic properties against the P-388 mouse cell line compounds **1** and **8**, and lower cytotoxicity of compounds **2**-**7** against the same cell line. However, in comparison to other human tumor cell lines, compounds **1**-**8** were characterized by moderate cytotoxic properties.

Conclusion

Plants of the genus Viburnum are very popular among medicinal plants. They are a rich source of compounds with biological activity, especially anti-proliferative, against numerous human tumor cell lines, which is confirmed by the results of scientific research presented in our article. This suggests that isolated compounds from Viburnum may be an important element in the prevention and treatment of cancer, of course after a positive assessment of their interaction with the recommended drugs. It is necessary to conduct further research on the identification of further active components of Viburnum extracts to assess their cytotoxic activity and anti-cancer properties, as well as to explain the course of changes at the cellular level.

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REVIEW PAPER

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Carcinoembryonic antigen as a tumor marker in lung cancer – is it clinically useful?

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ABSTRACT

Introduction. Lung cancer is the most common cancer in the Western world. Annually there are approximately 1.8 million new cases worldwide. It is characterized by poor prognosis with a 5-year survival of 10-17% depending on the country. Contributing to this poor prognosis is a mainly late diagnosis, as well as a fairly frequent recurrence despite radical surgery. Over the years, scientists have been searching for a tumor marker that would be useful for patients with lung cancer.

Aim. The aim of this study is to discuss the significance of carcinoembryonic antigen (CEA) in the diagnosis, prognosis of the disease course, and monitoring patients with lung cancer.

Methods. Review of the literature using the PubMed database, Termedia, Via Medica and the key issue: carcinoembryonic antigen as a tumor marker in lung cancer.

Conclusions. Serum CEA level can be a reliable complement to the diagnosis of lung cancer. It can be helpful in preoperative prediction of disease course and qualification for adjuvant treatment of non-small cell lung cancer especially adenocarcinoma. Trends and normalization of CEA during chemotherapy have an impact on progression-free survival and overall survival (OS) of patients. Various available publications describe CEA as a marker for metastatic lung cancer, which is the most specific for metastasis in the liver and brain.

Key words. carcinoembryonic antigen, lung cancer, prognostic factor, tumor marker

Introduction

Lung cancer is the most common malignant tumor in highly developed countries, but it is also becoming a major health problem in developing countries. According to the data published in 2012 by the International Agency for Research on Cancer (IARC), around 1.8 million new cases of lung cancer are diagnosed worldwide, which constitutes 13% of all malignant tumors. This cancer is the leading cause of neoplastic death in men and is the second most common cancer in women[1]. It is estimated that the number of lung cancer cases has increased by 51% worldwide since 1985 (44% in men and 76% in women)[2], and WHO indicates that the number of deaths will continue to increase, which is mainly the consequence of a significant increase in tobacco smoking. In Poland, according to the National Cancer

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Registry (*Krajowy Rejestr Nowotworów* in Polish), about 21,000 new cases are detected annually, which accounts for 14% of all new cancer cases[3]. A distinctive feature of lung cancer is a poor prognosis with a 5-year survival rate of 17% in the US, 12.3% in Europe, and 10% in the UK[4]. In Poland, it is around 11% for men and 16% for women[5].

Lung tumors are characterized by different microscopic structures requiring different clinical courses and treatment methods. Pathomorphological classification distinguishes two most common types of lung cancer: small cell lung cancer (SCLC) (about 15%) and non-small cell lung cancer (NSCLC), which includes: adenocarcinoma (40%), squamous cell carcinoma (30%), and large-cell carcinoma (10%)[6]. With regard to the numerous subtypes of lung cancer, the most important is the differentiation between SCLC and other types of NSCLC. This distinction is important due to clinical differences regarding the course of the disease, the presence of metastasis and the response to treatment. Small cell cancer is a tumor with high growth dynamics, which is characterized by high sensitivity to chemotherapy and radiotherapy, and because of the early metastatic cancer, the prognosis is bad. Although the cancer cells are small, they show the ability to grow and multiply extensively, which leads to early blood-borne dissemination. Non-small cell lung cancer is moderately susceptible to chemotherapy and radiotherapy. Therefore, surgery plays a major role in radical therapy. Individual subtypes of non-small cell carcinomas also differ. Adenocarcinoma develops from glandular cells located in epithelium lining the airways. It is most often located on the periphery of the lung and sometimes (at an early stage) metastases to the lymph nodes or distant metastases may develop. In addition to chemotherapy, molecularly targeted drugs are used. Squamous cell carcinoma usually locates centrally and is characterized by slower growth and subsequent metastases than other types of lung cancer.[6,7]

A poor prognosis in patients with lung cancer is caused mainly by the late occurrence of clinical symptoms. In 80% of cases, it is diagnosed at the stage of regional spread or distant spread, which results in much less effective treatment. Lung cancer at the dissemination stage is characterized by a 5-year survival rate of around 4%.[4] Moreover, according to various authors, 25–55% of patients suffer relapse after radical surgical treatment.[8-10] A relapse usually occurs in the form of metastases. This suggests the presence of micrometastases, not detected at the time of diagnosis and qualified for treatment [11]. This proves that current conventional diagnostic tests such as X-ray, chest CT scan and bronchofiberoscopy are not sufficient to estimate accurately the stage of the disease. Therefore, over the years there have been opportunities to study markers helpful in diagnostics, and particularly in monitoring treatment.

A tumor marker is a macromolecular substance produced in a tumor cell or by normal host cells in response to a developing tumour, and then excreted to the circulation or other body fluids[12]. An ideal marker is one that meets the following criteria: it can be used in population screening, assessment of the disease, monitoring the treatment and post-treatment control.

In the case of lung cancer, a sufficiently sensitive and specific factor has not been found so far that could become an ideal cancer marker. Regarding the markers that could be helpful for patients with lung cancer it is worth mentioning: CEA, CYFRA 21-1, Ca125, NSE, SCC, Pro-GRP, Ca19-9. With reference to these markers, the most promising but also controversial idea is raised by the carcinoembryonic antigen (CEA).

CEA is one of the most frequently used tumor markers in the world. It was first described by Gold and Feedman in 1965. It is a glycoprotein from the family of membrane proteins with a molecular weight of about 180–200 kDa, produced in the fetal period by cells of the digestive tract and pancreas, and after the birth by cells of the intestines, pancreas and liver. In healthy people, CEA concentration is below 5.0 ng/ml, whereas in people who smoke tobacco, it is higher, but usually does not exceed 10 ng/ml. T1/2 CEA is 2-8 days.[13,14] The increase in CEA in the blood or other body fluids is caused by various factors. This may be an increase in the number of cells producing CEA, an increased synthesis in tumor cells, or a reduction in the possibility of excretion from the body. Although the elevated concentration of CEA was first described in the case of colon cancer which is the leading marker in this case, it is also produced and released into the circulation in other various cancers. Generally, it is most likely produced by adenocarcinomas which are developed in the intestine, pancreas, stomach, thyroid, cervix, endometrium, prostate, urine bladder, breast, lung and it can be also produced by ovarian cancer. An elevated level of CEA may occur in such cancers as: neuroblastoma, sarcomas, lymphomas as well as in the case of cirrhosis of the liver, hepatitis, pancreatitis, peptic ulcer disease, chronic lung diseases, inflammatory diseases of the large intestine, nicotinism, and also during pregnancy.[13] However, these disorders are usually temporary and cause only a slight increase in CEA, rarely above 10 ng/ml.

The aim of this study is to discuss the significance of carcinoembryonic antigen in the diagnosis, prognosis and monitoring of patients treated for lung cancer on the basis of available publications.

CEA in small cell lung cancer

There is no relationship between serum CEA concentration and disease progression, progression-free survival (PFS), overall survival (OS), and no evidence has been found that its level is correlated with an objective re-

sponse to chemotherapy.[15,16] Although in the 1980s, the usefulness of this marker in small cell lung cancer was described, current studies have not confirmed the significance of this marker.

CEA in non-small cell lung cancer

Diagnostics

Due to a low level of sensitivity and specificity, the determination of CEA level is not applicable in screening tests. So far, no marker has been found that is appropriately sensitive to lung cancer, which would be useful in diagnosis.

It is very important to differentiate between benign and malignant lung diseases properly. Making an early diagnosis gives a chance for effective treatment of one of the most dangerous cancers in the world, however, based on the available basic tests, targeting the diagnosis to detect cancer early is difficult. Clinical symptoms of lung cancer appear late, which together with diagnostic difficulties result in the fact that only 20% of patients can undergo surgery. Therefore, a lot of research was done in order to search for a marker that would help in distinguishing malignant lung diseases. It was proved that serum CEA concentration is significantly higher in the case of malignant neoplasms of the respiratory system and it was found that their much higher values were observed in adenocarcinomas.[17,18] Therefore, CEA may be a useful indicator in the diagnosis of lung cancer, especially adenocarcinoma. The results of the above studies indicate that the increase in serum CEA concentration may become a reliable complement to computed tomography in the diagnosis of lung cancer, where CEA may appear as a single tumour marker or in a panel with other markers such as CYFRA 21-1, NSE. The combination of several markers increases the clinical effectiveness of diagnostics, but it also increases its costs.[17-19]

It was observed that elevated concentrations of CEA in bronchoalveolar lavage (BAL) fluid may also be useful in the diagnosis of lung cancer. Therefore, Ghosh, Charalabopoulos and Dąbrowska, based on performed analyses, indicate that in regards to non-small cell lung cancer, the CEA concentration in bronchoalveolar lavage fluid is significantly higher than in mild lung diseases.[20-22] However, it should be noted that it is also higher in people who smoke, which means that tobacco causes cell changes in the bronchial cells resulting in an increase in CEA secretion.[20,21,23] Charalabopoulos suggests that cigarette smokers diagnosed with mild lung diseases and high levels of CEA in BAL may be predisposed to develop lung cancer in the future.[21] Based on the above studies, it can be concluded that the measurement of carcinoembryonic antigen in BAL may be useful in the diagnosis of non-small cell lung cancer, but not as a single test, but as a complement to standard tests.

