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ORIGINAL ARTICLE / PRACA ORYGINALNA

Iwona Adamska-Kuźmicka[1], Anna Ksykiewicz-Dorota[2]

DEVELOPMENT DETERMINANTS OF INDEPENDENT DECISION-MAKING OF MIDWIVES

UWARUNKOWANIA ROZWOJU SAMODZIELNOŚCI DECYZYJNEJ POŁOŻNYCH

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S u m m a r y

Worldwide organizations uniting midwives, i.e. EMLC, ICM, EMA and those dealing with healthcare, i.e. WHO, FIGO have established major worldwide trends for obstetric care. The main subject of the guidelines of the institutions mentioned above is medicalization in obstetric care of a mother and her baby by increasing midwives' autonomy.

This work aims at specifying factors contributing to the development of midwives' decision-making independence.

The study was conducted among 134 midwives between year 2005 and 2007 in the wards in the Lublin Province. The following research methods were utilized in this work: the working time study by means of direct observation, the ergonomic work analysis method and a technique of assessing a level of independence in decision-making according to Kordaszewski.

On the basis of research results, it was found that independent decisions were more often made by midwives whose work experience had not exceeded 5 years ($p<0.05$) and who were younger than 25. Considerable statistical differences were found between the number of decisions made independently by midwives who had done various forms of professional training and the decisions made independently by nurses without any specialization at pregnancy pathology wards and labor wards ($p<0.05$), the former predominating over the latter.

S t r e s z c z e n i e

Światowe organizacje zrzeszające położne EMLC, ICM, EMA oraz zajmujące się opieką medyczną: WHO, FIGO wyznaczyły trendy rozwoju światowej opieki położniczej. Wiodącym tematem zaleceń w/w instytucji jest zmniejszenie medykalizacji opieki położniczej nad matką i dzieckiem poprzez większą autonomię położnych.

Celem pracy jest określenie czynników przyczyniających się do rozwoju samodzielności decyzyjnej położnych.

Badania przeprowadzono wśród 134 położnych, w okresie od 2005 do 2007 roku w oddziałach opieki położniczej województwa lubelskiego. W pracy zastosowano metodę badania czasu pracy z techniką obserwacji bezpośredniej, ergonomicznej analizy pracy z techniką szacunkowej oceny pomiaru obciążenia informacjami, decyzjami i wykonywaniem czynności oraz technikę oceny stopnia samodzielności decyzyjnej wg Kordaszewskiego.

Na podstawie wyników badań stwierdzono, że samodzielność decyzją częściej podejmują położne, których staż pracy nie przekroczył 5 lat ($p<0,05$) i nie ukończyły 25 roku życia. Stwierdzono istotne różnice statystyczne pomiędzy czynnościami samodzielnymi wykonywanymi przez położne posiadające różne formy doskonalenia zawodowego a czynnościami samodzielnymi wykonywanymi przez położne bez specjalizacji na oddziałach patologii ciąży i sali porodowej ($p<0,05$), na rzecz tej pierwszej grupy.

Key words: decision making independence, nursing, midwives
Słowa kluczowe: samodzielność decyzyjna, pielęgniarstwo, położne

INTRODUCTION

Organisations uniting midwives worldwide, EMLC, ICM, EMA as well as those dealing with medical care: WHO and FIGO have established worldwide trends for the development of obstetric care. Recommendations of the aforementioned organizations specify expectations for "back to nature" tendencies in obstetric care provided by midwives and doctors. Decreasing medicalization in obstetric care of a mother and her child by granting greater autonomy to midwives is the key issue [1, 2, 3, 4, 5, 6]. These guidelines may be applied owing to changes occurring within the scope of a definition of midwives' professional roles. These changes primarily involve extending the range of professional tasks, obtaining greater autonomy to undertake numerous tasks and improving relations between a midwife and the person being taken care of and other members of the interdisciplinary team who provide care. Other aspects which must be also considered include changing midwives' working models, customization of care and focusing on the person being taken care of, which results in using the nursing process method with its key component i.e. decision making [7, 8].

Jonesem et al. defined clinical decisions in nursing as solutions which are to reach their aims through the smallest amount of information, supported with logical thinking and, at a given time, guaranteeing the greatest certainty or the smallest number of mistakes in the result of making a decision [9]. The definition is widely used in nursing. It is important that decisions, both in nursing and in obstetric care, are seen in the light of clinical treatment and it is the treatment which determines the scope of the research into decisions made by nurses and midwives [10, 11].

This work aimed at attempting to specify the factors contributing to the development of midwives' decision independence.

SPECIFIC ISSUES

1. Was there any correlation between making independent decisions and education of the people involved in the research?
2. Did making independent decisions depend on midwives' age?
3. Did making independent decisions depend on midwives' work experience?
4. Did midwives' postgraduate education affect independence of their decisions?

MATERIAL AND METHODS

The research was carried out among 134 midwives between year 2005 and 2007 in three obstetric care wards: pregnancy pathology ward (PP) or perinatology, labour ward (LW), obstetric ward (OW) in six hospitals of the Lublin Province. Different levels of hospitals' preparation and equipment for administering care procedures as well as hospitals' territorial range of activities made up the key for the selection of hospitals. Selected hospitals were divided into three groups: poviat hospitals, provincial hospitals and clinical hospitals. Each of the groups featured two hospitals with similar ranges of obstetric care they administered. The research was conducted after obtaining written agreements from the Hospital Authorities, Heads of hospital departments, Ward Midwifes and personal agreements from the midwives participating in the observation of their work. Patients, who were looked after by midwives, were also asked to grant their approval for the conducted research.

The research material was collected throughout a fortnight on all weekdays during the 12-hour day shifts in each of the wards of the selected hospitals. Specifying the number of observation days was based on methodological premises concerning photography taken during a working day. There were 252 days (3024 hours) of constant observation during which activities involving decision making as well as the decision-free ones were specified among 28 715 activities which were noted from all working day photography presenting fractions administered by midwives.

Midwives with secondary education comprised the majority of the investigated group in all hospital units where investigations were compared. In all three wards of the clinical hospitals, there were growing tendencies concerning midwives education. Based on Pearson's correlation coefficient, considerable statistical correlations were found between education and hospital types ($p<0.005$), here again, a higher number of people with higher education were found in the clinical hospitals. The data is complemented according to the number of patients in all wards throughout the period covered by the investigation. There were 1.043 patients in total, the majority of whom were in the clinical hospitals. During the research, the lowest number of patients were in poviat hospitals - 586 females in total, whereas there were 723 patients in the provincial hospitals.

The following work experience figures were collected within the researched group. In the group of clinical hospitals, the majority of midwives from labour wards and obstetric care wards had work experience exceeding 20 years (LW – 5 people and OW – 9 people). In the pregnancy pathology ward , the same number of midwives with work experience brackets of over 20 years and 11-15 years were found (both brackets consisted of 5 people). In the provincial hospitals, the majority of midwives from the labour ward and obstetric care ward had work experience exceeding 20 years (LW – 6 women, OW – 6 women) The most numerous group in the pregnancy pathology ward comprised of midwives whose work experience ranged from 16 to 20 years – i.e. 6 respondents. Midwives working in the poviat hospitals were most numerous in the work experience bracket of 16-20 years. The figures were as follows: LW and OW – 5 people in each, PP – 4 people. Midwives with the shortest work experience, i.e. up to 5 years, were employed in all units of clinical hospitals only. Statistical relation of $\chi^2 = 26.43$, $p<0.005$ was found in all units involved in the research, clinical hospitals predominated over the others.

Another feature characteristic for midwives was postgraduate development concerning their professional specializations. Unfortunately, the majority of midwives in all types of obstetric wards had not obtained any specialisation. In the group of people who did develop their professional skills, more respondents had finished postgraduate specializations than were in the course of obtaining them at the time of the investigation. No statistical correlations were found in the above scope ($p>0.05$).

Midwives with secondary education comprised the majority of the investigated group in all hospital units where investigations were compared. In all three units of clinical hospitals, there were growing tendencies concerning midwives' education. Based on Pearson's correlation coefficient, considerable statistical correlations were found between education and hospital types ($p<0.005$). Here again, more people with higher education were found in the clinical hospitals. The data is complemented according to the number of patients in all wards throughout the period covered by the investigation. The majority of patients were in the clinical hospitals – 1,043 people in total. During the research, the fewest patients were in the poviat hospitals - 586 females in total, whereas there were 723 patients in the provincial hospitals.

The following research methods were used in the investigation: examining the working time by means of the direct observation method (photography of a working day), the ergonomic work analysis method according to Franaszczuk and Gadomska with the assessment technique of measuring the burden with information, decisions and taking actions as well as the assessment of the decision independence level according to Kordaszewski and finally the analysis of patients' documentation and midwives' work regulations. Sheets of constant observation, reception analysis, processing information and undertaking actions by a midwife from a given ward, which are extensively described in worldwide literature and have been modified for the sake of this investigation, were the major research tools used in the research.

Working day photography is based on taking measurements of working time and breaks in a given post. This way a full picture of the layout of individual activities during the whole shift of a working day is obtained. The observation is a method used in investigating decision processes because it allows to combine content observed between obtaining information and using it in the decision process (Martyniak, 1999).

The final decision making act is taken in the central nervous system and therefore, and because of a lack of direct research methods within the scope of decision processes, it is recommended to use indirect methods. An assessment of decision processes may only be conducted indirectly by assessing independence, information sources and the analysis of undertaken activities. According to Franaszczuk and Gadomska, the ergonomic analysis of work method with the technique of evaluating the information load, the decision load and burdening with activities, is the most adequate to achieve this aim (1970).

This method was first drafted in the 60s of the previous century by Polish authors – Irena Franaszczuk and Helena Gadomska. It was later used in the research of work ergonomics into physical and psychological strains in various posts conducted by other researchers. The research aimed at specifying the level of information load, activity load in a given post, which makes up a basis for drafting requirements concerning qualifications, modifying tasks trusted to an employee and for casting errors that may occur in the process of work (Franaszczuk, Gadomska, 1970). A modified working day photography sheet was a research tool presenting activities undertaken by sector midwives

together with the time of their duration , information sources, decision types, undertaken actions, levels of difficulty and types of care.

Another technique used in the research was an assessment of the level of decision independence according to Kordaszewski. These levels allow to undertake a very simple analysis of decision independence in midwives' actions.

Information obtained from an analysis of patients' documentations and midwives' work regulations completed data collected in the course of observations. Data gathered in this way was used in the investigation as a confirmation of undertaking actions, making decisions and transferring information.

Research results were analysed statistically by means of the STATISTICA v. 6.0 (StatSoft Polska) computer software. Appropriate tests were selected depending on the type of data that examined interactions of specific features. The following statistical tests were used: the Kolmogorov-Smirnoff test with Lillefors' amendment, the Leven's test, the Mann-Whitney test, the Wilcoxon's (Z) test, Spearmann's (Rs) rank correlation coefficient, the t-Student's test, Pearson's ($\chi 2$) correlation coefficient. Numerical data which was obtained, either failed to meet the criterion of normality or quality variation, therefore, nonparametric tests were employed.

RESULTS

Pursuant to the methodological assumptions adopted for this paper, fractions and subfractions were specified while assessing midwives' work. Six subfractions were identified for the sake of this paper, all of which reflected the nature of activities undertaken while taking care of a woman in labour and puerperium. They were classified as the following diagnostic activities: 1. Basic Diagnostics Activities, 2. Help with Maintaining Patient's Personal Hygiene, 3. Help with Maintaining the Hygiene of Patient's Environment, 4. Helping Patients within the Scope of Excreting, 5. Helping Patients within the Scope of Feeding and Communicating, 6. Communicating with Patients. These were analysed from the perspectives presented below.

Midwives' education considerably influenced independent decisions they took in the pregnancy pathology wards, labour ward ($p<0.01$) and obstetric care wards ($p<0.05$), table I. Independent decisions were most often taken by labour room midwives with higher education (Me = 14.67). Independent decisions were also relatively frequent in case of midwives with higher education working in the pregnancy pathology wards (Me = 10.33). In the obstetric care wards, independent decisions were most often made by studying midwives (Me = 8.50).

Tab. I. *Autonomous decisions vs. midwives' education*

Midwives' education	*Pregnancy pathology ward (PP)*		*Labour ward \(LW)*		*Obstetric ward (OW)*	
	Autonomous decisions					
	Me	SD	Me	SD	Me	SD
Higher education	10.33	3.01	14.67	2.31	8.43	3.55
Studying	7.56	4.69	8.58	6.03	8.50	4.19
Secondary education	6.16	3.29	3.88	5.36	5.63	3.29
$\bar{x}$	6.61	3.58	4.94	5.89	6.20	3.57
The ANOVA Kruskal-Wallis test	H = 7.87 p< 0.01 (**)		H = 11.37 p< 0.01 (**)		H = 7.17 p< 0.05 (*)	

An analysis into the research material proved the respondents' age to considerably influence making independent decisions, $p<0.05$, table II. Having been identified, the subfractions were aggregated in order to present the complete picture of tasks undertaken by midwives taking care of patients. The influence of the aforementioned variable on decisions made independently in the obstetric wards was statistically highly significant ($p<0.001$). A correlation of dependent decisions made in the labour wards and midwives' age ($p<0.05$) proved statistically significant. The greatest number of independent decisions were made by midwives from the specific wards who were in the 'over 51 years old' group. (PP – Me = 22.29; LW – Me 87.38; OW – Me = 12.33) and those under 30 (PP – Me = 19.14; LW – Me 70.88; OW – Me = 8.70).

Another research problem dealt with in this paper was to specify the influence of work experience on making decisions by midwives, table III. With respect to the labour room, no statistical correlation was found between work experience and decisions involving independent activities.

In perinatology and obstetric wards, where statistical correlations were reported between these two variables, younger midwives were found to undertake more independent activities and activities involving decisions than their colleagues with a longer work experience. The tendency concerned diagnostic activities in perinatology wards $\bar{x}$=22.25; helping patients with feeding $\bar{x}$=6.14; helping patients with

excreting: $\bar{x}$=45.29; and with communicating $\bar{x}$=72.50. Furthermore, in obstetric care wards, similarly to previous wards , an additional correlation was recorded within the scope of the hygiene of patient's environment: $\bar{x}$=25.50 and within other subfractions.

Tab. II. *Autonomous decisions vs. midwives' age*

Midwives' age	*Pregnancy pathology ward (PP)*		*Labour ward (LW)*		*Obstetric ward (OW)*	
	Autonomous decisions					
	Me	SD	Me	SD	Me	SD
< 30 age	19.14	7.63	70.88	72.38	8.70	5.89
31-40 age	16.64	5.05	46.52	30.86	6.44	4.76
41-50 age	16.71	5.47	48.27	45.10	5.40	4.37
>51 age	22.29	2.75	87.38	34.11	12.33	5.25
$\bar{x}$	17.35	5.49	53.58	44.58	7.12	5.29
The ANOVA Kruskal-Wallis test	H = 8.04 p< 0.05 (*)		H = 9.43 p< 0.05 (*)		H = 16.92 p< 0.001 (***)	

A number of activities undertaken by people with various forms of postgraduate training was specified as well as the influence of this number on the type of tasks these people undertook, table IV. An analysis into the research material revealed a great number of independent activities requiring independent decisions made in the labour wards during a 12-hour day shift and these were predominantly made by people who had completed various forms of postgraduate training ($\bar{x}$ = 311.76). Statistical analysis carried out by means of the Mann-Whitney test ($p<0.05$) pointed out statistically significant differences between activities undertaken by midwives in the labour wards and in the pregnancy pathology wards. The smallest number of activities were recorded in the obstetric wards for nurses before ($\bar{x}$ = 91.53) and after their postgraduate training ($\bar{x}$ = 107.87). No statistical correlations ($p>0.05$) were recorded in these units.