Pre-operative CEA concentration

Numerous reports indicate that elevated pre-operative serum CEA concentration is associated with more advanced cancer disease and the risk of recurrence.[24-26] A study conducted by Tomita et al. indicated that CEA level is an independent prognostic factor in patients with adenocarcinoma of the lung and confirming its growth before the operation suggests a worse prognosis despite an early diagnosis.[24] Matsuoka et al. observed a relationship between elevated levels of CEA for stage I lung adenocarcinoma, and a shorter progression-free survival (PFS), total survival (OS) and an early recurrence. This correlation was not confirmed in the case of squamous cell carcinoma.[25] Buccheri noted that the CEA level> 10 ng/ml at stage Ia to IIb was associated with a 67% risk of early recurrence of lung cancer after a radical surgical treatment.[26] Okada et al. emphasized that not only the elevated concentration of CEA is significant in the prognosis, but also the lack of normalization of the marker after the surgery was characterized by a worse outcome.[27] Muley et al. found evidence, after analyzing the significance of the TMI index (tumour marker index) presenting the geometric mean of normalized values of CEA and CYFRY 21-1, that elevated TMI values are a prognostically negative survival rate in non-small cell lung cancer in stage I.[28] Both Buccheri and Okada together with Muley did not observe differences between histological types, i.e. adenocarcinoma and squamous cell carcinoma. On the other hand, other authors stated that neither the evaluation of CEA level nor the TMI index are statistically significant in a prognosis regarding the course of the disease, and elevated CEA is not associated with a worse prognosis.[29]

In the course of publishing subsequent works, the question has appeared whether there is a relationship between elevated CEA concentration, histological type of lung cancer, and a worse prognosis. Some researchers showed a relationship between elevated levels of CEA and a worse prognosis only for adenocarcinoma of the lung.[25] However, there were also studies showing that in the case of squamous cell carcinomas, the CEA concentration> 5 ng/ml concerned 26% of patients in stage IIIB and 53% in stage IV.[30] Many authors have not found a significant difference between histological types of lung cancer. In some publications, the researchers analyzed patients with non-small cell lung cancer without division into particular histological types. Moreover, the opinion of some researchers that CEA plays a role in predicting metastases to mediastinal lymph nodes[31,32] has also been an issue of concern, while others have denied this statement.[24]

Monitoring the treatment

Several studies have suggested that CEA levels may become a predictor of response to the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib in patients with adenocarcinoma of the lung. Some authors claim that an elevated concentration of CEA before starting therapy may indicate a better response to TKI treatment, longer PFS and OS.[33-36] In their work Romero-Ventosa et al. reported that patients with CEA> 5 ng/ml indicated a significantly better response to treatment with inhibitors, and the median overall survival was 10.2 months.[35] The mechanism of this phenomenon has not been clarified. Jin et al. noted that the frequency of EGFR mutations was significantly higher in a group of patients with elevated levels of CEA, compared to the group with proper levels. In the group of patients with CEA <5 ng/ml, the frequency of EGFR mutations was 55%, whereas in the group with CEA> 20 ng/ml – 82%. On this basis, it can be concluded that elevated serum CEA concentration may help to predict the presence of EGFR mutations.[37] Moreover, it is worth mentioning that there are other studies that contradict the above data, indicating the opposite conclusions. Kappers et al. argue that in patients treated with gefitinib or erlotinib, it is observed that low concentration of CEA indicates a better prognosis.[38] Moreover, Chen et al. claim that patients with CEA levels> 32 ng/ml have a shorter PFS and OS, but not only the antigen output level is important, but also the tendency and normalization during treatment of the first TKI line. Those patients who had a decrease in CEA by more than 35% during the month with normalization had the longest PFS and OS.[39] Other studies also demonstrated the prognostic significance of CEA during standard chemotherapy (the inclusion criterion was the output concentration CEA> 10 ng/ml in patients in III-IV stage) and it was observed that the decrease by 14% correlated well with OS and PFS, while the increase by more than 18% might be a measure of disease progression.[40]

CEA as a metastatic marker

The carcinoembryonic antigen is helpful in detecting recurrences or distant metastases, mainly in colorectal cancer, but it may concern many cancers. The positive predictive value of CEA increase for confirmation of progression is over 90% and it can be considered as a universal marker of tumour metastases.[41] In many metastatic patients, regardless of the location of the primary disease, an increase in the marker is observed, even if it was normal before the treatment. This dependence has led to many studies to explain the phenomenon of carcinoembryonic antigen in metastatic processes. We know that neoplastic transformation induces intense CEA production. Tumour cells released from the primary tumour have significant amounts of the cellular form of CEA on their surface, which undergoes exfoliation and creates a free soluble form of the antigen. It is the content of the free form that increases at the onset of the neoplastic process or after its treatment - as an indicator of recurrence.[41] The relationship between CEA concentration and a poor prognosis forced scientists to conduct studies on the participation of the CEA cellular form in metastasis. It has been proven that as a result of homotypic interactions, it can aggregate tumour cells circulating in the blood, thus it increases their survival and makes them easier to remain in the bloodstream.[41,42] Researchers found receptors presenting the ability of bounding CEA on Kupffer cells in the liver and alveolar macrophages.[43] In conclusion, CEA acts as an adhesion molecule and "chemo-attracting", it can activate Kupffer cells, stimulate IL-1β, IL-6 and TNF-α and thus promotes adhesion of tumour cells to endothelial cells and facilitates the migration process resulting in tumour spread.[44,45]

It should be noted that many researchers confirmed the fact that high concentration of CEA is much more frequently related to patients with the M1 feature compared to M0. On this basis it seems reasonable to claim that CEA is associated with the development of metastases and a worse prognosis in advanced lung cancer.[46-50] There was a clear relationship between the CEA level and liver metastases, then the highest serum CEA concentrations were also observed.[46] Lee et al., based on the analysis of the history of 377 patients newly diagnosed in stage IV of non-small cell lung cancer, reported that elevated levels of CEA were strongly associated with generalized metastases in advanced lung cancer. This correlation was evident in adenocarcinoma type, with bone metastases, CNS, lungs and mediastinal lymph nodes. Very high concentrations of CEA (above 100 ng/ml) indicated a relationship with metastases to the abdomen and pelvis.[46] Arrieta et al. claim that the high concentration of this antigen is an independent prognostic factor for the development of CNS metastases in advanced non-small cell lung cancer. In his study, the specific factors were adenocarcinoma type and CEA concentration > 40 ng/ml. He showed that within 2 years of diagnosing lung cancer in stage IIIB-IV, 67% of patients with CEA> 40 ng/ml and 20% with CEA <40 ng/ml had brain metastases. In addition, he suggested that surface expression of CEA in tumour cells may be a pathophysiological mechanism of invasion of neoplastic cells to the CNS by bounding with immunoglobulins and transport across the blood-brain barrier.[47] It should also be taken into account that CEA is usually measured at higher concentrations in the cerebrospinal fluid of patients with brain metastases.[48] Other authors also emphasize the relationship between this marker and metastases in the CNS.[49] In turn, other researchers who also demonstrate the prognostic signif-

icance of CEA in predicting neoplastic dissemination, did not observe the relationship between it and the site of metastases. They were found in the bones, OUN, liver, adrenal glands. However, it was indicated that the concentration of CEA is significantly higher in the case of adenocarcinoma of the lung.[50]

Summary:

The carcinoembryonic antigen as a cancer marker in lung cancer has been analyzed by many researchers since around 1980. On the basis of numerous publications, the following conclusions can be drawn:

1. CEA can become a reliable complement to imaging tests and bronchofiberoscopy in the diagnosis of lung cancer, especially in doubtful cases when differentiated with benign lung diseases.

2. The initial CEA assessment may be helpful in pre-operative prognosis of the course of the disease. A high serum CEA concentration is associated with more advanced cancer, early recurrence and a worse prognosis after a primary resection. Prognostic factors such as CEA or TMI can help distinguish patients with NSCLC who may benefit from adjuvant therapy.

3. The role of CEA in predicting the course of TKI treatment is controversial, but it has been proven that a higher level of CEA correlates with the presence of EGFR mutations necessary for qualifying for TKI treatment and conditioning the response to treatment. Trends and normalization of CEA level during chemotherapy have an effect on PFS and OS of patients with adenocarcinoma of the lung.

4. Some authors emphasize the clinical significance of elevated CEA concentration only for adenocarcinoma of the lung.

5. CEA can be considered as a universal marker of neoplastic metastases also in the case of lung cancer. It is the most specific for the metastases appearing in the liver and in the CNS.

Despite these reports, CEA has not been included in pre-operative assessment, chemotherapy monitoring and follow-up standards. The guidelines of the American Thoracic Society and the European Respiratory Society in lung cancer do not recommend the routine determination of any markers, as well as the search for distant metastases in imaging studies in asymptomatic patients.51

However, on the basis of the aforementioned publications, it is worth considering performing imaging tests in order to exclude metastases in patients with elevated serum CEA concentration, even in the absence of clinical symptoms. This would allow the researchers to identify a group of patients with an increased risk of disease spreading. Despite so many studies and publications, there is still no results that could unambiguously confirm the usefulness of establishing serum CEA concentration in patients with lung cancer and would convince the American Thoracic Society and the European Respiratory Society to change their position.

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CASUISTIC PAPER

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Ramsay Hunt syndrome with deep hearing loss and meningitis

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ABSTRACT

Introduction. Ramsay Hunt syndrome is a clinical manifestation of varicella zoster virus reactivation. It is characterized by an erythematous vesicular rash in the external auditory canal and pinna with otalgia, vertigo and ipsilesional facial palsy. Symptoms develop over a few days with prodromal signs of facial weakness, tingling, facial numbness. Usually, cranial nerves VII and VIII are involved in the inflammatory process. Possible consequences of Ramsay Hunt syndrome are hearing loss, encephalitis and meningitis.
Description of the case report. The authors present the case of a 63-year-old woman with a vesicular rash, earache, vertigo and left-sided facial paralysis who was treated with antiviral drugs and analgesics. These symptoms were complicated by conductive hearing loss in the left ear and meningitis. After treatment facial paralysis decreased. Unfortunately, hearing loss was permanent.
Discussion. Rapid administration of antivirals and corticosteroids limited facial paralysis and improved facial expression. The prognosis for facial palsy is poorer in Ramsay Hunt syndrome than in idiopathic forms.
Conclusions. A past history of vertigo and hypertension could been a predisposing factor for the severe manifestation of Ramsay Hunt syndrome and subsequent complications.
Key words. facial palsy, hearing loss, meningitis, Ramsay Hunt syndrome

Introduction

Ramsay Hunt syndrome (RHS) is characterized by an erythematous vesicular rash in the external auditory canal and pinna with severe otalgia and ipsilesional facial palsy. It is a clinical manifestation of varicella zoster virus (VZV) reactivation. Neurological complications include changes in cerebrospinal fluids, peripheral motor neuropathy, aseptic meningitis, and cranial polyneuropathy. Both cranial nerves VII and VIII are commonly involved in the inflammatory process, producing vestibulocochlear symptoms such as vertigo, hearing loss, and tinnitus and symptoms of peripheral facial paralysis. Among these, vertigo, hearing loss, and tinnitus most commonly occur in patients with RHS.[1-4]

Description of the case report

A 63-year-old woman with a past medical history of vertigo and hypertension first presented a left-sided headache and burning for the past few days. One day later she had developed multiple vesicles on the left side of her face. She complained of earache and vertigo. ENT examination did not reveal any inflammatory process

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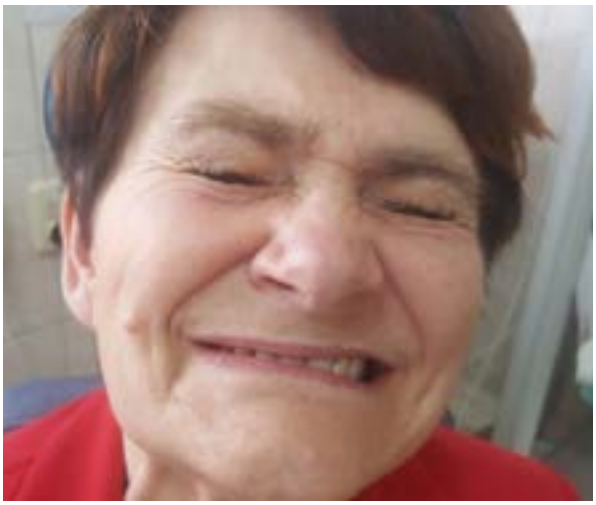

Figure 1. Facial paralysis on the left side (3rd degree according to the House-Brackmann grading scale) after six month

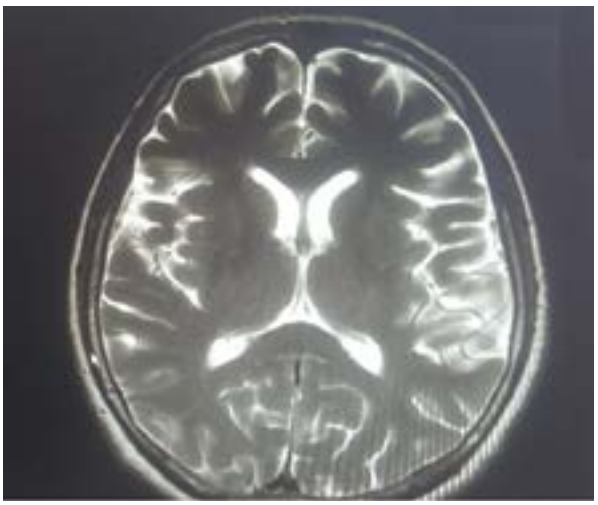

Figure 2. Changes in MRI examination

of the left ear. Computed tomography (CT) of the head revealed no changes. An ENT examination revealed a vesicular rash in the pinna of the left ear, peripheral paralysis of nerve VII on the left side (V degree according to the House-Brackmann grading scale) and deep conductive hearing loss (60-70 dB in audiometric tests). Brain magnetic resonance imaging (MRI) detected a high signal intensity lesion in both a T_2-weighted image and fluid-attenuated inversion recovery (FLAIR) apparent in white in both parietal patches, most likely vascular-related. Intraventricular fluid spaces were normal. In addition, the brain structure was without focal changes and with the correct signals. After intravenous administration of the paramagnetic agent, there was no focus of pathological contrast enhancement. On the other hand, there was a slightly more intense contrast enhancement of the mid-range and the front of the skull, which could be attributed to the inflammatory process of the meninges. Air entrapment of the pneumatic pyramidal cells of the left temporal bone was significantly reduced- probably by inflammatory lesions. Laboratory tests detected WBC 7.2 HGB 12.6 g/dl, CRP 2.35 mg/l. She was treated with antiviral drugs: acyclovir 800 mg 5 times daily every 4 hours with a night break. Tramadol hydrochloride (37.5 mg) and acetaminophen (325 mg) were administered 4 times daily to alleviate pain, carbamazepine 200 mg daily and ketoprofen 100 mg and metamizol 0.5 mg intravenously once ad hoc in the case of severe pain. Six months later, facial weakness on the left side (3rd degree according to the House-Brackmann grading scale) was seen. Conductive hearing loss of the left ear (60-80 dB) was without improvement and required the use of equipment.

Discussion

The incidence of hearing loss in patients with RHS has been shown to range from 6.5% to 85%. According to Chang et al., the incidence of hearing loss in patients with RHS is 76% and is more severe in the high frequency range than in the low frequency range.[1] Transmission of VZV infection from a dehiscent facial canal to the inner ear and organs through the oval or round window has been suggested as a potential route for inner ear involvement. For interneural transmission, the spread of VZV across the perineural tissues inside the internal auditory canal has been proposed as a possible route of infection. Cerebrospinal fluid infection is more likely to be a source of cochlear deficit rather than nerve anastomosis and direct connection between cranial nerve VII and VIII. Hearing loss and facial nerve paralysis are the predominant permanent consequences of VZV infection. The permanent character of this complications are caused by deep tissue processes. Histopathological examinations reveal pronounced segmental or partial atrophy and degeneration of cranial nerve VII and/or VIII.[2] Vertigo and facial nerve palsy depend on each other, because severe vestibular symptoms can be related to the severity of facial paralysis after the onset of herpetic symptoms.[5] The prognosis for facial palsy is poorer in Ramsay Hunt syndrome than in idiopathic forms.[6] Only 10% of patients with complete facial palsy are totally cured, with full recovery in as few as 20% of cases.[7] Facial nerve involvement is initially due to inflammation caused by the viral neuronitis and secondarily to the facial nerve edema.[8,9] Advanced age, elevated arterial blood pressure, vertigo and diabetes can be factors of poor recovery in Ramsay Hunt syndrome.[10] Hypertension and vertigo, which might have been the causes of poorer recovery from facial paralysis. Early administration within 72 hours of antivirals and corticosteroids improves the prognosis.[8,9,10,11] Recovery rates in patients with RHS are higher following treatment with steroid plus famciclovir than with steroid plus acyclovir, especially in patients without hypertension and diabetes mellitus.[12] A past history of vertigo is

a predisposing factor for hearing impairment. Hearing impairment is more severe in patients with vertigo than in those without vertigo in both the high and low frequency ranges, even though the degree of hearing impairment is not significantly different between patients with and without facial palsy. These findings indicate that the mechanisms of viral spread from CN VII to CN VIII may differ between vestibular and audiologic deficits.[1] The coexistence of Ramsay Hunt syndrome and varicella zoster encephalitis and meningitis is rare. Concomitant diseases such as diabetes and chronic renal failure may lead to an aggressive course of infection and can predispose to encephalitis and meningitis.[13,14] Organ transplant recipients also had herpes oticus more frequently. The incidence of VZV infection is reported as 11.2% at 4 years after kidney transplantation, an incidence that is approximately nine times greater than that in the general population.[8] HZ develops in 12% of liver transplant recipients.[15] For a transplant surgeon, it is imperative to remember that viral prophylaxis is essential in the follow-up of the transplant patients.[16] The possible mechanism of VZV spreading to CNS is reactivated viruses, which establish latency in geniculate ganglia, upward thread porus acusticus internus along with the facial canal, and eventually enter intracranially and first invade the basis pontis. Meanwhile, VZV may also spread downwards along with general somatosensory fibers to the skin of external auditory canal, resulting in herpes zoster formation.[17] Some data indicate that vasculitis might also be involved in other VZV CNS manifestations, such as herpes zoster-associated encephalitis. For this reason, VZV CNS infection must be suspected in several CNS syndromes and diagnostics should be based on CSF analysis for the detection of VZV DNA by PCR and/or intrathecal antibody production.[18,19,20]

Conclusions

Meningitis is possible but rare complication of herpes oticus. Hearing loss is quite often consequence of Ramsay Hunt syndrome. Favourable facial nerve paralysis recovery depends on rapid administration of antivirals and corticosteroids. The prognosis for facial palsy is poorer in Ramsay Hunt syndrome than in idiopathic forms. A past history of vertigo can be a predisposing factor of hearing impairment.