Tab. III. *Autonomous decisions vs. midwives'work experience*

Midwives'work experience	*Pregnancy pathology ward (PP)*						*Labour ward (LW)*						*Obstetric ward (OW)*					
	Subfractions ($\bar{x}$)																	
	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**
0-5 age	22.25	5.25	4.25	6.00	44.75	72.50	40.00	10.40	22.80	6.60	4.00	70.00	14.00	5.50	25.50	4.17	42.67	47.33
6-10 age	18.43	4.14	4.43	6.14	45.29	64.86	69.09	18.63	29.63	7.00	3.64	94.64	6.56	4.89	19.11	4.33	29.33	36.11
11-15 age	15.88	4.24	4.59	6.06	37.65	64.65	44.21	18.50	24.93	6.21	5.00	65.86	6.75	4.31	18.75	4.19	30.38	28.63
16-20 age	15.84	3.52	4.32	4.68	32.26	63.03	51.29	18.29	25.29	5.96	3.50	73.92	4.48	4.30	16.65	4.17	24.22	22.17
> 20 age	19.12	4.60	4.64	5.72	38.64	68.72	56.37	15.87	24.00	6.27	2.73	77.03	8.13	4.70	19.00	4.30	28.27	29.50
$\bar{x}$	17.34	4.12	4.48	5.45	36.93	65.65	53.58	17.04	25.19	6.28	3.52	76.17	7.12	4.60	18.79	4.24	28.70	29.31
The ANOVA Kruskal-Wallis test	H = 9.52 p< 0.05	p> 0.05		H = 11.74 p< 0.05	H = 15.84 p< 0.01	H = 10.57 p<0.05	p> 0.05						H = 14.39 p< 0.01	p> 0.05	H = 12.36 p< 0.01	p> 0.05	H = 11.46 p< 0.05	H = 17.31 p<0.001

Tab. IV. *Independent activities undertaken during a 12-hour shift vs. midwives' professional training*

Subfractions:	**Number of midwives' independent activities.**					
	Pregnancy pathology ward (PP)		*Labour ward (LW)*		Obstetric ward (OW)	
	Graduated or during Professional training					
	Before	After	Before	After	Before	After
1. Basic diagnostic activities as well as nursing and care activities.	16.90	18.00	50.57	72.14	6.70	10.88
2. Helping patients' in their personal hygiene.	3.88	4.44	16.84	18.71	4.49	5.22
3. Helping in hygiene of patients' environment.	4.28	4.82	24.36	30.96	18.94	18.33
4. Helping patientswithin the scope of excreting .	5.58	5.15	6.01	8.03	4.14	4.58
5. Helping patients with respect to feeding and communicating.	36.73	34.53	3.19	5.83	3.89	4.41
6. Communicating with a patient.	64.48	68.22	74.53	89.21	28.49	35.58
Activities in general	143.33	149.96	241.32	311.76	91.53	107.87
Average number of activities during the 1st working hour:	11.94	12.50	20.11	25.98	7.62	8.99
Mann-Whitney Test	Z = 2.64 p<0.05		Z = 2.04 p<0.05		Z = 0.58 p>0.05	

The greatest number of independent activities were undertaken by midwives who had graduated from their specialization courses (PP: $\bar{x}$ = 68.22; LW: $\bar{x}$ = 89.21; LW: $\bar{x}$ = 35.58) and those who did not have any postgraduate training (PP: $\bar{x}$ = 64.48; LW: $\bar{x}$ = 74.53; PŁ: $\bar{x}$ = 28.49).These activities were recorded in all units of the "Communication with a Patient" subfraction. An increase in independent activities was also recorded in the "Basic Diagnostic Activities" subfraction in the labour wards for nurses who had graduated from their specialization courses ($\bar{x}$ =72,14). In other subfractions, there was a considerable predominance of independent activities undertaken in labour rooms by nurses with a specialization over those undertaken by nurses in the course of their postgraduate training ($p<0.05$). In pregnancy pathology units, midwives who had done some postgraduate training hardly undertook more activities than their colleagues who were not raising their professional qualifications ($p<0.05$). Furthermore, the labour ward midwives who were not raising their professional qualifications undertook slightly more activities in the "Help with the Patient Environment's Hygiene" subfraction, which was different than in other subfractions ($p>0.05$).

DISCUSSION

This paper attempted to specify factors contributing to a development of nurses' independence in their decision making. Presented material proved these were the factors which were frequently described as basic and generally agreed upon to be the determinants of individual development.

The main research issue analysed in this paper was an occurrence of correlations between making decisions and midwives' age. An analysis of the collected material proved age to considerably influence decision making. It was surprising to find such a high tendency for making decisions in the group of youngest nurses. This situation might be a reflection of current midwife training programmes for undergraduate courses, which focus on professional autonomy and independent undertaking a number of decisions. Another significant factor might be a need to run graduate courses allowing midwives to obtain their degrees. As far as an increase in the number of decisions made by the group of oldest midwives is concerned, the most considerable factor seemed to be professional experience as well as life experience, both of which had been obtained for a number of years. The research undertaken by O'Connor et al. among Canadian women, which concerned a perception of health behaviours, proved that younger respondents tended to be more independent in their decisions [16]. It seems surprising that younger respondents seemed to find any kinds of choices easier to make. The issue may be concluded by quoting results of English research made by Fraser, which were obtained after self evaluation organised among obstetrics students and their mentors. A leading tendency of students' expansive and trouble-free attitude towards difficult clinical activities was observed, however, teacher's attitude to their behaviour proved rather evasive and they tended to belittle students' skills. Interestingly, both groups claimed to be aware of the consequences and the responsibility that their actions entailed [17].

Another research issue concerned specifying the influence that midwives' education had on the decisions they made. An analysis which was made proved there was a statistically significant influence of midwives' higher education on decisions they made independently. The research carried out by Lauri et al. in five countries (Sweden, Finland, Canada, Switzerland and USA) also proved there was a statistically significant influence of geriatric and surgical nurses' knowledge and experience on decision involving activities, especially within the scope of educational activities [18].

The influence of work experience on decisions made by midwives was also specified. Work experience had statistically significant influence on decisions made independently by nurses in relatively few subfractions. Interestingly, the youngest nurses took the effort of being independent and responsible for complex activities, such as: assessing a state of a pregnant woman and her child, and informing patients about activities to be taken and the course of actions. Decision making was recorded at similar levels in nurses' work experience brackets according to the research by Lauri et al., where the majority of decisions were taken by young people whose work experience ranged from 5-10 years [18]. This fact probably referred to a higher quality of midwives' education obtained exclusively from medical universities. Furthermore, the trend might have been reinforced by implementing the programme propagating individual approach to tasks and solving various types of problematic situations.

An influence of midwives' various forms of postgraduate training on their decision making was also checked. The research which had been obtained indicated significant differences between decisions made in two groups of midwives at statistically significant levels in pregnancy pathology units and in labour rooms ($p<0.05$). Such differences were not recorded in obstetric units ($p>0.05$). Midwives' differentiating their decisions with respect to the form of their postgraduate training seemed to be a very favourable trend. However, differences in decisions made by two groups were not absolutely clear-cut, which might confirm that specialist activities were undertaken by midwives without relevant qualifications. An insufficient number of midwives with specialisations did not allow to schedule shift staffing in such a way so as to guarantee a presence of specialists in every single shift. Writers' own research failed to reveal any influence of midwives' postgraduate training on making independent decisions ($p>0.05$). It must be pointed out that numerous activities requiring special qualifications (obtaining a specialisation or a specialist course) were undertaken by nurses who did not have relevant qualifications. Similarly, relatively few Swedish nurses had graduated from specialist courses and training. Hence the majority of activities were undertaken by nurses with lowest qualifications [19].

Writers' own research proved specialist activities to have been undertaken by unqualified nurses. Paradoxically, they could not refrain from undertaking these activities because it would disturb a continuity of work with a patient and her child. Consequently, it might be concluded that the management ought to intensify their efforts to encourage midwives to graduate from appropriate training. Having researched English midwives, Lavender pointed out to this professional group awareness of a significance of their postgraduate training, which was a condition of their autonomy and independence as well as improving midwifery services [20].

CONCLUSIONS

1. Midwives' higher education influenced their decision making ($p<0.05$).
2. Midwives from the youngest and the oldest age brackets tended to make decisions most often ($p<0.05$).
3. Decisions concerning obstetric care were most often made by nurses whose work experienced was below 5 years ($p<0.05$).
4. Significant statistical differences were found between independent activities undertaken in pregnancy pathology units and labour rooms by midwives with various forms of postgraduate training, and independent activities undertaken by midwives without a specialization ($p<0.05$), the former predominated.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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BMI VS OVERWEIGHT IN THE YOUNG PEOPLE OF BYDGOSZCZ

BMI A NADWAGA BYDGOSKIEJ MŁODZIEŻY

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S u m m a r y

I n t r o d u c t i o n . Overweight and obesity affect more and more children and young people. In many regions of Poland, a percentage of obese children and young people ranges from 2.5% to 12% of the population at their developmental age and demonstrates a clear upward trend. The aim of this work was to evaluate the frequency of overweight and obesity in young people with the use of two research tools.

M a t e r i a l a n d m e t h o d s . The research included 177 students (71 boys and 106 girls) aged 13 to 19. The frequency of overweight and obesity was evaluated with Polish BMI centile chart for sex and age, and fat content obtained by the SBIA method.

R e s u l t s . The data based on BMI shows that the excess body mass (overweight and obesity) occurred in 19.2% of the subjects. The evaluation of obesity on the basis of fatty tissue proved higher values in girls (over 38%)

C o n c l u s i o n s . For girls, BMI is not a reliable index for the evaluation of the risk of obesity.

S t r e s z c z e n i e

W s t ę p . Nadwaga i otyłość w coraz większym stopniu dotyka dzieci i młodzież. W różnych regionach Polski procent dzieci i młodzieży otyłej wynosi od 2,5% do 12% populacji w wieku rozwojowym i ma wyraźną tendencje zwyżkową. Celem pracy była ocena częstości występowania nadwagi i otyłości u młodzieży z użyciem dwóch różnych narzędzi badawczych.

M a t e r i a ł i m e t o d y . Zbadano 177 uczniów (71 chłopców i 106 dziewcząt) w wieku 13-19 lat. Do oceny częstości występowania nadwagi i otyłości zastosowano polskie tabele wartości centylowych BMI dla płci i wieku oraz zawartość tkanki tłuszczowej uzyskaną metodą SBIA.

W y n i k i . Dane uzyskane na podstawie wskaźnika BMI pokazują, że nadmiar masy ciała (nadwaga i otyłość) wystąpił o 19,2% badanej młodzieży. Oceniając otyłość na podstawie tkanki tłuszczowej uzyskano znacząco wyższe częstości u dziewcząt (ponad 38%)

W n i o s k i . Dla dziewcząt BMI nie jest miarodajnym wskaźnikiem do oceny stopnia zagrożenia otyłością.

Key words: overweight, obesity, young people
Słowa kluczowe: nadwaga, otyłość, młodzież

INTRODUCTION

Overweight and obesity are a serious problem in relation to health, psychosociology and economics both in Poland and in the world. The research conducted in 2000 by the National Food and Nutrition Institute (IŻŻ) shows that 50% to 60% of adults suffer from overweight and obesity [1]. This health problem affects more and more children and young people, too. In many regions of Poland, a percentage of obese children and young people ranges from 2.5% to 12% of the

population at their developmental age and demonstrates a clear upward trend.

The all-Polish research conducted by the IŻŻ shows that overweight and obesity affect 12.6% of boys and 12.2% of girls aged 1 to 18, whereas obesity occurred in 4.6% of the subject population [1].

Obesity is a medical condition distinguished by accumulation of body fat and increased accumulation of lipids in liver, muscles and spleen. An excessive increase in cells in childhood may contribute to the development of obesity in adulthood, since growth in cells is an irreversible process. For this reason, preventive actions should be taken as early as possible not to allow for metabolic and organ complications. Obesity is a disorder of a nutrition state which results from taking more energy in food than a person uses. Incorrect nutrition and little physical activity are two main obesity risk factors. Children spend a few hours a day in front of TV or computer, often snacking. Their physical activity is very little. These habits, established in childhood, remain in adulthood. According to the diabetes specialist Prof. Tatoń, obesity is a medical problem in merely 25% and a civilization problem in 75% [2]. In Poland, medical care of overweight and obese children is insufficient, since only 27% of school children with such disorders are covered by such care. In 2006, Poland signed the European Charter on Counteracting Obesity, part of which was the National Programme for Obesity Prevention POL-HEALTH. The specialists participating in the 2nd Convention of the Polish Association for the Study of Obesity in Szczecin in 2009 predict that, even with a lot of effort, the obesity epidemic may last from 5 to 10 years. Due to the importance of this problem, the tendencies of changes in the frequency of obesity in children and young people should be strictly monitored.

The aim of the work was to evaluate the frequency of overweight and obesity in young people with the use of two research tools.

MATERIAL AND METHODS

The research was done in 2009 and included 177 students (71 boys and 106 girls) aged 13 to 19. The body composition of the subjects was calculated with segmental bioelectrical impedance analysis (SBIA) using the Biospace *In Body 3.0* apparatus. Their body height was calculated with an anthropometer, and on that basis the apparatus calculated the BMI. A detailed description of the analyser's operating principles is presented by Kichul *et al.* [3].

The frequency of overweight and obesity was evaluated by the following tools:

1. Polish BMI centile charts for sex and age drawn up in 2004 [4], taking BMI centile values ≥ 85 as overweight and ≥ 95 as obesity.
2. The amount of fatty tissue in kilograms and percentage obtained by SBIA method and using the division of excess fat (%) according to Tompson [5]: normal range (♀< 26%; ♂ < 19%), obesity risk (♀ 26-31%; ♂19-24%), obesity (♀ ≥ 32%; ♂ ≥ 25%).

RESULTS OF THE RESEARCH

The frequency of overweight and obesity in the subjects, obtained with two different methods, is presented in Tables I and III. The results obtained on the basis of BMI (Table I) show that excessive body mass (overweight and obesity) occurred in 19.2% of the subjects (♂ 19.7%; ♀ 18.9%). Out of 177 students, persons with the correct BMI were singled out (Table II). It turned out that in this group the fat level in girls ranged from 13.5% to 32.1%. The fat level in boys ranged almost within normal range (6.3-20.6%). Evaluating obesity on the basis of fatty tissue, the values in girls were significantly higher (Table III). Obesity risk and obesity occurred in more than 38%. In boys, the results obtained by both methods were similar (18.9% and 18.3%, respectively).

Table I. *Frequency of overweight and obesity in young people aged 13-19 according to BMI 85th – 95th centile*

	N	Overweight (%)	Obesity (%)
Total	177	12.4	6.8
Boys	71	12.7	7.0
Girls	106	12.3	6.6

Table II. *Body composition in young people with correct BMI*

Feature	Girl (n=68)			Boys (n=49)		
	Min	Max	M	Min	Max	M
BMI [kg/m^2] 15th-85th centiles	17.8	23.0	20.5	18.4	23.8	21.3
Fat [%]	13.5	32.1	24.2	6.3	20.6	12.7
Fat [kg]	7.2	22.3	13.8	4.0	16.1	8.8
Lean Body Mass [kg]	34.1	53.2	42.8	44.7	73.4	60.1

Table III. *Frequency of obesity risk and obesity in young people aged 13-19 according to fatty tissue*

	N	Obesity risk (%)	Obesity (%)
Total	177	22.6	7.9
Boys	71	11.3	7.0
Girls	106	30.2	8.5

DISCUSSION

It is not easy to evaluate excessive body mass in children and young people. Monitoring and comparing overweight and obesity are reliable when the same criteria are applied. The most precise obesity index is the evaluation of fatty tissue. In mass research, the nutrition state is evaluated by BMI (kg/m^2), which is a proportion of mass to body height. It is assumed that people with the same height have similar lean body mass (LBM), therefore differences in their total body mass result from different contents of fatty tissue. Although the evaluation of body mass index is not a precise method of diagnosing obesity, this index is still recommended by WHO for evaluation of the level of obesity. For adults, the BMI values between 25.0 and 29.9 kg/m^2 are assumed as overweight, and over 30 kg/m^2 as obesity [6]. As per the recommendations of the WHO Expert Committee, overweight in people aged 10-19 is determined by BMI $\geq 85^{th}$ centile, and obesity by $\geq 95^{th}$ centile, which are based on the research into the American population [7]. For a short time now, the IOTF's international standard (International Obesity Task Force) has been in use, determining the so-called cut-off points. This standard, called the Cole's standard, is based on the measurements of children and young people in 6 countries [8, 9]. For more than ten years now, the European Childhood Obesity Group (ECOG) has been recommending using the body mass index (BMI) in the diagnosing overweight and obesity [10]. In 2005, European researchers published a report of the International Obesity Task Force, in which they announced that 16-22% of children and young people aged 4-18 were affected by overweight or obesity, including 4-6% of obese people [11]. In Poland, based on population research, percentile charts for BMI were drawn up by the Institute of Mother and Child in 1999 [12] and more recent ones – in 2004 by the Department of Epidemiology of the University of Medicine in Poznań [4].