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CASUISTIC PAPER

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Fibromuscular dysplasia – a case description

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ABSTRACT

Introduction. Fibromuscular dysplasia is an idiopathic, non-inflammatory and non-atherosclerotic disease that affects the walls of arteries (mostly renal and carotid arteries). Histological classification distinguishes three main types of the disease, depending on the structural changes occurring in one of the three layers of arterial vessel walls.

Objective. We present here a case of fibromuscular dysplasia affecting the internal carotid arteries.

Case description. This article describes the case of a 52-year-old female patient with hypertension, hyperlipidemia, and a cardiac pacemaker in whom computed tomography angiography revealed a narrowing of the internal carotid arteries without atherosclerotic symptoms. We describe the diagnostic methods and various types of treatment that the patient suffering from fibromuscular dysplasia was subjected to.

Conclusions. Due to a low detection rate of fibromuscular dysplasia, if the disease is suspected, all available diagnostic methods should be employed. Taking into account the unknown etiology of the disease, it is not possible to use a preventive therapy, or a therapy focused on stalling the progression of the disease.

Keywords. fibromuscular dysplasia, arteries, computed tomography

Introduction

Fibromuscular dysplasia (FMD), according to the definition, involves changes in the structure of one or all layers of the large and medium arterial vessel walls. These changes consist of an overgrowth of the fibrous connective as well as the smooth muscle tissues that do not result from inflammatory or atherosclerotic changes and cause the narrowing of the arterial lumen.[1] The main locations of FMD involve: renal arteries, vertebral carotid arteries, celiac trunk, upper mesenteric artery and coronary arteries.[2] The frequency of occurrence of FMD is estimated at 4-6% in renal arteries and 0.3-3% in carotid arteries.[3]

The frequency occurrence of FMD in Poland is equal to 0.05% and the changes more often concern women. The average age at FMD diagnosis is 55 years.[4] Clinical symptoms are usually identical with the symptoms of atherosclerotic narrowing of the internal carotid

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artery that cause the symptoms of acute ischemia of the brain as well as the retina.[4,5] FMD leads to severe consequences, especially in the cervical-cerebral area where it may lead to hypoperfusion, subarachnoid hemorrhage, artery dissection or occlusion.[6]

Diagnosis

FMD can be diagnosed by arteriography, in which the changes start a few centimeters along the middle section of the internal carotid artery resembling beads on a string, or aneurysmal dilatations separated by annular fibrous narrowings.[3] In 65% of cases, the changes are diagnosed on both sides. Additionally, they may cause the development of the internal carotid artery aneurysm.[7]

Classification

Histological classification of FMD:

Type 1 – the changes are associated with the intima tunica and characterized by a circular growth of mesenchymal cells as well as subendothelial connective tissue, which leads to significant hardening of the intima tunica, leading to a narrowing of the artery lumen – mainly in the renal arteries of children and youth.

Type 2 – the changes are associated with the tunica media and characterized by loosening of the smooth muscle cells and progressing fibrosis of the fibrous connective tissue, especially in the external section of tunica media – the most common dysplasia type including 60-70% of the cases.

Type 3 – the changes are associated with the tunica adventitia and characterized by the overgrowth of the fibrous connective tissue in the adventitia as well as in the external elastic lamina – often coexistent with the changes in the tunica media; most commonly occurring in women over the age of 50; associated with 10% of the cases.

The above classification is not significant when it comes to the selection of treatment, as all types may coexist simultaneously in one patient, or may be associated with the same artery.[1,3,8]

Case description

The patient (B.P.CH) (52) was diagnosed with hypertension, hyperlipidemia, and had been implanted with a single chamber pacemaker due to bradyarrhythmia in the course of a permanent atrial fibrillation; the patient is currently undergoing therapy with oral anticoagulants that are not vitamin K antagonists (dabigatran). She was initially hospitalized in the Neurology Department due to a double episode of right-hemisphere transient ischemic attack. The computed tomography (CT) scan of the head was performed at that time, and revealed multifocal angiogenic brain damage. Computed tomography angiography revealed a significant narrowing of the internal carotid arteries and did not reveal the presence of atheromatous plaques nor any vascular malformation. The neurological examination performed on the day of discharge showed a trace left-sided deficit. The patient underwent cardiologic consultation to arrange the planned closure of the left cardiac auricle. A month later the patient was hospitalized again in the Neurology Department with symptoms of ischemic stroke of the right hemisphere. The neurological examination revealed a central paresis of nerve VII on the left side, as well as a moderate paresis of the left limbs. A CT scan of the head revealed a wide hypodense area with features of right hemisphere edema; additionally, the examination showed scattered malacic foci in the left hemisphere. During hospitalization, fluctuating heterochronous intensification of the paresis and speech disorder was observed together with a significant increase in the National Institutes of Health Stroke Scale (NIHSS) score. The patient was discharged for the planned left cardiac auricle closure procedure with a recommendation for treatment with a 2×150mg dose of dabigatran. Neurological symptoms on the day of discharge: upper left limb monoplegia and moderate lower left limb paresis. Moreover, the date for the planned admission to the Neurological Rehabilitation Department was established. After the procedure of surgery-free closure of the left cardiac auricle with the Amulet 25mm occluder, an antiplatelet treatment therapy with acetylsalicylic acid and clopidogrel was recommended. After the procedure, the patient was transferred again to the Neurological Department in a neurological state similar to that on the day of discharge. A head CT scan revealed a presence of heterochronous angiogenic changes in the external capsules, the subcortical nuclei, and in the right frontal lobe with the possibility of hemorrhagic conversion at the border between the frontal and temporal lobes. During the 6th day of hospitalization, a degradation of the neurological state (intensification of left limb paresis with the paresis of the lower right limb – 21 points in the NIHSS scale) as well as a deterioration of the patient's mental condition with the presence of productive symptoms was observed. A control head CT scan did not show any new ischemic changes. Generalized tonic-clonic cluster epileptic seizures were observed. A neuroleptic agent as well as anticonvulsant drugs were introduced into the treatment. In accordance with the cardiologist's recommendations, antiplatelet drugs were also included (low-molecular-weight heparin at the therapeutic dose). The patient was transferred to the Neurological Rehabilitation Department for further rehabilitation in the following neurological state: triparesis, plegia of the left limbs as well as of the lower right limb. Due to the neurological state at the time of admission to the Rehabilitation Department (no verbal contact, psychomotor retardation, dozing off, selective instruction fulfillment, central paresis of nerve VII on the left side, weakened palatal and pharyngeal reflexes, quadriparesis with increased muscle tone in the left limbs, monoplegia of the lower right limb, severe paresis of the

upper right limb, no defense in the Baniewicz test, bilateral Babiński reflex, right foot clonus, lying) and based on a CT image of the head showing intracerebral hematoma of the right hemisphere, a decision was made to admit the patient to the Neurological Department. Magnetic resonance imaging of the head was performed in cooperation with the cardiologist (pacemaker reprogramming) – the examination revealed changes that did not correspond with the expected widespread damage – ischemic stroke within the limit of the frontal and parietal lobes of the right hemisphere as well as the left frontal lobe with the presence of small hematomas (petechiae) in the cortical layer (subacute phase), hematoma in the area of subcortical nuclei of the right hemisphere (subacute phase). A lumbar puncture was carried out – the examined cerebrospinal fluid did not demonstrate inflammatory features. Due to the fact that the ultrasound of the internal carotid and vertebral arteries confirmed a decrease of the flow velocity in both internal arteries to 30-35cm/s, an angio-CT examination from the aortic arch was performed to reveal a significant/critical narrowing of the lumen in the internal carotid arteries throughout their whole length, and significant limitation of the flow in the arterial circle arteries accompanied by a lack of atherosclerotic features. During the previous hospitalizations, the patient had undergone a wide range of "stroke in young people" examinations where no deviations had surfaced. The patient was examined by a rheumatologist and the antinuclear antibodies were collected (the results were correct), as well as by a vascular surgeon – she did not qualify for any intervention. Due to patient's deteriorating mood, sertraline was introduced in the treatment. The patient was rehabilitated with clinical improvement. A neurological examination on the day of discharge: logical contact, speech with features of dysarthria, slow, understandable, patient follows instructions, fed using a spoon, seated in a wheelchair, put in an upright position, residual triparesis of the left limbs and the lower right limb, increase of physical strength in the upper right limb. Subsequent image examinations showed a correct evolution of the hematoma. A new oral anticoagulant was introduced into the treatment. The patient was transferred to the Rehabilitation Department for further rehabilitation.

Table 1. Patient's laboratory examination results

		Referential norms
CRP	0.19 [mg/dl]	0-0.5 [mg/dl]
ESR	5 [mm/h]	0-15 [mm/h]

Discussion

What draws attention in the abovementioned case description is the significant, rapidly progressing and recurring character of the symptoms similar to the case described by Langis et al.[9] The key examination turned out to be the most common and readily available one – the USG Doppler examination of the internal carotid and vertebral arteries, which revealed a decreased blood flow in the internal carotid arteries.[10] Owing to this result, a decision was made to expand the diagnostics with the angio-CT examination of the vessels from the aortic arch, which confirmed the diagnosis. Given the magnitude of the changes, the patient was not qualified for further surgical intervention. Artery atherosclerosis was considered during differential diagnosis, however, the factor that distinguished atherosclerosis from dysplasia the most was the location of the vessel disease – in atherosclerosis the initial sections of the arteries are affected, whereas it's their middle and final sections in FMD.[11] Due to the non-inflammatory character of FMD, another disease unit considered in the diagnosis was vasculitis.

Cases where the already heightened inflammation indexes remain within the norms have been reported[12-16] Similarly as in the FMD case described by Altun et al. as well as Sarinen and Palomäki, the patient's ESR and C-reactive protein concentration remained within the referential norms.[15,16] (Table 1). Currently, due to the unknown pathogenesis of the disease, it is not possible to develop any therapy that would prevent or stall the progress of the disease.[3] It is difficult to determine the risk factors - the only one that is established is cigarette smoking.[17,18] Most of the decisions made in relation to the treatment must be based on the analysis of the particular case, as the literature examples are associated with individual reports based on small retrospective patient groups (the incidence is not known exactly, and is associated mainly with renal arteries). The analysis of literature data performed by Mettinger, which encompassed 1197 patients, has shown that 58% of FMD cases are associated with renal arteries, 32% with carotid arteries, and approximately 10% with other arteries (including the mesenteric artery and the intracranial arteries).[19] The low detectability of the disease still remains the main diagnostic problem. The incidence associated with carotid, vertebral, and intracranial arteries is still underestimated, and is related with the fact that its occurrence in these locations is rare and does not have the characteristic symptoms (frequent and common vertigo, tinnitus, headache), and sometimes its course is asymptomatic for a prolonged period of time.[20,21] Currently there is no known cure for FMD, and the pharmacological treatment focuses on alleviating the symptoms associated with the disease. Moreover, the treatment is hindered and limited due to the lack of randomized clinical studies. The antiplatelet and antithrombotic treatment – the antiplatelet therapy – is applied in patients with ischemic episodes, as in all cases of non-cardiogenic ischemic brain stroke (aspirin in the 75-325 mg/d dose). [22,23] However, the antiplatelet drugs only act

as factors diminishing the risk and do not treat the disease. The patients after stenting or after angioplasty are treated in the same way as the ones that experienced these procedures due to atherosclerosis.[24] The patients suffering from artery dissection in the extracranial section are treated with heparin and warfarin, or with antiplatelet drugs (aspirin or clopidogrel) for 3-6 months.[25]

Conclusions

Due to the unknown pathogenesis of FMP, it is not possible to develop a therapy that would prevent or stall the progress of this disease.

Due to the non-atherosclerotic character of pathological changes, the effectiveness of statins has not been proven.

It is difficult to determine the risk factors of FMP - the only one that is well established is cigarette smoking.

Given the significant dominance of women and the possibility of the hormonal influence on the disease, one should consider discontinuation of hormonal contraceptive drugs or, if a substitutive hormonal therapy is necessary, a decrease of dosage to the lowest effective level. If the patient is symptomatic (transient ischemic attach or brain stroke), the therapy should not be used.

Vessel dissection or anticoagulation treatment contraindications constitute an indication to the procedure associated with the carotid arteries in patients with recurring brain symptoms.

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Foundation/American Heart Association Task Force on Practice Guidelines, and the American Stroke Association, American Association of Neuroscience Nurses, American Association of Neurological Surgeons, American College of Radiology, American Society of Neuroradiology, Congress of Neurological Surgeons, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of NeuroInterventional Surgery, Society for Vascular Medicine, and Society for Vascular Surgery. *Circulation*. 2011;124(4):489-532.

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CASUISTIC PAPER

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Physiotherapeutic management of a patient with patellofemoral pain syndrome – a case report

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ABSTRACT

Introduction. Patellofemoral pain syndrome (PFPS) is a disorder of the front compartment of the knee joint with incompletely investigated, probably multifactorial pathogenesis. It mostly affects young people and runners. In patients with PFPS conservative management is a therapy of choice with fundamental importance of physiotherapeutic procedures. Therapy should be highly individualized and considering all possible factors that may cause PFPS symptoms.

Aim. The aim of this report was presentation of management of a 23 year old female patient with PFPS that developed secondary to a knee sprain. The medical history, diagnostic and therapeutic procedures were thoroughly described, then obtained results were presented and thereafter discussed.

Methods. Clinical assessment included functional and provocative tests of the patellofemoral joint as well as thigh and calf muscles tests, range of motion measurement of the knee joint and pain assessment using the VAS scale. Therapeutic management included 5 sessions of post-isometric muscle relaxation (PIR), mobilizations of the patella and applications of elastic tapes.

Results. After 5 sessions of therapeutic management PFPS symptoms were significantly reduced. Pain did not occur during normal activity, whereas in heavy joint loading, it occurred later and was of lower intensity. Range of motion as well as subjective sense of joint stability was also improved.

Conclusions. Individually adjusted conservative management based on PIR techniques, mobilizations of patella and kinesiotaping seems to be effective form of therapy for PFPS of functional nature.

Keywords. patellofemoral pain syndrome, excessive lateral pressure syndrome, runner's knee, physiotherapy, kinesiotaping

Introduction

Patellofemoral pain syndrome (PFPS) also called excessive lateral pressure syndrome (ELPS) is a disorder of the front compartment of the knee joint with incompletely investigated, probably multifactorial pathogenesis. It mostly affects young, active people while in women it appears twice often than in men. Also, due to frequent appearance in persons practicing running, the term "runner's knee" is commonly used with reference to this syndrome.[1,2]

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PFPS together with habitual subluxation of patella and its recurrent dislocation sets a group of disorders with one characteristic feature - patellofemoral malalignment. PFPS is highlighted in this group only with chondromalacia which is always present in PFPS while it is not regular in both others.[2] PFPS may occur as patellofemoral instability, pain that can be assisted with patellofemoral malalignment or isolated pain.[1]

External risk factors of PFPS include patellofemoral joint overloading i.e. training errors (accumulation of micro-trauma), sudden increase of training intensity or frequency, improper footwear and also knee joint traumas/surgery which may lead to damage of cartilage and/or change in distribution of forces in patellofemoral joint. Internal risk factors of PFPS involve various changes of knee joint structures (e.g. trochlear dysplasia, asymmetry of patellar joint surfaces, hypertrophy of infrapatellar fat pad), changes of lower limb mechanical axis (e.g patella alta, valgus knee, foot hyperpronation) and soft tissues imbalance (e.g. contracture of quadriceps m., calf muscles, iliotibial band or hamstrings, also impairment of lateral retinaculum of patella)[1,3]

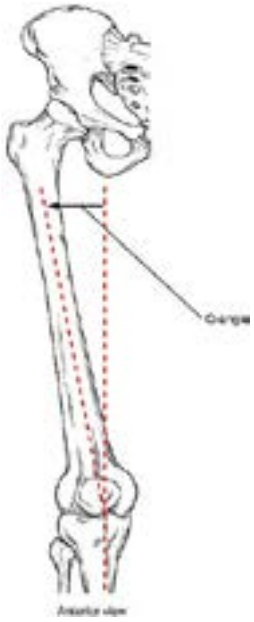

Figure 1. Q-angle
(source: *https://commons.wikimedia.org/wiki/File:818_Femur_Q_Angle.jpg*)

Primary symptoms of PFPS are often non-specific with a tendency to be ignored by patients. The onset can be acute or chronic and typical symptoms are: uni- or bilateral dull pain located under or around the patella, knee stiffness and clicking/cracking or crepitus inside the joint.[1,2] The characteristic feature of PFPS is increase of pain during long time maintained knee flexion ("theater sign") and also during activities forcing the joint e.g. climbing/descending the stairs, running, squatting, kneeling etc. Nocturnal pain can also occur in case of sleeping with flexed knees. Secondary PFPS symptoms can be subjective knee instability (weakness of quadriceps m.). Complete blockage of knee joint is not specific for PFPS and require differentiation with meniscus damage or presence of loose articular bodies.[1,2]

The diagnosis of PFPS is mainly based on assessment of present and former clinical symptoms. Specific examination that assess deviation of the patella from lower limb axis is measurement of Q-angle which is set between the line connecting front upper iliac spine with the center of patella and line connecting center of patella with front tibial tuberosity (Fig.1.). The value of Q-angle over 16° indicates the increase of force that pulls the patella laterally.[1,4]

Another specific examination for patellofemoral malalignment is so called "J-sign" that characterizes the trajectory of movement of the patella which is pulled laterally at the end of extension by excessively tensed lateral stabilizers (Fig.2).[2]

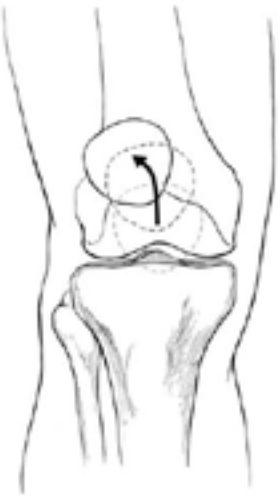

Figure 2. "J-sign"[2]