The research was based on the Polish centile charts drawn up in 2004. We followed the example of the research by Jodkowska *et al*. [13] who examined more than 8000 children aged 13-15, comparing 3 research criteria. The researchers concluded that the most useful tool was the limit values of BMI $\geq$ 85th centile for overweight and $\geq 95^{th}$ centile for obesity, using the standards dating from 2004. Mazur *et al*. applied different limit values, 90th and 97th centiles, respectively [14]. Due to the inconsistent criteria for the evaluation of overweight and obesity, and due to the diversity of age of the children, it is not possible to precisely compare the results of the works of the Polish authors [15]. In the presented research, about 19% of young people are overweight. Obesity occurred in 7% of boys and 6.6% of girls. In the research conducted by Jodkowska, overweight and obesity affected 13% of teenagers, including obesity in 3.3% and 5.7% of them, respectively.

The BMI shows a significant correlation (0.8) with an amount of fatty tissue [16]. In our research this correlation was 0.75 for boys and 0.83 for girls. When we analysed the group of boys and girls with the correct BMI (between 15th and 85th centiles), it turned out that this group included persons with a high content of fatty tissue in the total body mass, mainly in girls. When evaluating obesity due to fatty tissue, we discovered that the number of obese girls increased from 6.6% to 8.5%, and the number of people at risk of obesity from 12.3% to 30.2%. In boys, the results obtained with two methods were similar. According to Chrzanowska [17], the IOTF obesity criteria are too tolerant for the European population and may be a reason for an 'oversight' of obesity cases in mass research. When analysing BMI values, it should be borne in mind that this index does not differentiate fat mass from muscle mass and bones. The earlier research into a group of female students aged 18-25, showed that a content of fatty tissue was 22.1 ± 5.2% on average, while LBM 46.5 ± 5.6 kg [18]. The girls with correct BMI values (17.8-23 kg/m^2) had little content of lean body mass (LBM) and large content of fatty tissue. This may mean that a large group of girls at risk of obesity may not be detected by the way of screening. Among girls the vogue is to be slim, so they lose weight using different diets. Perhaps a small amount of proteins in food as well as insufficient physical activity cause their insufficient muscle mass. Consumption of an increased amount of carbohydrates in food may lead to excessive fat in the body.

Examination of the body composition by screening is rather impossible, therefore it should be considered to introduce measurements of skin-fat folds. Subcutaneous fatty tissue constitutes about 50% of total fat [19]. Our research was not conducted on numerous material. In order to be able to generalize the observations, the research should be done on a more numerous group of students.

CONCLUSIONS

1. On the basis of BMI, the frequency of excessive body mass in the group of subjects was 19%.
2. Based on percentage of fat overweight and obesity values were 18.3% in boys and 38.7% in girls.
3. For girls, BMI is not a reliable index for the degree of obesity risk.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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AFFINITY OF OCHRATOXIN A TO HUMAN SERUM ALBUMIN DETERMINED BY FLUORESCENCE ANISOTROPY MEASUREMENTS

OKREŚLANIE POWINOWACTWA OCHRATOKSYNY A DO ALBUMINY SUROWICY LUDZKIEJ ZA POMOCĄ POMIARU ANIZOTROPII FLUORESCENCJI

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Summary

Ochratoxin A (OTA) is a fluorescent toxic metabolite produced by several strains of *Aspergillus* and *Penicillium* fungi. The fluorescence anisotropy measurements provide useful information about the binding of OTA to human serum albumin (HSA).

On the basis of fluorescence anisotropy measurements of OTA diluted in fluids with different HSA concentrations, the affinity of this mycotoxin to HSA is determined.

Streszczenie

Ochratoksyna A (OTA) jest fluoryzującym toksycznym metabolitem produkowanym przez grzyby pleśniowe z gatunków *Aspergilus* i *Penicillium*. Pomiary anizotropii fluorescencji dostarczają użytecznych informacji o wiązaniu OTA do albuminy surowicy krwi.

Na podstawie pomiarów anizotropii fluorescencji ochratoksyny A, rozpuszczanej w płynach o różnych stężeniach albuminy, jest określane powinowactwo tej mikotoksyny do albuminy.

Key words: ochratoxin A, fluorescence anisotropy, binding to HSA
Słowa kluczowe: ochratoksyna A, anizotropia fluorescencji, wiązanie do HSA

INTRODUCTION

Ochratoxin A (OTA, $C_{20}H_{18}ClNO_6$, Mw=403.82 g/mol) is a mycotoxin produced by some species of *Aspergillus* and *Penicillium* which are widespread in food and feed [1-4]. Its chemical structure is presented in Fig. 1A. OTA is a colourless and crystalline compound, relatively heat stable. Baking and roasting of contaminated food reduce the toxin content by about 20 %, while boiling has no effect on it [5]. OTA can occur in a variety of plant products, mainly in cereals, grains, coffee as well as in grape juice, wine, beer and bread. It has been also found in the blood and kidney of pigs fed with contaminated feed [4]. This mycotoxin is a causative factor of many disease states in both humans and animals. It is nephrotoxic, carcinogenic, teratogenic, neurotoxic, immunotoxic and hepatoxic [4]. It is most probably the causal agent in the development of nephropathies (Balkan Endemic Nephropathy, and Chronic Interstitial Nephropathy) and urothelial tumors [5].

Several studies have proved that OTA exhibits an extremely high affinity to human serum albumin (HSA) and other macromolecules in the blood. The bond with serum albumin results in the generation of a mobile reservoir of ochratoxin, from which it can be slowly released, and hence rendered bioavailable over extended periods of time and furthermore, it can retard the elimination of OTA from the body [5]. OTA is a serious threat for human health; hence its interaction with human serum albumin requires a detailed investigation. Ochratoxin A exhibiting very high binding to HSA has very long half-life (about 1 month) in human plasma [2, 4]. Compounds being able to displace OTA from serum albumin are desirable. It is expected that such compounds can be efficient antidotes of OTA.

OTA is a fluorescent compound, so a method of fluorescence spectroscopy can be used to determine some biophysical properties of this compound. Steady state fluorescence anisotropy measurements were used to determine the association constants for OTA to HSA. For small and fast rotating fluorophores like free ochratoxin molecules, steady-state fluorescence anisotropy is small, close to zero. The fluorescence emitted by large and slow rotating molecules like proteins, or small molecules bound to proteins or membranes, exhibits the enhanced steady-state anisotropy close to limiting anisotropy. One can use this fact to determine the binding constant of ochratoxin A to HSA molecules. The method of fluorescence anisotropy measurements is also used to study the behavior of OTA in HSA solution in presence of selected competitive compounds.

EXPERIMENTAL PROCEDURE

Ochratoxin A (*N*-{[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl] carbonyl}-3-phenyl-L-alanine) was obtained from Sigma-Aldrich. 1.2 mM stock solution of this toxin was prepared in ethanol. The stock solution for fluorescence measurements was added to phosphate buffered saline (PBS) and HSA solutions. The final toxin concentration was 1μM.

The HSA was purchased from Sigma–Aldrich. The HSA solutions with the desired concentration were obtained by dilution of adequate amount of HSA in PBS. The pH of HSA solution was kept at 7.4. Competitive compounds - ibuprofen (IBU, $C_{13}H_{18}O_2$) and flurbiprofen (Flurbi, $C_{15}H_{13}FO_2$) were purchased from Sigma-Aldrich.

The fluorescence spectra and fluorescence anisotropy of OTA diluted in PBS and HSA solutions were recorded, and next the binding properties of OTA to HSA were determined. A PTI (Photon Technology International, Birmingham, NJ, USA) spectrofluorometer in "L-format" was used for recording the fluorescence spectra and steady-state fluorescence anisotropy. Details of measurements were described previously [6, 7].

RESULTS AND DISCUSSION

Fig. 1B presents the steady-state fluorescence emission spectra of 1μM OTA solution in PBS and in HSA solution. The 385 nm wavelength of light was used for excitation and maximum of emission was observed at 445 nm for both solutions (PBS, HSA).

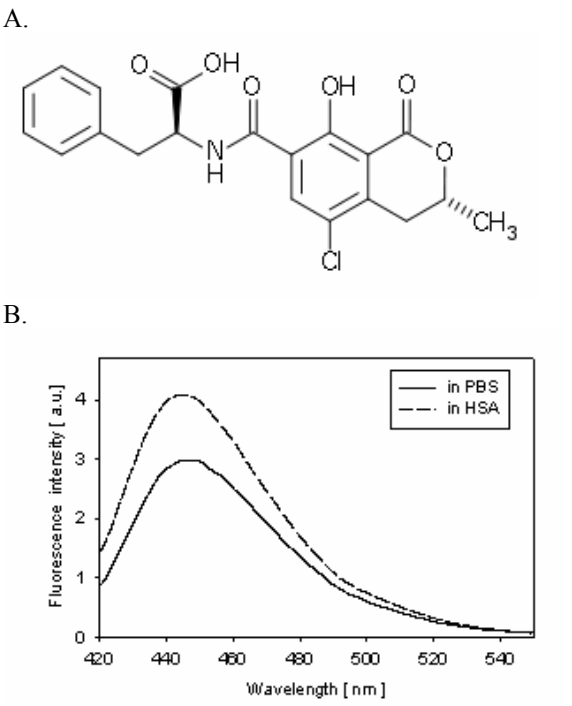

Fig. 1. *(A) Chemical structure of OTA. (B) Fluorescence emission spectra of 1 μM OTA diluted in PBS and in 10 μM HSA solution. pH of each solution equaled 7.4*

Ryc. 1. *(A) Wzór strukturalny OTA. (B) Widma fluorescencji (emisyjne) 1 μM OTA rozpuszczonej w PBS oraz w 10 μM roztworze HSA. pH każdego roztworu wynosiło 7,4*

Fig. 2A shows the changes of fluorescence anisotropy of OTA diluted in HSA solutions over HSA concentration. A very fast increase of fluorescence anisotropy with increasing HSA concentration is observed. It proves a very high affinity of this compound to HSA. For comparison, in the same figure

the changes of fluorescence anisotropy of another compound – carboxylate form of camptothecin (CPT-C) over HSA concentration are depicted. Carboxylate form of camptothecin exhibits very high affinity to HSA, but as it shows in the Fig 2A OTA demonstrates much higher affinity to HSA. It exhibits the highest affinity to HSA among compounds studied by our group [6]. Quantitative measure of binding properties of toxin or drug to HSA is association constant defined as

$$K = \frac{A_B}{A_F[HSA]}$$

where A_B represents the concentration of bound toxins, A_F represents the concentration of free toxins and [HSA] represents total concentration of HSA. On the basis of experimentally determined anisotropy using previously described methods, the concentration of free and bound toxin/drug in HSA solution was determined and then the double-reciprocal plots were drawn. These plots for OTA and reference compound i.e. CPT-C (camptothecin carboxylate) are shown in Fig. 2B. Slope of lines fitted to experimental values determine the inverse of association constants (1/K). Fig. 2B shows that association constant of OTA to HSA is equal $(2.2 \pm 0.2) \cdot 10^6$ M^{-1}, while this constant determined for reference compound (CPT-C) is equal $(3.3 \pm 0.5) \cdot 10^5$ M^{-1}.

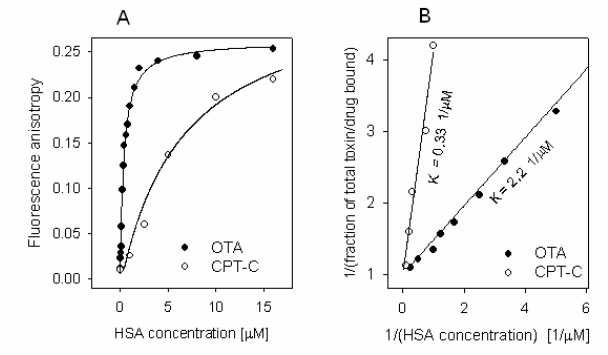

Fig. 2. *(A) Fluorescence anisotropy of 1 μM OTA and 1 μM CPT-C over HSA concentration. (B) Double-reciprocal plots for binding of OTA and CPT-C to HSA*

Ryc. 2. *(A) Anizotropia fluorescencji 1 μM OTA and 1 μM CPT-C w roztworach HSA w zależności od stężenia HSA. (B) Zależność odwrotności udziału molekuł związanych OTA i CPT-C od odwrotności stężenia albuminy*

OTA exhibiting very high binding to HSA has very long half-life in human plasma what is a serious threat for human health. Therefore, the compounds being able to displace OTA from serum albumin are desirable. Fig. 3A shows how compounds binding easily with HSA, such as ibuprofen and flurbiprofen, introduced into HSA solution, decrease the OTA binding with HSA. On the basis of a change in fluorescence anisotropy presented in Fig. 3A, the concentrations of free and bound toxin in HSA solutions and in HSA solutions containing competitive compounds were determined, and then the double-reciprocal plots were drawn. They are presented in Fig. 3B. Slope of lines fitted to experimental values determine the inverse association constant (1/K). Values of association constants of OTA to pure HSA, and HSA contained competitive compound are summarized in Table I.

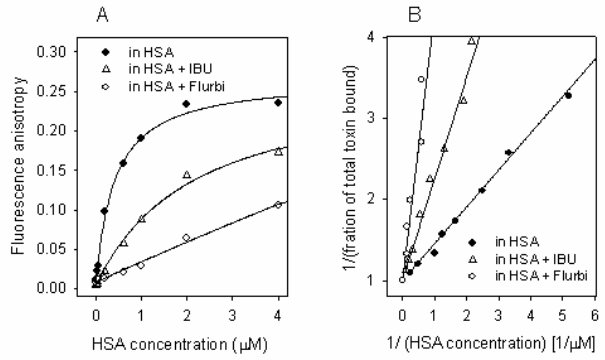

Fig. 3. *(A) Fluorescence anisotropy of OTA in HSA solutions and HSA solutions containing competitive compounds - ibuprofen and flurbibrofen over HSA concentration. Concentration of OTA equal 1μM. Concentrations of competitive compounds equal 1mM. (B) Double-reciprocal plots for the binding of OTA to pure HSA and to HSA contained competitive compounds – ibuprofen and flurbiprofen*

Ryc. 3. *(A) Anizotropia fluorescencji OTA w roztworach HSA i w roztworach HSA zawierających konkurujące związki – ibuprofen i flurbiprofen w zależności od stężenia HSA. (B) Odwrotności udziału molekuł OTA związanych z czystą HSA i z HSA zawierającą konkurujące związki (ibuprofen i flurbiprofen) w zależności od odwrotności stężenia HSA*

Table I. *Values of association constants of OTA to pure HSA and to HSA contained competitive compounds - ibuprofen and flurbiprofen. Concentration of competitive compounds was 1mM*

Tabela I. *Wartości stałych powinowactwa OTA do czystej albuminy i do albuminy zawierającej konkurujące związki – ibuprofen i flurbiprofen. Stężenie konkurujących związków wynosiło 1 mM*

Solution	K_{OTA} [M^{-1}]
HSA	$(2.2 \pm 0.2) \cdot 10^6$
HSA + IBU	$(0.8 \pm 0.1) \cdot 10^6$
HSA +FLURBI	$(0.3 \pm 0.05) \cdot 10^6$

On the basis of the results presented in Fig. 3A, 3B and Table I, one can conclude that it is very difficult

to inhibit OTA binding to HSA. In the performed experiment, concentration of competitive compounds (ibuprofen, flurbiprofen) was 1 mM, i.e. 1000 fold higher than concentration of OTA (1 μM). High concentrated competitive compounds are not able to prevent OTA binding to HSA. In HSA solutions containing competitive compounds the association constant of OTA to HSA decreased only few folds – from $2.1 \cdot 10^6$ M^{-1} in pure HSA to $8 \cdot 10^5$ M^{-1} in HSA solution containing 1 mM ibuprofen and to $3 \cdot 10^5$ M^{-1} in HSA solution containing 1 mM flurbiprofen. The obtained results suggest that OTA, in comparison with ibuprofen or flurbiprofen, exhibits preferential binding properties to HSA. The search of nontoxic compounds able to inhibit strong OTA binding with HSA is a big challenge.

CONCLUSION

Steady-state fluorescence anisotropies of ochratoxin A diluted in HSA solution were measured and next, association constants to HSA was determined. Performed measurements proved that OTA exhibits extremely high affinity to HSA. The preferential OTA binding to HSA makes it difficult to eliminate this toxin from the body. Fluorescence anisotropy measurements showed also that inhibition of OTA binding to HSA by competitive compounds such as ibuprofen and flurbiprofen is not efficient.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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EDUCATIONAL LEVEL AND PHYSICAL ENDURANCE OF PHYSIOTHERAPY STUDENTS

POZIOM EDUKACYJNY A SPRAWNOŚĆ FIZYCZNA STUDENTÓW FIZJOTERAPII

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S u m m a r y

Socio-economic development in the last decade caused numerous changes in the quality of life of many societies of the widened European Union. One of its indications in Poland are the changes in the education system, which are manifested in significant increase of both young people choosing extramural studies on different education levels and two-cycle realization of their higher education studies.