The presence of chondromalacia can be assessed by joint surface pressure test in lying position, patella moving test and by checking the influence of quadriceps m. on pain in the patellofemoral joint (Zohlen sign). Medial patella glide should also be examined as it may show shortening of lateral retinaculum – the result is positive if examiner cannot move the patella medially by about a half of its width (Fig.3).[2]

The comprehensive diagnostics of PFPS should consider other aspects that could determine its occurrence e.g. elasticity of m. quadriceps and assessment of iliotibial band, hamstrings and calf muscles length since their shortening may increase pressure of the patella to femur by permanent initiation of flexed position.[1,2,5]

Additional imaging (X-ray, MRI) are secondary to functional assessment in diagnostic of PFPS because

positive results of these examinations neither determine the presence of this disorder nor the risk of its occurrence in future. They are applicable in excluding other possible causes of patellofemoral pain, especially in patients with trauma in medical history.[1,2]

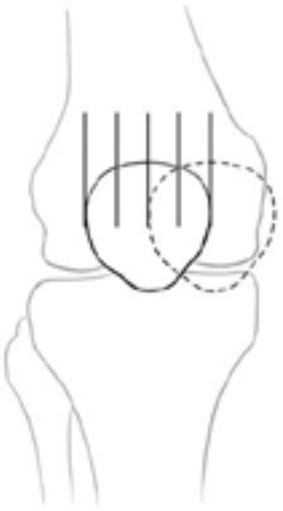

Figure 3. Patella glide test[2]

Because PFPS is most often of functional nature thus the therapy of choice is individualized comprehension management that consider all structural and biomechanical dysfunctions. It provides mainly stretching of shortened structures (mostly iliotibial band, quadriceps muscle, hamstrings and calf muscles) and strengthening of weakened groups of muscles (extensors and rotators of the hip joint).[7] The core stability, sensomotoric and proprioceptive exercises on unstable basis and correction of postural faults should also be considered. The manual therapy techniques are likely to be used for restoring of correct mobility of the patellofemoral joint.[1,2] The correction of the patella position can also be achieved by using Kinesiotaping applications. Secondary prevention should include reduction of patellofemoral joint loading, for example, by changing the type of previous activity for that generates smaller joint compression (e.g. riding the bike cycloergometers, swimming), using proper training footwear or reduction of body mass.[1,3,7]

The surgery (i.a. lateral retinaculum release, reconstruction of medial retinaculum, patella chondroplastics) is introduced in case of lack of conservative treatment effects. It should be considered for those patients who didn't improve after conservative treatment that lasted a minimum of 6 months and other possible causes of PFPS excluded.[2]

The aim of this report was the presentation of physiotherapeutic management of young female patient with PFPS that developed secondary to a knee sprain.

Case report

The patient is a 23 year old accountant leading moderately active lifestyle – hiking in her spare time and occasionally dancing and participating in group fitness activities.

The original trauma occurred during physical education class. A knee joint sprain was diagnosed in a hospital emergency room on the basis of an x-ray picture. After the fitting of plaster cast, the patient was discharged with a recommendation of a complete unloading of the injured leg. The cast was removed after three weeks and patient was advised to progressively return back to normal function with continuous assistance of crutches. The patient regained the ability to perform normal daily activities without knee pain in about 4 months after trauma.

About 7 months after the original trauma during physical education class symptoms returned. After orthopedic consultation, ultrasound imaging revealed changes in the patellofemoral joint described as chondromalacia of first grade. Simultaneously, damage of other joint structures was excluded. The patient was administered to 10 sessions of laser and local cryotherapy and was advised for taking supplementation of glucosamine sulfate, hyaluronic acid and collagen which diminished the symptoms for short time with subsequent recurrence with variable intensity until present time.

Functional diagnostics.

The patient sought physiotherapeutic consultation about 7 years after the original trauma due to recurrent ailments of the right knee. The symptoms were described as a feeling of crushing and pressuring inside the joint mostly localized medially nearby lower part of the patella and on the level of the joint fissure. Pain was intensified by long term flexion or excessive loading of the knee joint and was of dull and continuous character depending on the patient's activity level. Its intensity at rest was evaluated as low (0-2 in VAS scale) but was aggravating during climbing stairs, long walking or hiking (5-8 in VAS scale). Sometimes pain was accompanied by a feeling of clicking or friction inside the joint, especially in maximal knee flexion. An evident lateral shift of the patella without features of valgus or varus knee was found visually. The affected right knee joint was found to be 1 cm larger in circumference by comparative measurement. The medial part of right knee joint fissure was also found touch-sensitive. The results of functional tests and range of motion (ROM) measurement clearly stated contracture of quadriceps and gastrocnemius muscles. Lateral patella subluxation with shortening of lateral retinaculum was also noticed. Performance of McConnel test evaluating the influence of patella medialization on knee pain was impossible because of large lateral pressure in patellofemoral joint.

Table 1. Details of the patient's functional assessment

Test/Examination	Result	Interpretation
Dancing patella	positive	effusion in right knee joint
Facet tenderness	positive	damage/degenerative changes of right patella cartilage
Zohlen's sign	positive	
Patella moving test	positive	
Crepitation test	positive	patellofemoral joint chondromalacia
"J-sign" (R/L)	positive /negative	increased forces pressuring right patella laterally
Q angle (R/L)	21°/17°	
Lateral Subluxation Suppression Test	positive	lateral subluxation of right patella
Ober Test	negative	no iliotiobial band shortening
Patella Glide test	limitation of medial glide	shortening of lateral retinaculum
Thomas Test (R/L)	positive /negative	contracture of right rectus femoris muscle
Drawer test posterior/anterior	negative/negative	efficient cruciate ligaments
Apley Distraction/Compression Test	negative	undamaged menisci
Chaitow's Discriminatory Test for gastrocnemius m. (Fig.4)	positive	gastrocnemius muscle contracture
Knee flexion ROM (active/passive)	R 117°/120° L 128°/131°	limitation of right knee joint extension
Sagittal plane ROM of ankle joints (active/passive)	R 40-0-15°/47-0-22° L 45-0-18°/45-0-30°	minor limitation of right ankle joint mobility

captions: R – right, L – left

All dysfunctions revealed in functional assessment presented a range of symptoms typical for PFPS of functional character (Table 1).

Physiotherapeutic management

The goal of the therapy was reduction of exertional knee pain to the extent that allows covering moderate mountain hikes and elimination of pain occurring during daily activities. There were 5 physiotherapeutic sessions performed daily or in a maximal 3 day interval which lasted on average 35-45 minutes. Each one session included Swedish massage of whole right lower limb, and post-isometric muscle relaxation techniques (PIR) described by Chaitow on quadriceps and gastrocnemius muscles.[5] Following this, patella mobilizations and Kinesiotaping applications were performed. After the last session complete functional diagnostics were repeated.

Post-isometric relaxation techniques (PIR)

In order to prepare the muscles for PIR, each session was started with Swedish massage of whole right lower limb. Then the PIR technique for gastrocnemius muscle was performed. The patient was lying supine on a

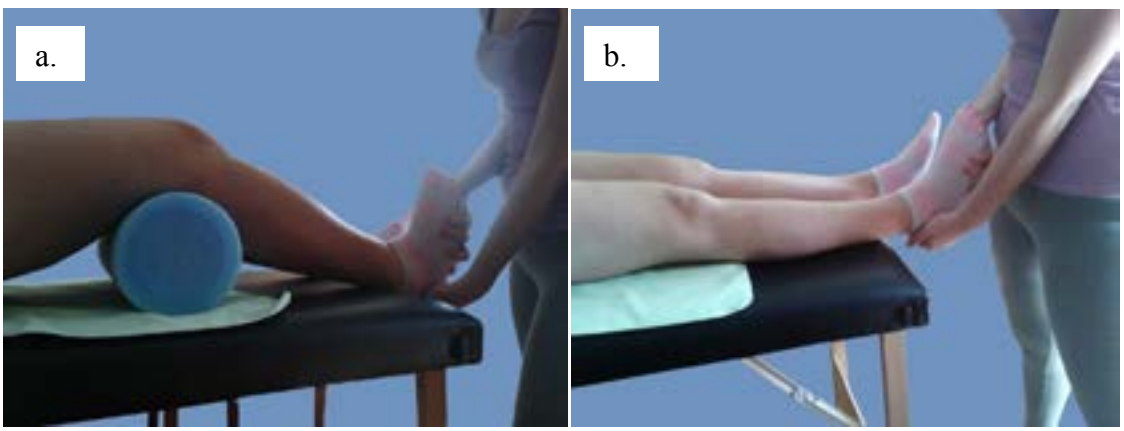

Figure 4. Chaitow's Discriminatory Test for gastrocnemius m.: a. correct elasticity of soleus m., b. contracture of gastrocnemius m. (own material)

therapeutic table. Her lower limbs were straightened with feet off the table. One of the therapist's hands supports the heel grasp Achilles tendon and the other was placed on foot dorsally with the thumb medially on the plantar side. With passive movement as used in stretching, the therapist found the limit of dorsiflexion and positioned the foot just before this limit with muscle partly relaxed (Fig.5a.). The patient was then asked for isometric plantar flexion against a constant resistance of 25-30% of maximal force applied by the therapist for 7 seconds. After muscle relaxation and 5 seconds of rest, the foot was positioned in maximal painless dorsiflexion within the new ROM limit and held for a minimum of 30 seconds (Fig.5b.). To increase the effectiveness of this technique, a special breathing cycle was introduced that consisted of breathing in during isometric contraction increase, and holding breath during maximal contraction. Then during rest before muscle stretching, a long breathing out was performed in order to facilitate full relaxation.

Subsequently, the same technique was used for the rectus femoris muscle. The patient was lying prone with right knee bent. The therapist was supporting the leg with one hand on the level of ankle joint and stabilizing the pelvis with other hand on the sacrum (Fig. 6a.). Then the patient was asked to perform isometric knee extension against constant resistance applied by the therapist with simultaneous attempt of hip flexion by pushing the thigh against the table. The contraction was maintained for 7 seconds and involved about 25-30% of the patient's maximal power. After relaxation and rest that lasted up to 5 seconds, the shin was positioned in maximal painless knee flexion and held for at least 30 seconds (Fig. 6b.). The special breathing cycle described above was also adopted in this procedure.

Each session provided three repeats of the complete sequence separately for both muscles while each repeat was started just before a new ROM limit.

Patella mobilizations

To restore correct elasticity of lateral retinaculum, a passive medial mobilization of the patella of second degree by Maitland was used.[8] The second degree of mobilization was explained by pain occurring before first resistance during glide. The patient was lying supine with both legs straightened. The therapist held the patella

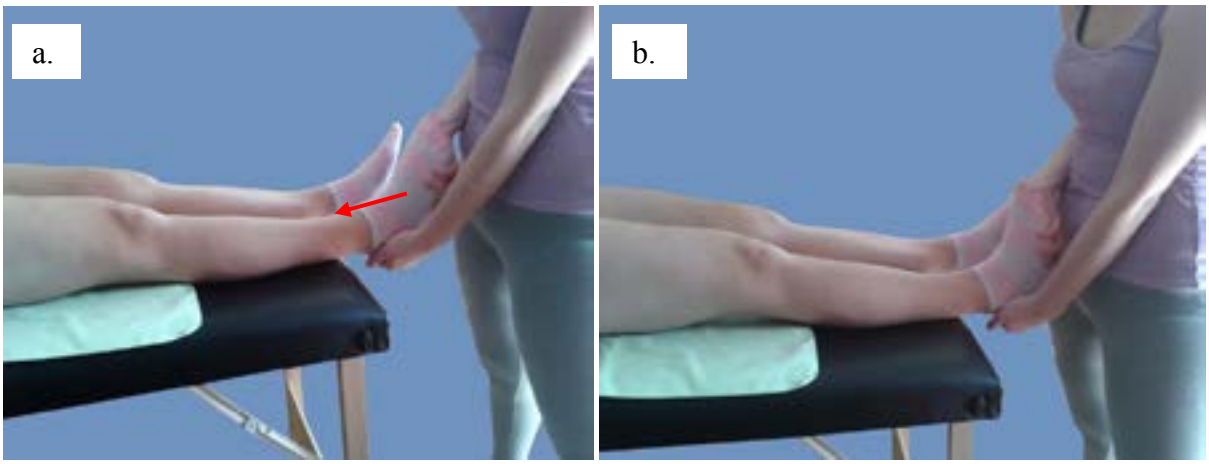

Figure 5. PIR for gastrocnemius muscle: a. Starting position, b. Final position (own material)

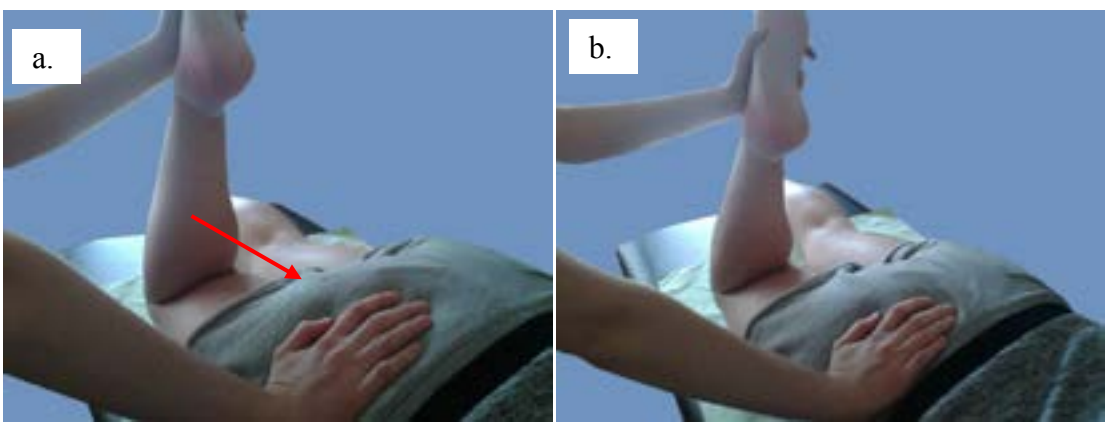

Figure 6. PIR for rectus femoris muscle: a. Starting position, b. Final position (own material)

with thumbs along the lateral margin with forefingers on the opposite side (Fig.7a.). Afterwards, the patella was moved medially up to the movement limit without any pain (Fig.7b.). Patella was held in this position for 15 seconds and then moved back to the starting position. This procedure was repeated 5 times with several seconds intervals.

Kinesiotaping

In order to maintain the therapeutic effects obtained being the stretching of lateral retinaculum and unloading of lateral femur condyle, the correction of patella alignment by elastic tapes application was used. The application applied was of mechanical correction type in which positional stimulus induces required resting position. It enables the maintenance of a full and functional ROM, simultaneously inhibiting pathological compensation patterns. In the patient described, this application was assumed to limit excessive lateral glide observed mainly at the end of knee extension.

The patient was lying supine with both knees bent. Three parts of tape each 15 cm long were prepared. First "I" shaped and 5 cm wide tape was applied arch-like to hold the patella from its lateral side and pushing medially. During tape application the patient was asked for active knee flexion while the medial 1/3 part of tape was applied with 50% tension along the lateral margin of the patella. In maximal knee flexion, both bases of tape were stuck without tension (Fig.8a.). Afterwards in the same position of the patient, both "V" shaped parts of tape were applied with bases put without tension one below the other on the medial side of the knee joint. Application was performed in knee flexion, sticking particular parts of tape so as they grasp the patella between them. The tension of tape was 30% while all ends were stuck without any tension (Fig.8b).

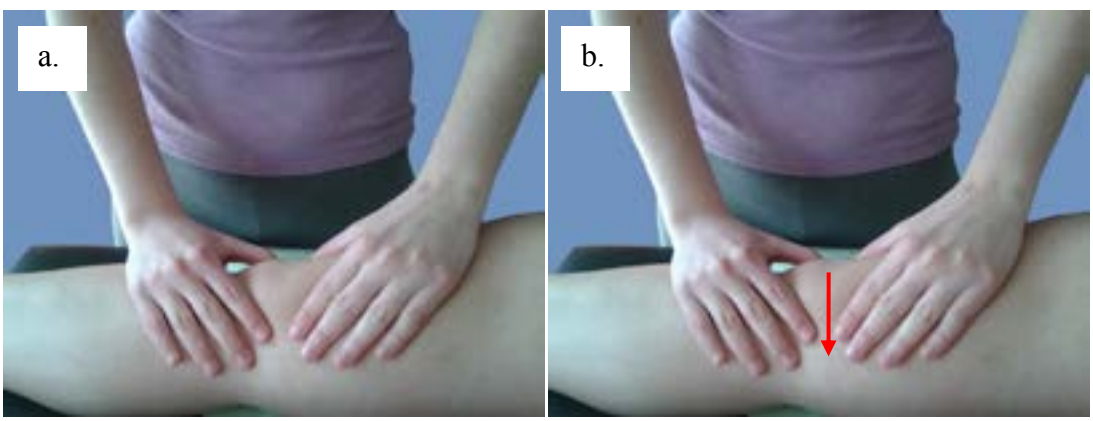

Figure 7. Medial patella mobilization by Maitland: a. Starting position, b. Final position (own material)

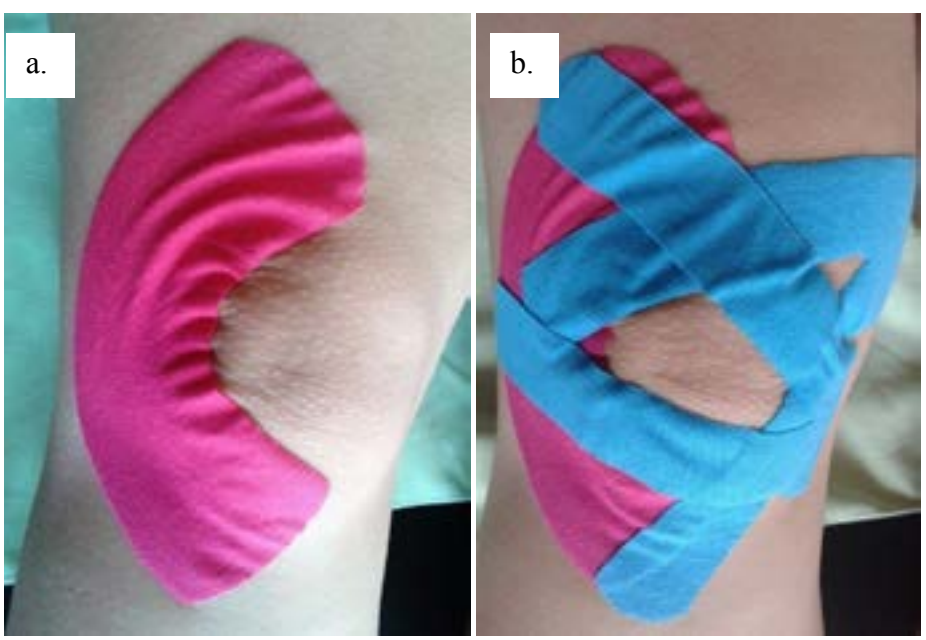

Figure 8. Kinesiotaping of patellofemoral joint: a. Basic mechanical correction, b. Full application correcting lateral dislocation of the patella (own material)