The aim of this paper was to try to estimate the differences in physical fitness of physiotherapy students of extramural master's and bachelor's studies.

141 female and male students of the third year of extramural master's studies and of the second year of extramural bachelor's studies were placed under observation. Basic morphological characteristics were measured and physical fitness was determined according to the results of the Eurofit test trials and W. Starosta test of global motor coordination.

Based on the results of comparative analysis realized by means of the "U" test, lack of mean differences in almost all tested characteristics was established. It was observed only in the mean difference of endurance test in men (D = 4.48 and U = 6.78), which was more in favour of the extramural students of bachelor's studies.

The conclusions that were formed, related to a confirmation of the fact that the studies were undertaken by morphologically and motorically shaped subjects, and that the educational system did not influence development and physical fitness of extramural physiotherapy students. Moreover, the results showed there was a reason to search for probable differences in full-time students.

S t r e s z c z e n i e

Rozwój społeczno-gospodarczy ostatniej dekady spowodował liczne zmiany w jakości życia wielu społeczeństw państw rozszerzonej Unii Europejskiej. Jednym z tego przejawów w Polsce są zmiany w systemie edukacyjnym charakteryzujące się znacznym zwiększeniem liczby młodych osób studiujących niestacjonarnie na różnych poziomach edukacyjnych oraz dwustopniowa realizacja studiów.

Celem pracy uczyniono próbę stwierdzenia różnic w sprawności fizycznej osób studiujących fizjoterapię w trybie niestacjonarnym na poziomie studiów magisterskich i licencjackich.

Obserwacją objęto 141 studentów i studentek 3. roku niestacjonarnych studiów magisterskich i 2. roku niestacjonarnych studiów licencjackich. Zmierzono podstawowe charakterystyki morfologiczne, a sprawność fizyczną określono wynikami prób testu Eurofit i testem koordynacji obrotowej W. Starosty.

Na podstawie wyników analizy porównawczej zrealizowanej testem „U" stwierdzono brak różnic średnich niemal wszystkich badanych charakterystyk. Zaobserwowano je jedynie w różnicy przeciętnych testu wytrzymałościowego mężczyzn (D=1,42 i U=2,84) oraz próby równowagi ogólnej kobiet (D=4,48 i U= 6,78), których

bardziej korzystne wyniki cechowały studiujących w trybie studiów licencjackich.

Sformułowano wnioski dotyczące potwierdzenia faktu podejmowania studiów przez młodzież morfologicznie i motorycznie ukształtowaną oraz o braku wpływu poziomu edukacyjnego na rozwój i sprawność osób studiujących fizjoterapię trybem niestacjonarnym – także zasadności poszukiwania ewentualnych różnic wśród młodzieży realizującej naukę trybem stacjonarnym.

Key words: educational level, physical endurance, physiotherapy, students
Słowa kluczowe: poziom edukacyjny, sprawność fizyczna, fizjoterapia, studenci

INTRODUCTION

Socio-economic development in the last decade led to numerous changes in the quality of life of many societies of the widened European Union. One of its indications in Poland are the changes in the education system, which are manifested in significant increase of both young people choosing extramural studies on different education levels and two-cycle realization of their higher education studies.

From our own previous studies it was confirmed that most young people starting their studies in medical school are morphologically and motor shaped, while the main environmental factors do not cause significant differences in basic morphological and motor characteristics [1]. Traces of environmental differences were noted in differentiation when taking into account realization of payable studies [2, 3, 4]. Hence, it is likely that introducing the changes in the educational system, which are connected with its two-cycle realization, will influence the picture of physical fitness of the young people, who study to become physiotherapists and in this profession physical exercise appears at different levels of intensity and is a typical phenomenon [5, 6, 7] Moreover, high level of physical fitness is generally expected from such employees [8, 9, 10].

The aim of this paper was an attempt to establish differences in physical fitness of extramural master's and bachelor's students. It was assumed that due to their established and, at the same time, perhaps higher position in the educational hierarchy, subjects attending five-year master's studies will be characterized by more advantageous determinants of development and physical fitness.

MATERIAL AND METHOD

Subjects taking part in the study were male and female physiotherapy students of the third year of extramural master's studies and the second year of bachelor's studies of Faculty of Health Studies in Ludwik Rydygier Collegium Medicum in Bydgoszcz. By the time the study was conducted, students of both groups had realized the same number of motor education classes, which allowed for excluding its influence on the expected differences of researched characteristics. However, subjects who had undertaken five-year master's studies (male students 71%, female students 72%) started to work in the profession more often than subjects who had undertaken bachelor's studies (male students 45%, female students 21%).

Height and body mass were measured as basic morphological features, and Rohrer's index was calculated from their mean [11]. Physical fitness was determined based on the results of the Eurifit test fitness trials in limber, static force, trunk force, functional force and circular – respiratory endurance, which are thought to be conducive to health and general balance, arm movement speed, explosive force, running speed – components aiding in reaching motor goals [12]. Moreover, global motor coordination level was established – it was composed of the sum of left-sided and right-sided rotation [13], and the power measured by the result of vertical jump with left and right arm [14], both of which are included in the components of physical fitness which aid in reaching motor goals.

The results were obtained from 28 men and 44 women realizing their five-year master's studies and 22 male and 47 female students realizing bachelor's studies. The results were analyzed separately for male and female students in both differentiation groups, by means of basic statistical methods using ‘U’ test for determining significant differences in mean outcomes [15].

RESULTS

Table I shows basic morphological characteristics of the tested student groups.

The numeric data presented in the table shows a lack of significant differences in basic morphological characteristics of the tested student groups and their different directions in female and male groups – also an age difference larger than expected from the level of realization of studies.

Table I. *Comparative characteristics of morphological features of the tested student groups*

Tab. I. *Charakterystyka porównawcza cech morfologicznych badanych zespołów studenckich*

character	Category	M	σ	D	u
male students (bachelor's students n = 32 / master's students n = 48)					
Age (years)	bachelor's	23.18	2.1	2.07	2.72*
	master's	25.25	3.30		
Body height (cm)	bachelor's	179.72	6.26	1.19	0.67
	master's	178.53	6.14		
Body mass (kg)	bachelor's	78.90	11.35	1.38	0.43
	master's	80.28	11.06		
Rohrer index (n)	bachelor's	1.35	0.23	0.06	1.2
	master's	1.41	0.20		
Female students (bachelor's students n = 57 / master's students n = 76)					
Age (years)	bachelor's	22,63	2,43	4.82	5.73**
	master's	27.45	5.13		
Body height (cm)	bachelor's	167.06	6.10	0.48	0.39
	master's	167.54	5.71		
Body mass (kg)	bachelor's	60.51	9.04	1.31	0.74
	master's	59.20	7.74		
Rohrer index (n)	bachelor's	1.29	0.17	0.04	0.79
	master's	1.25	0.13		

* statistically significant difference p≤0.01 α = 2.68
** statistically significant difference p≤0.05 α = 2.01

Table II presents the physical fitness components characteristics which aid in reaching motor goals in the tested men groups.

Tab. II. *Comparative characteristics the of physical fitness components conducive to reaching motor goals in tested male groups*

Tab. II. *Charakterystyka porównawcza komponentów sprawności fizycznej sprzyjających osiągnięciom motorycznym badanych grup mężczyzn*

Component	Category	M	σ	D	u
General balance	bachelor's	8.45	3.80	0.51	0.47
	master's	8.96	3.78		
Speed of arm movement	bachelor's	9.26	1.10	0.34	1.06
	master's	8.92	1.22		
Explosive force	bachelor's	217.27	21.30	11.69	1.61
	master's	228.96	29.74		
Reaching jump (right arm)	bachelor's	50.21	9.53	0.59	0.22
	master's	49.62	8.36		
Reaching jump (left arm)	bachelor's	49.47	10.00	1.14	0.43
	master's	48.33	8.29		
Running speed	bachelor's	19.02	2.15	0.36	0.66
	master's	19.38	1.59		

** statistically significant difference p≤0.01 α = 1.99
* statistically significant difference p≤0.01 α = 2,65

As shown in the table, presented mean differences are insignificant, with slightly better fitness results of the two-cycle studies group.

Table III shows physical fitness components characteristics which aid in reaching motor goals in the tested women.

Tab. III. *Comparative characteristics the of physical fitness components conducive to reaching motor goals in tested female groups*

Tab. III. *Charakterystyka porównawcza komponentów sprawności fizycznej sprzyjających osiągnięciom motorycznym badanych grup kobiet*

Component	Category	M	σ	D	u
General balance	bachelor's	6.72	2.87	4.48	6.78*
	master's	11.20	3.46		
Speed of arm movement	bachelor's	10.12	1.04	0.18	0.81
	master's	10.30	1.15		
Explosive force	bachelor's	172.17	14.76	1.23	0.33
	master's	173.40	19.69		
Reaching jump (right arm)	bachelor's	34.07	6.81	0.44	1.03
	master's	34.51	6.81		
Reaching jump (left arm)	bachelor's	32.87	6.48	1.38	1.03
	master's	34.25	6.27		
Running speed	bachelor's	21.00	1.51	0.42	1.35
	master's	21.42	1.62		

* statistically significant difference p≤0.01 α = 2.65
** statistically significant difference p≤0.05 α = 1.99

It can be concluded that the group of women realizing two-cycle studies is characterized by significantly lower mean in general balance trial, marking a better result. As for the remaining women, similarly to the male students group, differences in their trial mean results are insignificant and multidirectional.

Table IV presents characteristics of physical fitness components supporting health of the tested men groups.

Tab. IV. *Comparative characteristics of the physical components conducive to tested male groups*

Tab. IV. *Charakterystyka porównawcza komponentów sprawności fizycznej sprzyjających zdrowiu badanych grup mężczyzn*

Component	Category	M	σ	D	u
Limber	bachelor's	22.36	5.97	3.85	1.75
	master's	26.21	9.46		
Static force-right arm	bachelor's	61.72	9.25	2.95	1.12
	master's	64.67	9.18		
Static force-left arm	bachelor's	59.45	10.17	0.87	0.31
	master's	60.32	8.89		
Trunk force	bachelor's	28.45	4.35	1.42	1.17
	master's	27.03	4.19		
Functional force	bachelor's	30.02	11.29	5.06	1.32
	master's	24.96	15.65		
Circulary-respiratory endurance	bachelor's	7.88	1.77	1.42	2.84**
	master's	6.46	1.8		

* statistically significant difference p≤0.01 α = 2.68
** statistically significant difference p≤0.05 α = 2.01

This compilation shows significantly greater mean of circular – respiratory endurance trial in students realizing two-cycle system. The differences in the remaining characteristics are insignificant and multidirectional.

Table V presents physical fitness components characteristics supporting health of the tested women.

Tab. V. *Comparative characteristics of physical fitness components conducive to health of tested women groups*

Tab. V *Charakterystyka porównawcza komponentów sprawności fizycznej sprzyjających zdrowiu*

Component	Category	M	σ	D	u
Limber	bachelor's	28.39	8.73	0.73	0.44
	master's	29.12	6.98		
Static force-right arm	bachelor's	36.04	8.45	1.71	1.11
	master's	37.75	6.08		
Static force-left arm	bachelor's	34.44	8.24	2.03	1.38
	master's	36.47	5.71		
Trunk force	bachelor's	24.04	3.35	0.75	0.87
	master's	24.79	4.76		
Functional force	bachelor's	14.35	16.9	1.58	0.44
	master's	15.93	17.15		
Circulary-respiratory endurance	bachelor's	4.94	1.54	0.48	1.71
	master's	4.46	1.17		

The numerical characteristics included in the table do not significantly differentiate tested women groups. Women realizing five-year master's studies are characterized by insignificantly better results.

DISCUSSION

The research conducted on extramural physiotherapy students realizing either a long-cycle or two-cycle educational system did not allow for verification of positive hypothesis concerning the influence of the educational level on physical fitness. Significant differences in means of single characteristics and a tendency of results of assumed differentiation, distinct for male and female students, were stated. Indirectly, it was observed that lack of employment in the profession did not influence physical fitness level of the subjects. At the same time, the tendency that had been observed in the usefulness of individual features in successful realization of physiotherapy profession studies, did not appear here, which could indicate that physical fitness, despite general expectations [8, 9, 10, 16], is not a directional feature in this profession. However, it is believed that such result is supported by the observed tendency of equalization of university students' biological value [1] or is a further consequence of previous motor identification for the studies.

The aspect negatively verifying the posed hypothesis is a clear difference in mean results of endurance trial in men and general balance trial in women. Subjects in their bachelor's studies were characterized by better results, what, on the one hand, may point to lower endurance level in the long-cycle system students, most of whom were employed as physiotherapist, or to lesser importance of this feature in male physiotherapists. However, such thinking would stand in contradiction with previously stated social view of this problem [8, 9, 10, 16], relating to a great meaning of motor coordination in successful realization of this profession. The observed differences in endurance trial in men groups could be explained by both: lower mean age of subjects following two-cycle education and the fact that subjects following long-cycle education more often start working, what is not conducive to keeping physical fitness on appropriately high level [17]. These conditions may also influence significant differences in mean results of motor coordination in women and a slightly higher mean Rohrer index of the subjects studying in the long-cycle system, which, according to previous studies, does not aid in the development of this motor feature [18, 19, 20].

Different tendencies in the groups of men and women establish an interesting aspect of the studies and, at the same time, a cause for further research in this direction. It seems that they may be in correlation with different predispositions of both sexes to become a physiotherapist, which are connected with distinct perception of professional nuisances in men and women who decide on this profession [16] and that might be observed at stage of studies.

CONCLUSIONS

1. Established lack of differences in most motor characteristics in groups of extramural physiotherapy students on different educational levels could be a result of previous fitness identification for the studies or it might imply an insignificant influence of the change of educational system on the physical fitness of the physiotherapy students.
2. Confirmed multidirectional differences of single components of physical fitness in tested groups of male and female students; moreover, distinct

tendencies in differences of the remaining characteristics that were under observation might attest to unequal biological effects of the changes of the studying system in the subjects of both sexes.

3. Obtained study results show insignificant consequences of changes in the educational system to the biological value of the subjects studying physiotherapy, which are observed in a slightly greater extent in the motor than morphological area. However, it is possible that in studies on more extensive trial and with additional use of other environmental differentiations, they may form a clearer picture.

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BONE TURNOVER MARKERS IN HEALTHY CHILDREN AND ADOLESCENTS FROM BYDGOSZCZ AND SURROUNDING AREA

WSKAŹNIKI PRZEBUDOWY TKANKI KOSTNEJ U ZDROWYCH DZIECI I MŁODZIEŻY Z BYDGOSZCZY I OKOLIC

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Summary

Biochemical bone turnover markers are specific bone-derived molecules that reflect the bone metabolic activity. Osteocalcin (OC) is classified as a marker of bone formation, and collagen type I cross–linked C–terminal telopeptide (CTX) as a marker of bone resorption. We examined fifty-two healthy children and adolescents 2-18 years of age from Bydgoszcz and surrounding area. OC and CTX were determined in serum by ELISA. The peak mean values of bone turnover markers were found in pubertal girls (OC 114.5±42.5 ng/ml; CTX 2,193±0,305 ng/ml) and boys (OC 106.9±36.5 ng/ml; CTX 2.401±0.279 ng/ml). The lowest mean concentrations were observed in both genders in postpubertal period. Significant correlations were found between OC and CTX in all studied children ($r=0.69$ $p<0.0001$) and in girls and boys ($r=0.77$ $p<0.0001$; $r=0.63$ $p<0.001$, respectively). The results of our study may be useful for determination of the reference values for OC and CTX in healthy Polish children and adolescents, which may then facilitate the diagnosis and monitoring of therapy or disease progression in children with bone diseases.