Results

Before therapy, the patient evaluated pain intensity of 2 at rest and an increase up to 3 on the VAS scale during stair climbing. Pain reduction was observed during the first therapeutic session while analgesic effects were increased and became stable after each session. As a final result, complete pain elimination during daily activities was achieved. Maximal pain intensity in heavy joint loading e.g. during long hiking times was evaluated as 2 on the VAS scale, moreover, symptoms occurred markedly later than before therapy. Clicking and cracking inside the joint occurred less often and became painless. Facet tenderness and patella moving tests were found negative. Pain during the Zohlen test was also diminished. The patient subjectively noticed a greater freedom of movement and stability during walking. Angular measurements showed significant improvement of ROM in the knee and ankle joint. Right knee flexion came to 130°, foot plantar flexion to 45°, and foot dorsiflexion to 20° and these values were comparable with those in left leg. Complete functional assessment was repeated 6 weeks later and the results were the same as just after the end of therapy.

Discussion

In this report we presented patellofemoral pain syndrome and an example of physiotherapeutic management in a patient with this disorder that developed as a late complication after a knee sprain. This program may be also applied in other patients in which limitation of ROM in the knee joint or patellofemoral joint occurred on the basis of muscle imbalance. The use of therapy that combines various interventions depending on individual factors determining PFPS occurrence is considered as an effective and recommended form of treatment.[1,2,3] One of the elements included in the presented management was Kinesiotaping (KT) of which effectiveness in PFPS was stated i.a. by Campolo et al. who compared two different methods of taping in group of 20 persons with unilateral PFPS. They reported significantly larger improvements in pain during stair climbing and getting up out of a squat position in a group with KT than in a control group without taping.[9] Chen et al. examining EMG of vastus medialis and vastus lateralis muscles in patients with PFPS noticed normalization of activation of both muscles after KT in comparison to placebo which directly corrected patella alignment and reduced pain.[10] In contrast, Kuru et al. comparing the effect of KT and electrostimulation combined with identical exercise programs ascertained the same level of improvement of both interventions.[11] Akbas et al. compared two groups of women who underwent therapeutic programs including exercises and muscle stretching with additional KT applied every 4 days in one group. The authors stated no significant differences between both groups in mobility and pain, however, in the KT group a faster improvement in hamstring elasticity was noticed.[12] These findings, regardless of differences, encourage us to consider Kinesiotaping as an effective method of mechanical correction of patella alignment that can be included in a comprehensive management of patients with PFPS.

Relatively little information has been issued about the use of manual therapy (MT) in therapy of PFPS. In the presented case, a glide mobilization described by Maitland was used of which effectiveness in the treatment of degenerative disease of the patellofemoral joint was confirmed by Kumar.[13] Van den Dolder et al. reported improvement in knee flexion after mobilization with movement in sagittal plane combined with deep friction massage.[14] Espi-Lopez et al. conducted a systematic review of reports about the use of MT combined with other physical modalities in PFPS treatment. The authors in appraising the results of 5 randomized clinical trials confirmed the effectiveness of MT in management of PFPS mainly in combination with muscle strengthening and stretching. They perceived, however, that better therapeutic effects can be expected after combination of these techniques is applied on both hip and knee joints than acting on knee joints only.[15]

In the presented case, the use of post-isometric relaxation techniques was motivated by the presence of rectus femoris and gastrocnemius muscle contracture of which relationship to PFPS was stated by Waryasz and McDermott.[16] High effectiveness of quadriceps stretching in PFPS treatment was stated before by i.a. Mason et al. who compared efficacy of stretching with muscle strengthening and taping. They noticed significant improvement in function and pain just after a one week session of muscle stretching.[17] In turn, Moyano et al. comparing classic stretching exercises with PNF stretching techniques (hold-relax), observed a bigger improvement in PNF group than in classic stretching group.[18]

Implementation of combined physiotherapeutic methods presented in this paper provided quick pain reduction and improvement of knee mobility. Persistence of these results was confirmed in clinical examination conducted 6 weeks after the end of therapy. Satisfactory outcomes of this management may hold promise for effective treatment for patients who didn't get proper physiotherapeutic assistance in the acute phase of this disorder. However, precise identification of individual functional deficits related to PFPS and, on this basis, the choice of relevant therapeutic techniques, seems to be crucial.

Conclusions

1. Patellofemoral pain syndrome is a common disorder of the knee joint with complex and individual

etiology that causes pain and severe functional impairment of the lower limbs.

2. Individually programmed conservative management based on post isometric relaxation techniques, mobilizations of patella and Kinesiotaping seems to be an effective therapy for patellofemoral pain syndrome of a functional nature.

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— References

Systematic review should contain the following elements:
— Introduction
— Description of the subject literature (a source of publication, data range)
— Analysis of the literature
— A summary
— References

Review article should contain the following elements:
— Introduction
— Body of the subject matter (the problem)
— Conclusion
— References

REFERENCES/ EXAMPLES OF CITATION

References should be prepared according to the AMA style. The list of references should be placed at the end of an article and prepared according to the order of citation in the text.
Citations in the article should be placed after a sentence ending with a full stop and edited as the so called 'superscript'. In-text citations should only be placed at the end of a sentence or a paragraph, not in the middle.
Examples:

— The degree of respiratory muscles fatigue depends on the applied exercise protocol and the research group's fitness level. [1, 2] The greatest load with which a patient continues breathing for at least one minute is a measure of inspiratory muscles strength.[3]
— Diabetes mellitus is associated with a high risk of foot ulcers.[4-6]

A citation should contain a maximum of 6 authors. Wnen an article has more than six authors, only the first three names should be given by adding 'et al.'. If the source

does not have any authors, the citation should begin with the title.

Journal titles should be given in brief according to the Index Medicus standard.

The number of sources cited for an opinion article/ a review article should be between 40 and 50, and from 20 to 40 for other articles. A minimum of 50 % of literature should come from the last 5 years.

The following are examples of individual citations made according to the required rules of editing and punctuation:

Article from a journal, number of authors from 1 to 6	Lee JC, Seo HG, Lee WH, Kim HC, Han TR, Oh BM. Computer-assisted detection of swallowing difficulty. Comput Methods Programs Biomed. 2016;134:79-88. de Kam D, Kamphuis JF, Weerdesteyn V, Geurts AC. The effect of weight-bering asymmetry on dynamic postural stability in people with chronic stroke. Gait Posture. 2016;53:5-10.
Article from a journal, number of authors more than 6	Gonzalez ME, Martin EE, Anwar T, et al. Mesenchymal stem cell-induced DDR2 mediates stromal-breast cancer interactions and metastasis growth. Cell Rep. 2017;18:1215-28. Jordan J, Toplak H, Grassi G, et al.Joint statement of the European Association for the Study of Obesity and the European Society of Hypertension: obesity and heart failure. J Hypertens. 2016;34:1678-88.
Article from an online journal	Coppinger T, Jeanes YM, Hardwick J, Reeves S. Body mass, frequency of eating and breakfast consumption in 9-13-year-olds. J Hum Nutr Diet. 2012;25:43-9. doi: 10.1111/j.1365-277X.2011.01184.x. Cogulu O, Schoumans J, Toruner G, Demkow U, Karaca E, Durmaz AA. Laboratory Genetic Testing in Clinical Practice 2016. Biomed Res Int. 2017;2017:5798714. doi: 10.1155/2017/5798714.
Websites	Cholera in Haiti. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/haiti-cholera/. Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012. Address double burden of malnutrition: WHO. World Health Organization site. http://www.searo.who.int/mediacentre/releases/2016/1636/en/. Accessed February 2, 2017.
Book	Naish J, Syndercombe Court D. Medical Sciences. 2nd ed. London, Elsevier;2015. Modlin J, Jenkins P. Decision Analysis in Planning for a Polio Outbreak in the United States. San Francisco, CA:Pediatric Academic Societies;2004.
Chapter in a book	Pignone M, Salazar R. Disease Prevention & Health Promotion. In: Papadakis MA, McPhee S, ed. Current Medical Diagnosis & Treatment. 54th ed. New York, NY: McGraw-Hill Education; 2015:1-19. Solensky R. Drugallergy: desensitization and Treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. Allergens and Allergen Immunotherapy. 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

NOTE: The editorial board requires consistent and carefully made references prepared according to the above-mentioned AMA standards. Otherwise, the work will be sent back to the authors.

TABLES AND FIGURES

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

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

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

If a given figure has already been published, one should give a source and obtain a written consent from a person having copyrights for reprinting the material, with the exception of documents constituting public interest.

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The Editorial Staff requires using only standard abbreviations. One should not use abbreviations in the title and in the abstracts. A full version of a term, for which a given abbreviation is used must be given before the first appearance of the abbreviation in the text, with the exception of standard units of measurement.

The abbreviation used for European Journal of Clinical and Experimental Medicine is Eur J Clin Exp Med.

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Papers written incompatibly with the rules determined in the hereby Instructions cannot be published in the European Journal of Clinical and Experimental Medicine.

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