Streszczenie

Biochemiczne wskaźniki przebudowy tkanki kostnej odzwierciedlają dynamiczny aspekt metabolizmu kości. Celem pracy było oznaczenie w surowicy stężenia markerów tworzenia: osteokalcyny (OC) i resorpcji kości: C-końcowego usieciowanego telopeptydu kolagenu typu 1 (CTX) u zdrowych dzieci z Bydgoszczy i okolic. Do badań włączono pięćdziesiąt dwoje dzieci i młodzieży w wieku 2-18 lat. Stężenie osteokalcyny i C-końcowego usieciowanego telopeptydu łańcucha α kolagenu typu I oznaczano metodą immunoenzymatyczną ELISA. Najwyższe wartości oznaczanych wskaźników stwierdzono w okresie pokwitania u dziewcząt (OC 114,5±42,5 ng/ml; CTX 2,193±0,305 ng/ml) i chłopców (OC 106,9±36,5 ng/ml; CTX 2,401±0,279 ng/ml). Najniższe wartości odnotowano zarówno u dziewcząt, jak i u chłopców w okresie popokwitaniowym. Wykazano istotną korelację między OC i CTX w całej grupie badanych dzieci ($r=0,69$ $p<0,0001$) oraz u dziewczynek i chłopców ($r=0,77$ $p<0,0001$; $r=0,63$ $p<0,001$ odpowiednio).Wyniki uzyskane w pracy mogą być pomocne w ustalaniu wartości referencyjnych wskaźników przebudowy tkanki kostnej u dzieci i młodzieży zdrowej oraz mogą być później przydatne w diagnostyce i monitorowaniu leczenia dzieci chorych.

Key words: osteocalcin (OC), collagen type I cross–linked C–terminal telopeptide (CTX), healthy children, adolescents
Słowa kluczowe: osteokalcyna (OC), C-końcowy usieciowany telopeptyd kolagenu typu 1 (CTX), zdrowe dzieci, młodzież

INTRODUCTION

Bone is a dynamic tissue that consistently undergoes remodeling. During formation and resorption processes, bone turnover markers are released. These markers are specific bone-derived molecules that circulate in the blood or are present in the urine reflecting the bone metabolic activity [1].

Bone turnover markers are usually classified as markers of bone formation and bone resorption. Bone formation markers are the products of osteoblasts in different stages of differentiation. Osteocalcin (OC), bone alkaline phosphatase (b-ALP) and type I procollagen propeptide are included in the bone formation markers. Except for tartrate resistant acid phosphatase (TRAP), bone resorption markers are the products of bone collagen degradation. The most common markers of bone resorption are peptide fragments deriving from collagen type I such as type I collagen cross–linked C–terminal and N-terminal telopeptides (CTX and NTX) and pyridinolines [2-5].

Osteocalcin (OC), a major noncollagenous protein of bone matrix, is synthesized and secreted by mature osteoblasts, odontoblasts and hypertrophic chondrocytes during the matrix mineralization phase under the control of 1.25-vitamin D_3. OC contains 49 amino acids, including three γ-carboxyglutamate residues which provide the point of interaction between OC and hydroxyapatite in the extracellular bone matrix [1, 3, 5-11]. Vitamin K acts as an indispensable cofactor for the formation of γ-carboxyglutamate residues and inadequate dietary vitamin K intake results in the synthesis of undercarboxylated (i.e. inactive) osteocalcin (ucOC). It has been established that in adults, low vitamin K status of bone is associated with low bone density and an increased risk of osteoporotic fractures. Findings suggest a pronounced low vitamin K status of bone during growth in healthy children [12]. Moreover, recent data demonstrates that in healthy, prepubertal children, modest supplementation with MK-7 (45 microg menaquinone-7; one of the vitamin K2 species) increases circulating concentrations of MK-7 and increases osteocalcin carboxylation [13].

C-terminal telopeptide of type I collagen (CTX) is an octapeptide derived from C-telopeptide of type I collagen. CTX is released by mature osteoclasts that may resorb bone. There are two forms of CTX: α and β. α epitopes are released during degradation of newly synthesized type I collagen, whereas β are released from mature collagen type I. It has been shown that α/β ratio can be a useful measure of the age of bone tissue. α/β ratio is three times higher in young than in old bones [2, 3, 5, 10, 14, 15]. Previous studies showed that serum CTX levels reflect the pediatric growth curve that is similar to patterns observed for other biochemical bone markers [2, 6, 7, 16-18].

The aim of our study was to investigate serum concentration of osteocalcin and CTX in healthy children and adolescents from Bydgoszcz and surrounding area.

SUBJECTS, MATERIAL AND METHODS

We studied fifty two healthy children and adolescents aged 2-18 years (23 girls and 29 boys) divided into three groups: prepubertal (girls 2-8 yrs, boys 2-9 yrs), pubertal (girls 8,1-14 yrs, boys 9,1-15,5 yrs), postpubertal (girls 14,1-18 yrs, boys 15,6-18 yrs). The inclusion criteria were as follows: lack of evidence of systemic illnesses, endocrine or renal disorders, chronic diseases, obesity and not taking drugs known to affect bone metabolism.

The study was approved by Ethical Committee of Collegium Medicum, Nicolaus Copernicus University. All parents and subjects were informed about the purpose of the study. Participation was voluntary. Written informed consent was obtained from all parents of the study participants and from the subjects who were over 16 years of age.

Fasting blood samples were collected between 7.00 and 9.00 am. After centrifugation serum samples were kept frozen at -70^0C until assayed for bone marker concentrations.

The concentration of osteocalcin was determined in serum by N-MID Osteocalcin One Step ELISA kit (Nordic Bioscience Diagnostics A/S (Denmark). The assay detects total osteocalcin (intact and N-MID fragment) released by osteoblasts to the circulation; detection limit 0.5 μg/L; interassay CV 3.6-6.4% (for concentrations 6.8-50.5 μg/L); intraassay CV 2.0-3.4% (for concentrations from 7.0-43.2 μg/L).

CTX concentration was measured using Serum CrossLaps One Step ELISA assay (Nordic Bioscience Diagnostics A/S (Denmark). This method is based on highly specific monoclonal antibody against a β-aspartate isomerized form of the sequence EKAHD-β-GGR derived from the C-terminal telopeptide region of

the type I collagen α-1 chain; detection limit 0.01 µg/L; interassay CV 5.4-8.1% (for concentrations 0.273-0.488 µg/L); intraassay CV 5.0-5.4% (for concentrations 0.242-0.476 µg/L).

The data we obtained was expressed as means (±SD) and compared by the Student's test. Normality of variables was tested by Shapiro-Wilk test. Pearson correlation test was used. P values ≤0.05 were considered statistically significant.

RESULTS

Prepubertal group consisted of 4 girls and 7 boys. The mean age in this group was 5.7±2.3 yrs. 10 girls and 18 boys were included in the pubertal group and their mean age was 11.8±2.0 yrs. The oldest group was formed by 9 girls and 4 boys. The mean age in postpubertal group was 16.0±0.7 yrs.

We observed the sigmoid regression curves of age-related change in serum OC and CTX values. Figures 1, 2, 3 and 4 show log OC and CTX concentrations in healthy children and adolescents in relation to age and gender. Significant variation with age was observed for both bone markers. Compared with prepubertal and pubertal groups together, serum levels of OC and CTX were lower in postpubertal children (girls and boys together) (OC 39.8±14.9 vs. 99.2±37.3 ng/ml, $p<0.002$ and CTX 1.346±0.626 vs. 2.217±0.341 ng/ml, $p<0.005$). Sex was significant only for CTX levels with higher concentrations observed in pubertal and especially postpubertal boys in comparison to girls at the same pubertal stage.

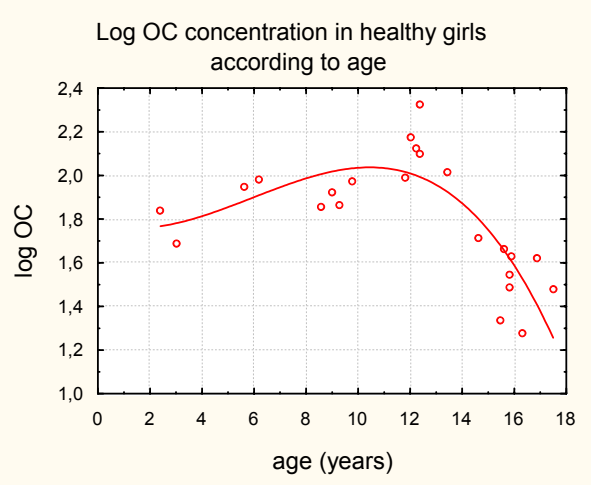

Fig. 1. *Log OC concentration in healthy girls according to age*

Ryc. 1. *Log stężenia OC u zdrowych dziewczynek w zależności od wieku*

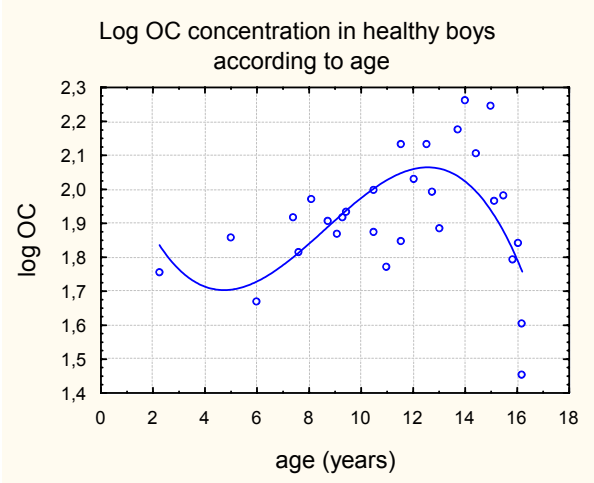

Fig. 2. *Log OC concentration in healthy boys according to age*

Ryc. 2. *Log stężenia OC u zdrowych chłopców w zależności od wieku*

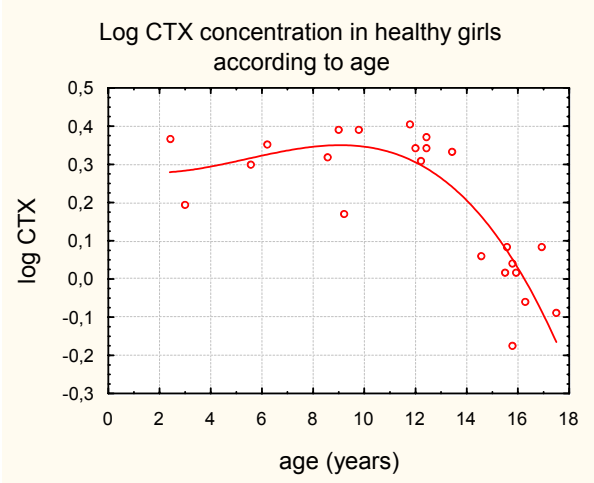

Fig. 3. *Log CTX concentration in healthy girls according to age*

Ryc. 3. *Log stężenia CTX u zdrowych dziewczynek w zależności od wieku*

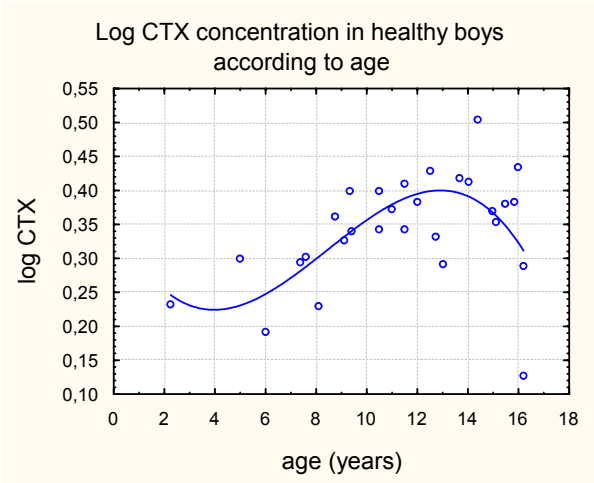

Fig. 4. *Log CTX concentration in healthy boys according to age*

Ryc. 4. *Log stężenia CTX u zdrowych chłopców w zależności od wieku*

CTX concentration was significantly higher in postpubertal boys than in girls (2.105±0.603 vs. 1.009±0.189 ng/ml, $p<0.03$) and a tendency for greater CTX values in boys from 9.1-15.5 yrs in comparison to pubertal girls (2.401±0.279 vs. 2.193±0.305 ng/ml $p<0.09$) was observed. However, in prepubertal children only slightly higher CTX concentration was observed in girls than in boys (2.031±0.338 ng/ml vs. 1.887±0.255 ng/ml).

Generally, in healthy girls OC levels were higher than in boys except for postpubertal group but these differences were not statistically significant. In prepubertal children a little higher OC concentration was observed in girls than in boys (75.5±21.1 vs. 71.2±16.1 ng/ml). The highest mean values were observed during puberty in both genders (114.5±42.5 ng/ml in girls and 106.9±36.5 ng/ml in boys). During puberty, mean OC values increased by about 150% in both genders, whereas in adolescents OC concentration gradually decreased. After pubertal period OC concentration decreased about 3-fold in girls and 2-fold in boys, as compared to the levels in the pubertal children (35.2±11.0 ng/ml and 49.9±18.9 ng/ml, respectively).

In general, girls showed decreased postpubertal values of OC and CTX about 2-3 years earlier than boys. The peak OC values in girls were observed at about 12 years of age and in boys from 12 to 14 years of age. The maximum concentration of CTX was observed from 12 to 14 years of age in girls and from 11 to 15 years of age in boys.

Significant correlations between OC and CTX were found in all studied children ($r=0.69$ $p<0.0001$) and in girls and boys between CTX and age ($r=-0.63$ $p<0.001$; $r=0.41$ $p<0.03$, respectively) and CTX and OC ($r=0.77$ $p<0.0001$; $r=0.63$ $p<0.001$).

DISSCUSION

At least two different markers reflecting bone formation and bone resorption are needed to evaluate the bone turnover. We determined two bone turnover markers from the same serum sample. Bone turnover in children and adolescents was evaluated by measuring osteocalcin (a bone formation marker) and CTX (a bone resorption marker).

Comparing our results to the results of the Ambroszkiewicz et al., we observed that osteocalcin concentration in children of both genders in puberty period is similar but in the pre- and postpubertal period is lower than the one obtained in children from the Institute of Mother and Child in Warsaw [2]. Osteocalcin concentration in the age group 2-9 years was 105.6 ng/ml, in the age of 9.1-13 years – 115.6 ng/ml and 50.3 ng/ml in the age group 13.1-18 years in girls, and respectively 96.6 ng/ml, 117.8 ng/ml and 92.3 ng/ml in the age group 2-10 years, 10.1-15 years and 15.1-18 years in boys [6]. It is worth to add that in our study the number of the youngest (11) and the oldest (13) children was twice lower than the number of pubertal (28) children what might have influenced mean osteocalcin concentration. Our results, similarly to other studies [6, 7, 18], confirm that there is a significant decrease of osteocalcin concentration in postpubertal children. We observed a peak of osteocalcin concentration appearing about 2-3 years earlier in girls than in boys, after that OC levels decrease, what is connected with the end of pubertal period. Similarly, in boys older than 15 years old the lowest osteocalcin values were found. This statement is consistent with the observations of other authors [6, 7, 18]. In Thai children the highest OC values in the serum were found at about 12 years of age in girls and a year later in boys [19].

According to Rauchenzauner et al. [18] osteocalcin level depends on pubertal degree expressed in Tanner stages and it is the lowest in Tanner stages 4 and 5. A similar stand is shared by other researches [19,20] who observed the significant differences in OC and b-ALP levels in serum of Lebanese and Thai children depending on the pubertal degree. On the contrary, Yilmaz et al. [21] found no significant differences in OC concentration depending on the pubertal stage in healthy pubertal Turkish children. Yilmaz et al. [21] indicated a significant influence of estrogens on bone mineral density in girls as well as in boys. In addition, they claimed that bone formation markers such as OC and b-ALP are good predictor factors of bone weight only in girls. The level of estrogens influences peak bone mass also in boys [21, 22]. On the contrary, the results of van Coeverden et al. [23] show that bone turnover markers OC and b-ALP are good predictors of bone mass in boys and of bone mass increase in both sexes. Our results indicate that the differences in OC levels between girls and boys are not noticeable until the postpubertal period. In postpubertal group a slightly higher OC concentration was observed in boys than in girls but these differences were not statistically significant.

Studies carried out among large groups of children and adolescents up to 19 years of age in Ireland and Austria and in the Institute of Mother and Child in Warsaw showed that concentration of bone resorption marker – CTX in children is even several times higher than in adults [2, 16-18]. Crofton et al [16] observed no significant differences in CTX values between girls and boys from 1 to 9 years of age. Our results confirm similar tendency in prepubertal healthy children. The highest CTX concentration in the serum was observed in pubertal period. Other authors also show increased CTX values in children at this age [2, 16-18]. The highest CTX levels in girls were found, like OC, 2-3 years earlier than in boys. Gajewska et al. [2] and other authors [16, 18] obtained similar results. Contrary to Crofton et al. [16] we observed a tendency for lower CTX values in girls comparing to boys in healthy pubertal children. There is a significant decrease of CTX concentration in postpubertal girls comparing to boys. Our results are consistent with the observations of other authors [2, 16-18]. Rauchenzauner et al [18] observed significantly lower CTX levels in the serum of adolescents over 17 years old in comparison to values obtained for children from 2 to 15 years of age.

One limitation of our study is a relatively small number of children, especially in prepubertal and postpubertal ages. However, we observed a similar pattern of age-related change in serum OC and CTX levels to the shape of curves reported in larger healthy pediatric Polish and foreign groups [7,16,18]. Finally, were compared mean CTX and OC concentrations in our study with other results obtained for Polish children and adolescents [2, 6, 17].

CONCLUSIONS

The results of our study may be useful in determination of the reference ranges for OC and CTX in healthy Polish children and adolescents, which may facilitate monitoring and the diagnosis of therapy or disease progression in children with bone diseases. Using both, formation and resorption markers may be better than a single marker in the longitudinal assessment of bone metabolism because sensitivities and predictive values of single markers are usually poor.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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METABOLISM OF EXTRACELLULAR-ADENOSINE AND EXTRACELLULAR-ADENINE IN GLIOMA C6 CELLS

METABOLIZM EKTO-ADENOZYNY I EKTO-ADENINY W HODOWLACH GLEJAKA C6

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S u m m a r y

Neurons and glioma cells are considered the main sources of ex-purines in the central nervous system of mammals. Among them, extracellular-adenosine (ex-Ado) acts as a neuromodulator participating in the regulation of the secretion and activity of neurotransmitters and trophic factors as well as in apoptosis, necrosis and cell proliferation. In this report, we identified two mechanisms responsible for ex-Ado metabolism in rat C6 glioma cells (GC6), adenosine transporters and ecto-enzyme breaking N-glycosidic bond in Ado extracellularly, which may cooperate in Ado removal. At physiological concentrations, the transported ex-Ado and its product extracellular adenine were used in the intracellular resynthesis of adenine nucleotides. The ecto-enzyme from GC6 revealed the high affinity for Ado (K_M = 1.08±0.12 μM, V_{max}= 0.38±0.03 pmol adenine/s x 10^6 cells). The activity of the enzyme with Ado as a substrate was inhibited by guanosine, inosine and 2'-deoxyadenosine but not by pyrimidines. The V_{max}/K_M ratios calculated for the ecto-enzyme and Ado transporters, being an indicator of the efficiency of transport or catalysis, were similar. To our knowledge, this is the first report on the presence of ecto-enzyme converting ex-Ado to ex-adenine in glial cells which can play an important role in Ado metabolism with intracellular adenine phosphoribosyltransferase responsible for AMP resynthesis.

S t r e s z c z e n i e

Neurony i komórki gleju są podstawowym źródłem puryn obecnych w przestrzeniach międzykomórkowych mózgu. Wśród nich zewnątrz-komórkowa adenozyna (ex-Ado) jest neuromodulatorem, który uczestniczącym w regulacji wydzielania i aktywności cząsteczek sygnałowych (neurotransmiterów) oraz czynników troficznych, jak również w procesach nekrozy i proliferacji komórek. W tej pracy opisujemy dwa mechanizmy odpowiedzialne za obniżenie stężenia adenozyny obecnej poza komórką: 1) aktywność ekto-enzymów uczestniczących w hydrolizie wiązań N-glikozydowych adenozyny i 2) transport tych związków do komórki oraz przemiany przetransportowanych puryn w komórkach glejaka. Wykazaliśmy, że adenozyna zlokalizowana poza komórką (ex-Ado) a także produkty jej degradacji są transportowane do komórek glejaka i wykorzystywane do resyntezy nukleotydów adeninowych. Ektoenzym glejaka C6 charakteryzował się wysokim powinowactwem względem Ado jako substratu (K_M =1,08±0,12 μM; V_{max}= 0,38±0,03 pmol adenine x s^{-1}). Współczynniki V_{max}/K_M określające wydajność transportu puryn i katalizy są podobne. Guanozyna, inozyna i 2'-deoksyadenozyna hamowała hydrolizę ex-Ado, podczas gdy pirymidyny nie wpływały na aktywności tego enzymu. Wyniki tej pracy po raz pierwszy wykazały obecność w błonach komórek glejaka C6 ekto-enzymu metabolizującego ex-adenozynę do ex-adeniny. Powyższe przemiany mogą w komórce glejaka pełnić istotną funkcję w resyntezie AMP z udziałem wewnątrzkomórkowej foforybozylotransferazy.

Key words: ex-purine, ecto-enzyme, nervous system, glial cells, purine transporters, N-glycosidic bond
Słowa kluczowe: zewnątrzkomórkowe puryny, ekto-enzymy, komórki glejaka C6, transportey puryn, wiązanie N-glikozudowe

ABBREVIATIONS

NBTI – nitrobenzylthioinosine
Ado – adenosine
Ino – inosine
TBA – tetrabutyl ammonium
NMG – N-methylglucamine

INTRODUCTION

In the central nervous system (CNS) ex-purines (ATP, ADP and adenosine) act as neurotransmitters, modulate the secretion/release and activity of other neurotransmitters and trophic factors, and participate in the activation of apoptosis, necrosis and cell proliferation [1-3]. The glial cells are among the main sources of ex-purines in the CNS [1,4]. In the mammalian brain, ex-Ado plays a role of neuromodulator. Its concentration outside the cell might be regulated by adenosine ecto-deaminase, which degrades ex-adenosine (ex-Ado) into inosine and ammonium ions [5] or by protein nucleoside transporters present in cells of many types that transport Ado across the cell membranes consistent with the concentration gradient [6]. Although the presence of adenosine ecto-deaminase was detected also on the astrocyte surface, the ex-Ado metabolism in the glial cells is much less known than in neurons and synaptosomes [7]. This research focuses on the metabolism of (ex-Ado) in the C6 glioma cell cultures. C6 glioma cells posses adenosine receptors[8-11,9,12] , ecto-enzymes hydrolyzing ATP and ADP into AMP (ENTPDase1, ENTPDase2) and ecto-5′-nucleotidase [13-14] as well as the nucleoside transporters [15-16]. Research on ex-guanosine metabolism showed that ex-nucleoside degradation by glial cells might be performed by specific N-glycosidases breaking the N-glycosidic bonds [7, 17].

Aims of the reported study were following: 1) identification and properties of the C6 glioma ecto-enzymes participating in the ex-Ado metabolism, 2) determination of the role of protein transporters in the regulation of the level of ex-Ado and its degradation products, and 3) identification of the intracellular compounds formed from ex-adenosine.

MATERIALS AND METHODS

MATERIALS

Rat C6 glioma cells were cultured in DMEM medium supplemented with 10% calf serum, penicillin (50IU/ml) and streptomycin (50μg/l) and BMCyclin2 (5 μg/ml) in 37°C in the atmosphere of 5% CO_2. Analytical grade reagents purchased from Sigma, ICN, GIBCO BRL, Fluka, Merck and POCH Gliwice were used. Purine isotopes [2,8-^{3}H]-Ado (35.9 Ci/mmol = 1.3 TBq/mol), [2.8-^{3}H]-adenine (24.2 Ci/mmol = 895.4 GBq/mol) and [2.8-^{3}H]-ATP (31.4 Ci/mmol = 1.2 TBq/mol) were purchased from Sigma.

METHODS

Assays were carried out at a cell density close to 4.5 x 10^5 cells/cm^2. Before the experiments, cells were washed three times with an appropriate buffer (Buffer A, B or C, see ‘Measurement of ecto-enzyme activity” section).

CELL INTEGRITY

Cell integrity was measured [18] using lactate dehydrogenase (LDH) as a marker. For the research on the purine metabolism and transport we used only those cultures of glioma C6 cells in which the activity of LDH, a marker of cells integrity, did not exceed 5% of the total activity of that enzyme after the experiments.

MEASUREMENT OF ECTO-ENZYME ACTIVITY

To determine the extracellular purine metabolism, the cells were grown on 3.5 cm culture dishes, washed and incubated with 1ml of appropriate incubation mixture consisting of Buffer A (25 mM Tris-HCl pH 7.4, 100 mM NaCl, 20 mM KCl, 10 mM $NaHCO_3$, 5 mM glucose, 4 mM $MgCl_2$, 2 mM $CaCl_2$), Buffer B (35 mM Tris-HCl pH 7.4, 250 mM sucrose, 10 mM glucose, 4 mM $MgCl_2$, 2 mM $CaCl_2$) or Buffer C (35 mM KH_2PO_4/K_2HPO_4 pH 7.4, 250 mM sucrose, 10 mM glucose, 4 mM $MgCl_2$, 2 mM $CaCl_2$). Each incubation mixture also contained appropriate substrates and the nucleotide uptake inhibitors (NBTI, dipyridamole, or papaverine). For liquid scintillation spectroscopy of 0.02-5 μCi/sample the following ^{3}H-isotope were applied: [2.8-^{3}H]-adenine (895.4

GBq/mol), [2.8-^{3}H]-Ado (1.3 TBq/mol) and [2.8 -^{3}H] ATP (1.2 TBq/mol). Qualitative and quantitative purine analysis was performed using the HPLC method. The samples were separated on SUPELCO Discovery column C18, 15cm x 4mm, 5µm using 50 mM KH_2PO_4/K_2HPO_4 buffer pH 5.0 containing 2.5 mM EDTA, 6.25 mM TBA and 1% MetOH as a solvent for hypoxanthine, xanthine, uric acid, adenine, inosine, IMP, adenosine, and AMP or 10% MetOH for ADP and ATP separation.

DETERMINATION OF EX-PURINE`S TRANSPORT INTO GLIOMA C6 CELLS

To determine the adenosine or adenine uptake we used ^{3}H-isotopes (0.02-5 µCi/sample). Cells were incubated with Buffer A with or without the appropriate transport inhibitors. To examine the influence of Na^+ on the purine transport, we used the Buffer A in which NaCl was replaced with N-methylglucamine (NMG). To terminate the uptake, the extracellular solution was removed and C6 cells were washed five times with the ice-cold Buffer A containing Na^+ or NMG^+ and the purine transport inhibitors. Then, the cells were incubated overnight with 1 M NaOH at 37°C and the aliquots of the dissolved cells were analyzed by liquid scintillation spectroscopy.

Quantitative and qualitative analysis of the intracellular purines in glioma C6 cells

To examine the intracellular pool of purines, the cells, after being washed five times with Buffer A, were incubated with ^{3}H-purines in an appropriate buffer. Subsequently, they were washed again, floated with 1 ml of hot (100°C) water, cooled immediately, homogenized, and treated with 5 M $HClO_4$ to remove proteins and be neutralized with KOH. Finally, samples were analyzed using HPLC and liquid scintillation spectroscopy.

STATISTICAL ANALYSIS

The data is expressed as mean ±SD. All experiments were done at least in triplicate.

RESULTS

Metabolism of ex-adenine compounds in the C6 glioma cell cultures

After 5-min incubation in a presence of the C6 glioma cells (GC6) and nucleoside or nucleobase transport inhibitors (10 µM dipyridamole, 1 µM NBTI and 250 µM papaverine), almost 65% of 10 µM ex-ATP was metabolized to adenine (18.7%), Ado (33.8%), AMP (3.3%), ADP (8.9%) and ATP (35,2%). The only product of Ado degradation by enzymes located on the C6 glioma cell surface was adenine (Fig. 1).

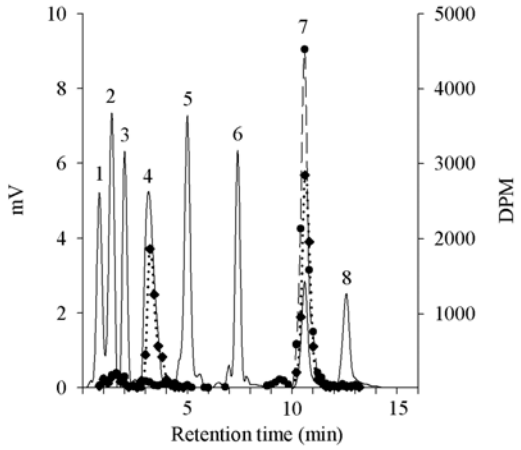

Fig. 1. *The extracellular metabolism of ex-Ado in the presence of intact C6 glioma cells measured by HPLC and liquid scintillation spectroscopy (1 - xanthine, 2 - hypoxanthine, 3 - uric acid, 4 - adenine, 5 - inosine, 6 - IMP, 7 - adenosine, 8 - AMP, (—) standards [25µM], (--- • ---) time 0, (··· ♦···) 5 min incubation). The ecto-N-glycosidase activity was determined in the presence of Buffer A containing 1 µM NBTI, 10 µM dipyridamole, and 250 µM papaverine*

The intact GC6 cells did not deaminate Ado to inosine (Ino) and in the incubation mixture we did not detect the presence of hypoxanthine as a product of Ado degradation. The intact glioma C6 cells did not reveal the adenosine ecto-kinase activity. Results presented in Figure 2 indicate that after 5-min incubation of glioma cells with Ado at physiological, nanomolar concentrations of adenosine, 70-80% of Ado present in the incubation mixtures was degraded. During the same period of time only 30% of 1 µM Ado, (a quantity that corresponds to the upper limit of the physiological concentrations:[19]) was degraded. At higher micromolar concentrations (30 and 100 µM), during the same time, the percentage of ex-Ado degradation was substantially smaller (Fig. 2).

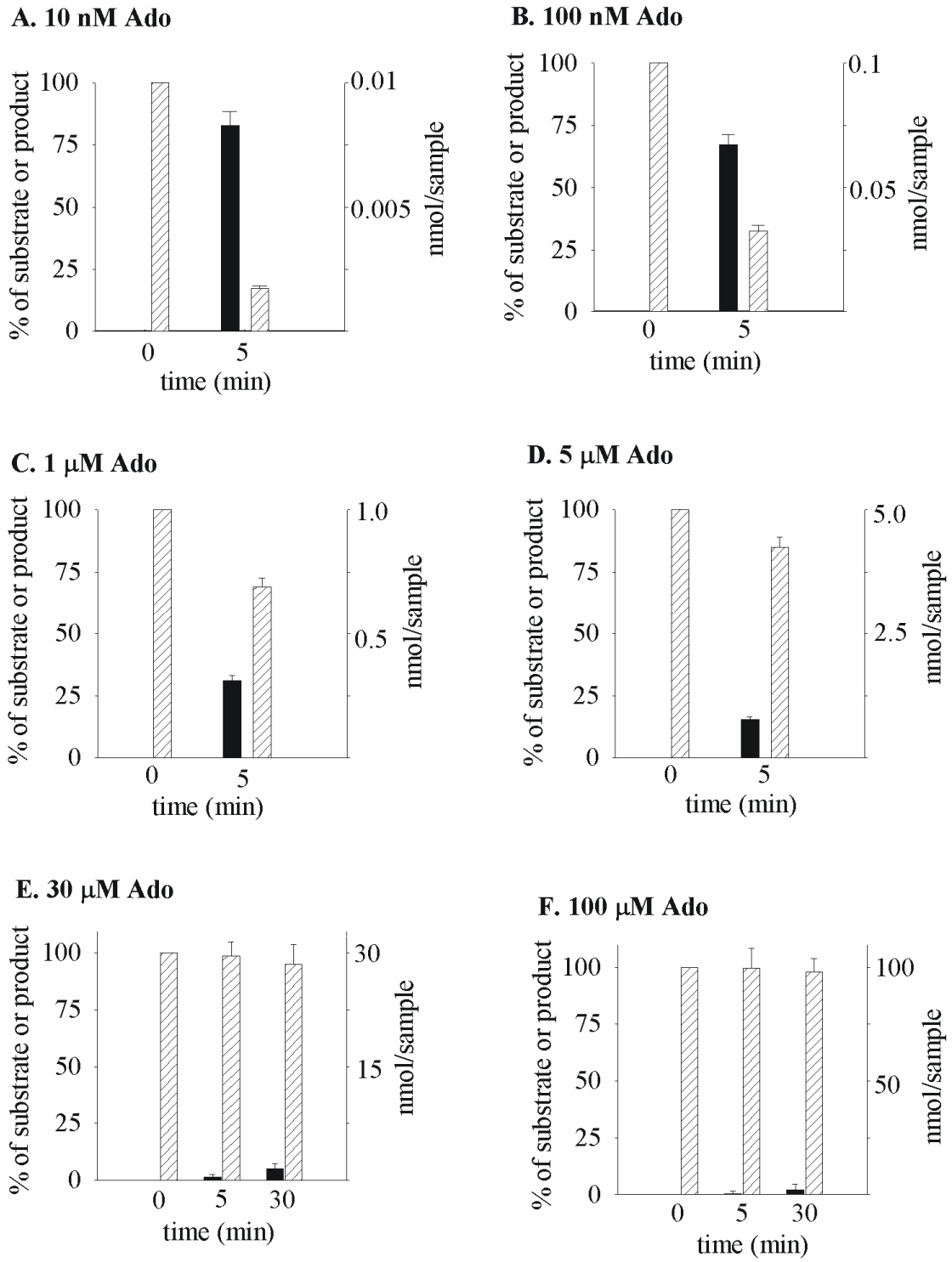

Fig. 2. *Metabolism of ex-Ado in the presence of intact C6 glioma cells. The cells were incubated in the presence of buffer A, 1 μM NBTI, 250 μM papaverine, and 10 μM dipyridamole; nmol/sample correspond to nmol/3.6 x 10^6 cells (▨ - Ado, ■ - adenine)*

The only product of Ado degradation by enzymes located on the C6 glioma cell surface was adenine (Fig. 1), so in the Ado molecule the N-glycosidic bond was broken. There are two possible mechanisms of breaking the N-glycosidic bond in the adenosine molecule. The first possible mechanism is phosphorolysis, which requires the presence of orthophosphate ions, and whose products are adenine and ribose-1-phosphate. The other possible mechanism is hydrolysis. In that case the reaction product would

be adenine and ribose. In the reported research, the presence of inorganic phosphate in the incubation mixture did not accelerate the Ado degradation by the intact glioma C6 cells (LDH activity < 1%) (Table I).

Table I. *The influence of inorganic phosphate on the degradation of ecto-adenosine in the presence of intact C6 glioma cells*[1]

Inorganic phosphate [μM]	*pmol adenine/min × 10^6 cells*
0	13.32±2.21
10	13.05±1.70
25	13.45±1.53

[1]in the presence of 1 μM adenosine, Buffer A, 250 μM papaverine, 10 μM dipyridamole and 1 μM NBTI

Therefore, the enzyme degrading the adenosine N-glycosidic bond might be a hydrolase belonging to an N-glycosidase subclass and we classified it as an adenosine ecto-N-glycosidase. The optimal pH for ecto-N-glycosidase is 7.0. The enzyme is not activated by Mg^{2+} or Ca^{2+} ions and reveals a high affinity for adenosine (K_M = 1.08±0.12 μM, V_{max}= 0.38±0.03 pmol adenine/s x 10^6 cells) (Table II). The activity of the enzyme with Ado as a substrate is inhibited by guanosine, inosine and 2'-deoxyadenosine (Table III).

Table II. *Some kinetic properties of ecto-N-glycosidase and adenosine transporters from C6 glioma cells*

Constant	*Ecto-N-glycosidase*	*Adenosine transporters*
K_M (for adenosine) [μM]	1.08±0.12	9.34±2.52
V_{max} (for adenosine) (pmol adenine /s × 10^6 cells)	0.38±0.03	3.54±0.77
V_{max} / K_M	0.35	0.38

Table III. *The influence of nucleosides on the ecto-N-glycosidase activity from C6 glioma cells*

Nucleoside [2μM]	*Activity (%)*
ADENOSINE	100[1]
Ado + Inosine	87
Ado + 2'-deoxyinosine	100
Ado + 2'-deoxyadenosine	89
Ado + Guanosine	63
Ado + Cytidine	100
Ado + Uridine	100

[1]100% of the ecto-N-glycosidase activity = 19.31 pmol adenine/min × 10^6 cells with 2 μM Ado as a substrate

Besides the enzymatic degradation, another mechanism that decreases the ex-Ado concentration is a transport of that nucleoside into the GC6 cells by the protein transporters. We found that ex-Ado, in 1 μM concentration, incubated for 10s with the glioma C6 cells was not degraded since 96% of the compounds transported, in that time period, into the cells was adenosine. Therefore, we investigated the influence of the inhibitors on the Ado transport into the glioma C6 cells under these conditions. The strongest inhibitor of the Ado transport was dipyridamole; papaverine was less efficient, while NTBI only slightly inhibited the Ado transport (Fig. 3). The determined K_M for adenosine transporters was 9.34±2.52 μM, V_{max} was 3.54±0.77 pmol adenosine/s x 10^6 cells. The V_{max}/K_M ratio calculated for adenosine transporters and adenosine ecto-N-glycosidase, being an indicator of the efficiency of transport or catalysis, are similar (Table III).

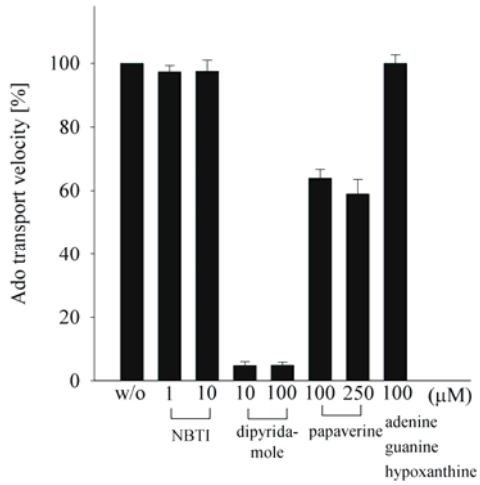

Fig. 3. *The influence of transport inhibitors and purine bases on the adenosine transport into C6 glioma cells. The amount of transported compound was measured after 10s incubation of C6 glioma cells with Ado (100% = 0.36 pmol Ado/s × 10^6 cells)*

NTBI in 10-μM concentration does not inhibit the adenine transport into C6 glioma cells while 250 μM papaverine inhibits 98% of the adenine transport (Fig. 4). Hypoxanthine and guanine, which do not inhibit the Ado transport, strongly (62%) inhibit the adenine transport into the cells (Fig. 4).

For times longer that 10s and in the presence of adenosine transport inhibitors (10 μM dipyridamole and 1 μM NBTI), only adenine produced in the hydrolysis of Fig. 3, 4 ex-Ado is transported into the cells (Fig. 5). During absence of these inhibitors, both Ado and adenine are transported into the cells (Fig. 6). Replacement of sodium ions by NMG^+ in media did not change the velocity of the adenine and Ado transport into the cells (data not shown). These results prove the presence of transporters independent of the sodium ions in the glioma plasma membranes.

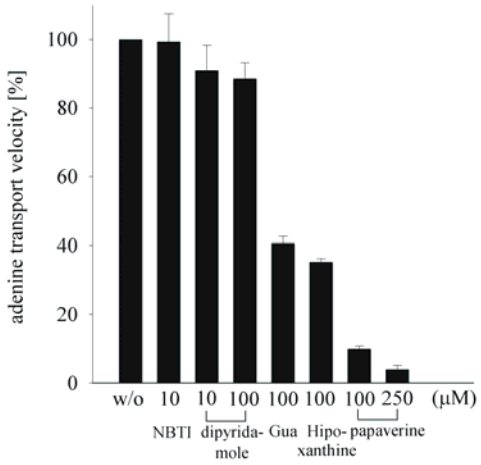

Fig. 4. *The influence of transport inhibitors and purine bases on the adenine transport into C6 glioma cells. The amount of transported compound was measured after 10s incubation of C6 glioma cells with adenine (100% = 0.42 pmol adenine/s × 10^6 cells; Gua – guanine)*

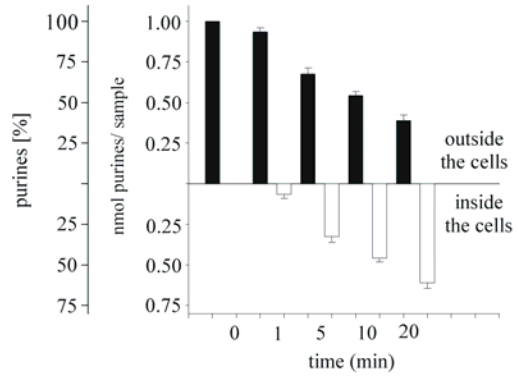

Fig. 5. *The influence of the incubation time on the ex-adenine transport into C6 glioma cells. Transport was detected in the presence of 1 μM ex-Ado and Buffer A containing 1 μM NBTI and 10 μM dipyridamole (■ - extracellular purines, □ - intracellular purines)*

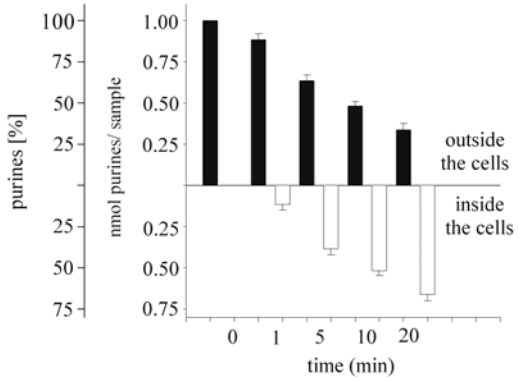

Fig. 6. *The influence of the incubation time on the ex-Ado and ex-adenine transport into C6 glioma cells. Transport was detected in the presence of 1 μM ex-Ado and Buffer A without transport inhibitors (■ - extracellular purines, □ - intracellular purines)*

METABOLISM OF ADO AND ADENINE TRANSPORTED INTO THE CELL

We have shown that Ado and adenine are transported into C6 cells by the specific protein transporters. Insight into the glioma C6 cells Ado and adenine has been included into the pool of adenine nucleotides (Tables IV and V).

Table IV. *Metabolism of ex-adenine transported into C6 glioma cells*[1]

Purine	*^{3}H-purines inside C6 cells [%]* [2] *after 1 min*	*after 10 min*	*after 20 min*
Xanthine	0	1.1±0.7	0.5±0.2
Hypoxanthine	0	1.6±0.9	0.5±0.4
Uric acid	0	0	0.5±0.1
Adenine	90.3±4.2	5.1±1.4	1.8±0.9
Inosine	0	2.5±0.8	1.1±0.5
IMP	0	0.7±0.4	0.6±0.2
Adenosine	0	4.2±1.1	1.3±0.3
AMP	8.5±0.9	29±2.3	23.1±2.1
ADP	0	26.7±2.1	33.2±1.5
ATP	0	24.1±1.6	32.9±2.5
Unknown	1.6±1.1	5.5±2.1	5.9±0.9

[1] C6 glioma cells were incubated in the presence of 1 μM Ado, Buffer A, and adenosine transport inhibitors (10 μM dipyridamole and 1 μM NBTI)
[2] 100% = total amounts of ^{3}H-purines inside C6 cells

In the absence of nucleoside transport inhibitors, after 20-min incubation of adenosine with the C6 cells, 66% of ex-Ado and its hydrolysis product (adenine) were inside the glioma cells. Of those, 0.4% was adenine, 13.1% was adenosine and 75.4% of purines were resynthesized into adenine nucleotides (Fig. 6, Table V).

Table V. *Intracellular metabolism of ex-adenosine and ex-adenine transported into C6 glioma cells*[1]

Purine	*^{3}H-purines inside C6 cells [%]* [2] *after 10 min*	*after 20 min*
Xanthine	0	0.3±0.1
Hypoxanthine	2.3±1.0	5.1±0.8
Uric acid	0	0.2±0.1
Adenine	0.7±0.2	0.4±0.2
Inosine	2.1±0.6	2.7±1.2
IMP	0.6±0.1	0.3±0.1
Adenosine	19.5±1.5	13.1±0.8
AMP	23.9±2.5	25.8±1.9
ADP	24.5±1.9	24.9±2.3
ATP	25.1±1.4	24.7±2.5
Unknown	1.8±0.3	2.5±2.4

[1] C6 glioma cells were incubated in the presence of 1 μM Ado without purine transport inhibitors
[2] 100% = total amounts of ^{3}H-purines inside C6 cells

However, after 1-min incubation of ex-Ado with GC6 cells in the presence of dipyridamole and NTBI, 90% of the transported purines were adenine, and 8.5% was converted into AMP. After 20-min incubation, 61% of ex-purines were transported into the cells. Of those 1.8% was adenine, while 89.2% was used in a re-synthesis of nucleotides (AMP, ADP and ATP) (Fig. 5, Table IV).

DISCUSSION

Our results proved that adenosine is metabolized by GC6 cells into adenine in times shorter than 1 min. Analysis revealed that 96% of all adenine derivatives transported into the cells during first 10s of ex-Ado incubation with GC6 cells were adenosine. Such conditions minimized the effects of the intracellular Ado metabolism on its transport velocity through the cell membrane. In previous papers describing transport of adenosine and adenine into the glioma C6 cells, authors did not report any enzymatic degradation of ex-adenosine in nanomolar or low micromolar concentrations by these cells even during a 1-min period [15-16]. We found that adenosine transporters present in the glioma C6 membranes are strongly inhibited by dipyridamole, while NTBI only slightly inhibits the Ado transport. These results prove that the glioma C6 membranes contain transporters belonging to the *ei* type, which are not sensitive to NTBI [16]. Ado transporters from the glioma C6 cells reveal high affinity for adenosine, since their K_M for adenosine is between 9.34±2.52 μM (our results) and 12±1 μM reported by other authors[16]. Our research indicates that glioma cells possess different transporters for adenine and adenosine. We have shown that dipyridamole strongly inhibits the transport of adenosine and only insignificantly inhibits the adenine transport, while papaverine strongly inhibits the adenine transport and to a much smaller degree the transport of adenosine. It is known that papaverine is an inhibitor of the nucleobase transport in erythrocytes [20]. The presence of different transporters for adenine and adenosine is also proved by results indicating that hypoxanthine and guanine (adenine competitors for the same transporters) inhibit the adenine transport and do not affect the transport of adenosine.

Transport of adenine and Ado was efficiently inhibited by 1 μM NBTI, 10 μM dipyridamole and 250 μM papaverine. These inhibitors did not affect the purine extracellular metabolism (data not shown). Therefore, the ex-purine degradation was determined in the above condition. Our research revealed no adenosine ecto-deaminase activity on the surface of the C6 glioma cells. However, the adenosine ecto-deaminase activity was reported for rat astrocytes [7]. Some reports also suggest the presence of adenosine deaminase binding protein CD26 in the C6 glioma cell membranes, which expression decreases with the increasing density of cells in the culture [21]. Research on glioma cell cultures of different density revealed that adenine is the only product of conversion of extracellular adenosine. These results indicate the presence of an extracellular enzyme degrading N-glycosidic bonds. Traversa et al. [17] reported the activity of an enzyme metabolizing guanosine into guanine in rat brain membranes. In that paper the authors suggested the presence of purine nucleoside ecto-phosphorylase, but did not detect the influence of acyclovir, the cytosolic phosphorylase inhibitor, on the guanosine degradation or binding to the membranes [17]. The presence of an ecto-enzyme metabolizing guanosine into guanine in astrocytes was also suggested. The authors showed that hypoxanthine is the product of the ex-Ado conversion in the presence of rat astrocytes. Therefore, they suggested that ex-Ado is deaminated into inosine and subsequently phosphorolysed into hypoxanthine on the surface of these cells. However, degradation is slow for both guanosine and adenosine.

Nucleosides might be metabolized into their respective nucleobases in either phosphorolysis or hydrolysis of the N-glycosidic bond between base and sugar moieties [22-25]. We found phosphorylase metabolizing inosine and guanosine into respective nucleobases only in the cytosol of C6 glioma cells. We also found that in the absence of phosphate ions, the degradation of adenosine in cells was negligible. Despite the presence or absence of EHNA (inhibitor of adenosine deaminase), we have not detected adenine among products of the adenosine degradation in cytosol. Therefore, we conclude that the enzyme metabolizing Ado into adenine is expressed only on the surface of C6 glioma cells. We analyzed the N-glycosidase activity using buffers that did not contain phosphate ions. Under these conditions, adenine was one of the main products of ex-ATP and ex-Ado degradation by the C6 glioma cells. The addition of phosphate ions to the reaction mixture did not affect the rate of the adenosine degradation into adenine (independent from that nucleotide concentration),

which led to the conclusion that the intact C6 glioma cells degraded ex-Ado into ex-adenine using adenosine N-glycosidase for hydrolysis of the N-glycosidic bond. The presence of N-glycosidases hydrolyzing the N-glycosidic bonds of purine nucleotides in the animal cells is as controversial as the presence of phosphorylases, for which adenosine is a substrate. Not long ago they were supposed to occur only in plants, protozoa, and other microorganisms [26-29, 22, 30]. In animal cells, only the presence of purine nucleoside phosphorylases was expected and only the N-glycosidases hydrolyzing NAD^+ and $NADP^+$ [31, 22] detected the activity of secretory N-glycosidases (EC 3.2.2.1) in the saliva and salivary gland of the *Aedes aegypti* mosquito. The N-glycosidases of C6 glioma have a pH optimum similar to the lupin nucleosidase (pH 7.5) [30] and saliva enzyme (pH 6.0-7.0) [22]. The identical optimum (pH 7.0) is found for the nucleosidase specific for inosine, guanosine, and adenosine (IAG-NH) from *Trypanosoma brucei brucei* [26]. Similar to the saliva nucleosidase, the magnesium and calcium ions and EDTA do not affect the activity of the C6 glioma ecto-enzyme.

The nucleosidase specific for inosine, guanosine, and adenosine (IAG-NH) was also isolated from *T. vivax* [26, 28]. In *Crithidia fasciculate,* the presence of two other nucleosidases was detected, one specific for inosine and guanosine (IG-NH) and the other specific for inosine and uridine (IU-NH) [27, 29]. The C6 glioma ecto-enzyme K_M value for adenosine is similar to that of the lupin enzyme (K_M=4.8 μM) [30]. A much higher value of K_M is found for the enzymes from *T. vivax* (K_M=8.5 μM) and *T. brucei brucei* (15 μM) [27, 26]. Nucleosidases from *Crithidia fasciculata* also hydrolyze adenosine, but their affinity for that nucleoside is much lower; for IG-NH enzyme the K_M is 106 μM, while for IU-NH it is 460 μM [27, 29].

The K_M of the ecto-enzyme from C6 glioma for adenosine is 1.08±0.12 μM. Activity for adenosine as a substrate was inhibited by guanosine, inosine, and 2'-deoxyadenosine, while uridine, cytidine, and 2'-deoxyinosine did not inhibit the adenosine hydrolysis. Based on these results, it might be expected that the first three compounds might also be substrates for adenosine ecto- N-glycosidase. Inhibition of C6 glioma adenosine ecto-N-glycosidase by guanosine and inosine and not by uridine suggests that this enzyme belongs to the group of nucleosidases specific for inosine, guanosine and adenosine [26, 28, 30]. The adenosine transporters and ecto-N-glycosidase of C6 glioma cells differ by K_M and V_{max} values. However, the V_{max}/K_M coefficients, being the efficiency measure of both transport and catalysis, are similar. The adenosine ecto-N-glycosidase, having an affinity for Ado 9 times higher than transporters, seems to be adapted to the efficient degradation of physiological concentrations of Ado in the CNS extracellular spaces, and therefore might participate in the regulation of signaling *via* adenosine receptors. The role of adenosine transporters would increase in the presence of higher nucleoside concentration. It should be stated that high micromolar concentrations of ex-Ado in brains occur due to pathologic changes strongly affecting the cell metabolism, in particular purine metabolism.

The results reported here support the previous suggestions of many authors on low activity of xanthine oxidase in brains and a more significant role of adenine kinase rather than adenine deaminase in the metabolism of intracellular adenosine that is supported by experiments showing that the K_M of cytosolic deaminase for Ado is at least an order of magnitude lower than that of adenosine kinase [32, 33]. Much less is known on the intracellular metabolism of adenine. Reports on the purine metabolism in brain show the presence of adenine phosphoribosyltransferase synthesizing AMP from adenine in the cytosol of astrocytes [34]. However, the role of that enzyme was questioned by many authors [34]. However, the results reported here revealed that adenine might appear in animal cells as a product of ex-Ado degradation and might be used in resynthesis of AMP. These experimental results confirm that adenine phosphoribosyltransferase can exist in the brain glia cells and play a substantial role in their purine metabolism.

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ORIGINAL ARTICLE / PRACA ORYGINALNA

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TAFI (THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR) AND PARAMETERS OF ITS ACTIVATION IN POSTMENOPAUSAL WOMEN TAKING ORAL AND TRANSDERMAL HORMONE REPLACEMENT THERAPY

TAFI (THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR) I PARAMETRY JEGO AKTYWACJI U KOBIET W OKRESIE POMENOPAUZALNYM STOSUJĄCYCH HORMONALNĄ TERAPIĘ ZASTĘPCZĄ DROGĄ DOUSTNĄ I PRZEZSKÓRNĄ

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Summary

The aim of the study was to assess concentration of TAFI: Ag and parameters of its activation in postmenopausal women taking oral and transdermal hormone replacement therapy (HRT).

The study was performed on 76 healthy, non –smoking postmenopausal women (1-2 years after menopause). 46 women aged 44 – 58 years (mean age 52 years) were taking oral (26) or transdermal (20) hormone replacement therapy. The control group consisted of 30 women aged 44 - 54 years (mean age 49 years) who did not take HRT. The following parameters of hemostasis were determined in plasma: concentration of TAFI: Ag, sTM (soluble thrombomodulin*)*, activity of protein S (measured by ELISA- Enzym Linked Immunosorbent Assay) and activity of protein C (measured using chromogenic and chronometric methods).

The study results show significant increase in the concentration of sTM as well as increased activity of protein S in women taking oral HRT in comparison to the control group ($p<0.02$, $p<0.003$, respectively). Among women taking transdermal HRT, statistically significant differences in concentration of TAFI: Ag, sTM and activity of protein S and protein C in comparison with women taking oral HRT and the control group were not observed. Moreover, we found a significant negative correlation between concentration of TAFI: Ag and activity of protein C in women taking HRT.

The occurrence of increased concentration of sTM in women taking oral HRT is most likely an effect of high-dose estrogen on activation and stimulation of endothelium. High-dose estrogen has probably an impact on increased protein S hepatic synthesis and secretion into the blood in women taking oral HRT.

Streszczenie

Celem pracy była ocena stężenia TAFI: Ag i parametrów jego aktywacji u kobiet w okresie pomenopauzalnym stosujących hormonalną terapię zastępczą (HTZ) drogą doustną i przezskórną.

Badaniami objęto 76 zdrowych kobiet, niepalących, 1-2 lat po menopauzie. 46 kobiet w wieku 44-58 (średnia 52 lat) stosowało doustną (26), przezskórną (20) hormonalną terapię zastępczą. Grupę kontrolną stanowiło 30 kobiet w wieku 44-54 (średnia 49 lat) niestosujące HTZ. Materiałem do badań było osocze cytrynianowe. Stężenie TAFI, Ag, sTM (frakcja rozpuszczalna trombomoduliny) oraz aktywność białka S oznaczono przy użyciu metody immunoenzymatycznej

(ELISA), aktywność białka C oznaczono metodą chronometryczną i chromogenną.

Zaobserwowano istotny statystycznie wzrost stężenia sTM oraz aktywności białka S w grupie kobiet stosujących HTZ drogą doustną w porównaniu z grupą kontrolną. Różnice te były istotne statystycznie ($p<0.02$, $p<0.003$-odpowiednio). W grupie kobiet stosujących HTZ drogą przezskórną nie stwierdzono żadnych różnic istotnych statystycznie w badanych parametrach w porównaniu z grupą kontrolną i grupą kobiet stosujących HTZ drogą doustną. Ponadto uzyskano u kobiet stosujących HTZ ujemną korelację pomiędzy stężeniem TAFI: Ag a aktywnością białka C.

Wyższe stężenie sTM u kobiet stosujących HTZ drogą doustną jest, prawdopodobnie wynikiem działania dużych dawek estrogenów na śródbłonek naczyniowy i dowodem jego pobudzenia. Podwyższona aktywność białka S u kobiet stosujących HTZ drogą doustną jest, jak się wydaje, efektem stymulującego działania dużych dawek estrogenów na jego wątrobową syntezę i wydzielanie do krwi.

Key words: TAFI, sTM, protein S, protein C, hormone replacement therapy
Słowa kluczowe: TAFI, sTM, białko S i białko C, hormonalna terapia zastępcza

INTRODUCTION

Hemostasis requires a balance between coagulation process and fibrinolytic system and their inhibitors [1]. In normal hemostasis three main components are involved: blood vessel wall, blood flow in the blood vessels and a blood composition. The disorders of any of these parameters can lead to thrombotic and embolic complications [2].

TAFI (Thrombin Activatable Fibrynolysis Inhibitor) was recognized as a link, which joins fibrinolytic process with coagulation [3, 4]. High concentration of thrombin can activate TAFI which, in turn, stimulates fibrinolysis [5]. TAFI was described in three independent laboratories at the same time and named as: carboxypeptidase U (CPU), carboxypeptidase R (CPR) and plasma carboxypeptidase B (CPB) [3, 6]. The main organ producing TAFI is liver, but TAFI was also found in the pancreas. The conversion of pro-TAFI to TAFI is regulated by thrombin, which impacts the peculiar endothelial receptor for thrombomodulin (TM). The complex of thrombin with receptor intensifies the activation effect by about 1250-fold. Therefore, it is acknowledged as a physiological activator of inhibitor [3]. Moreover, the complex TM-thrombin activates protein C, which can lead to transformation of pro-TAFI into TAFI [3, 6].

The antifibrinolytic mechanism of TAFI involves detaching a part of the α-chain of fibrin that is the amino acid residues of lysine and arginine from the C-terminal. Then, fibrin loses the capability of absorption of plasminogen and plasminogen activators on her surface. C-terminal lysine residues in the fibrin are essential for binding plasminogen and t-PA (tissue plasminogen activator) [3, 7]. Then, both plasmin generation and fibrin degradation to FDP (fibrin degradation products) decrease [7].

Menopause is caused by physiological decrease of ovarian endocrine function, which, in turn, leads to a loss of the influence of negative feedback of the hypothalamus-hypophysis-ovarium axis. Insufficiency of estrogen and progesterone production is a result of cessation of ovary function. The secretion of gonadotrophins FSH (Follicle-Stimulating Hormone) and LH (Luteinizing Hormone) by hypophysis is increased during and after the menopause [8, 9]. Inhibin b and FSH concentrations were recognized as menopausal markers [8, 10].

Changes in hormonal balance in perimenopausal women lead to considerable metabolic changes. The early symptoms of hormonal dysfunction are manifested as: hot flashes, mood swings, nocturnal sweating, palpitation, insomnia, atrophy of reproductive and urinary systems, osteoporosis, and higher risk of ischemic heart disease. The prolonged hypoestrogenism is one of the factors responsible for the atherosclerosis development, heart vascular complications, osteoporosis, increase of insulin resistance and frequency of hypertension in perimenopausal women [8, 11, 12].

Decreasing or minimizing menopausal symptoms in women is often possible only by starting pharmacological treatment. Hormonal Replacement Therapy (HRT) should be individually matched – 'tailored hormonal therapy'. The suitable kind of hormone, dose and route for administration of medications are very important. Determination of these parameters should lead to the best effectiveness of HRT and minimize side effects [13].

Recently published data showed many differences concerning the influence of oral estrogens and gestagens on hemostasis. This may depend on the dose, kind of estrogens and progestagens and route for administration of hormones [14]. The results have

shown the influence of fibrinolytic inhibitor activated by thrombin (TAFI) on the fibrinolytic activity, but TAFI's participation in the hemostasis disturbances in perimenopausal women has not been explained yet.

The aim of the study was to assess concentration of TAFI and parameters of its activation in postmenopausal women taking oral or transdermal hormone replacement therapy (HRT).

MATERIAL AND METHODS

Study group

The study was performed on 76 healthy, nonsmoking, postmenopausal women, who were 1 - 2 year after menopause. 46 women aged 44 – 58 years (mean age 52 years) took oral (26) or transdermal (20) hormone replacement therapy. HRT was taken daily continuously in the form of composite preparation: estrogen - progestagen combinations. 26 women from the study group used oral HRT [2 mg 17 beta – estradiol (E_2) and 1 mg norethisterone acetate (NETA)-(Kliogest, Novo Nordisk Pharma, Poland)] and 20 women took transdermal HRT [50µg E_2 and 170µg NETA (SYSTEN® Conti, Janssen-Cilag, Warsaw, Poland)]. Women took HRT for 6 - 14 months. The persistent climacteric symptoms like heavy and regular hot flashes with drenching sweat were the main conditions to start HRT.

The control group consisted of 30 healthy, nonsmoking, postmenopausal women, aged 44-54 years (mean age 49 years), who did not take HRT.

Blood pressure (BP) and body mass index (BMI) were measured at the beginning of the study in all the study groups. The systolic blood pressure (SBP) range was 120 ± 19 mmHg, diastolic blood pressure (DBP) range was 74 ± 15 mmHg, the BMI range was 21.0 ± 5.0 kg/m^2. The concentration of FSH was between 35.6-118.1 lU/l in women taking HRT. The concentration of FSH was also assayed in the control group and was found to be 37.4-117.3 lU/l.

Women in both groups had no diabetes mellitus or glucose intolerance. They never had any incident of thrombosis or systemic illnesses. None was taking any medication that might have interfered with fibrinolytic system. All women included in the study had a complete gynecological examination, cytology smear, palpable breast examination, mammography and biochemical examinations (lipid profile and hormone profile).

Venous blood for hemostatic tests (4.5 ml) was collected in a fasting state into cooled tubes *(Becton Dickinson* Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate *(the final* blood-anticoagulant ratio was 9:1) after 30 minutes of rest between 7^{30} and 9^{30} am after a 12-hour overnight fast. The blood samples were immediately mixed and centrifuged at *3000 g* at + 4°C for 20 minutes. The obtained platelet-poor plasma was divided into 200 µl Eppendorf-type tubes and then samples were frozen at -86 °C until assayed, but for not longer than 6 months.

Hemostatic assays

The concentration of TAFI: Ag was determined by Enzyme Linked Immunosorbent Assay (ELISA), (TAFI-IMUBIND® Tafia/ai*, American Diagnostica inc.), sTM was determined by ELISA (IMUBIND® Trombomodulin, American Diagnostica inc.), the activity of protein S was measured by ELISA (ASSERACHROM® Protein S, Diagnostica Stago Asnieres, France). The activity of protein C was performed on an automated coagulometer CC-3003 and with reagents produced by Bio-Ksel Co (Grudziadz, Poland).

FSH assay

Level of FSH serum was determined by MEIA (AXSYM®SYSTEM, Abbott laboratories, Diagnostics Division, Abbott Park, IL 60064, USA).

Statistical Analysis

Statistical analysis was performed using Statistica 6.0 software (StatStoft®). Shapiro-Wilk test was used to assess normality of the distribution. For variables, with normal distribution mean (X) and standard deviations (SD) were determined. The median (Me), lower quartile Q1 and upper quartile Q3 were used for values, which distribution were different from normal. Testing of the regularity of the distribution allowed us to use the classical t-Student test. We also used the U-Mann-Whitney rank sum test. Spearman correlation coefficients were calculated to determine if there were associations between concentration of TAFI: Ag and activity protein C. The *p*-values < 0.05 were considered statistically significant

Women in both groups were selected in the Outpatient Gynecology Centre of the University Hospital in Bydgoszcz. Written informed consent was obtained from each participant before entering the study. The study was approved by the Bioethics

Committee of Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun (no KB/305/2004).

RESULTS

Table 1. shows concentrations of TAFI: Ag, sTM and protein C and S activity in women taking oral or transdermal HRT and in the control group. We did not observe significant differences in TAFI: Ag concentration between the study and control group. In the group of women taking oral HRT higher concentration of sTM was observed than in the control group. This difference was significant ($p<0.02$). We also found significant increase of protein S activity in women using oral HRT in comparison to the control group ($p<0.003$). We did not find significant differences of protein C activity in women taking oral, transdermal HRT and in women from control group. Moreover, in women taking HRT a negative correlation between TAFI: Ag concentration and protein C activity was observed ($R=-0.48$, $p<0.002$) (Fig.1).

Table 1. *Concentration/activity of assayed parameters in women taking oral HRT, transdermal HRT and in control group*

Assessed parameters [units]	Oral HRT (n=26) **(I) M+/-SD Me/ Q1/Q3**	Transdermal HRT (n=20) **(II) M+/-SD Me Q1/Q3**	Control group (n=30) **(III) M+/-SD Me Q1/Q3**	Significance level **(p)**
TAFI: Ag [ng/ml]	0 0/27	20.25 0/44.25	0 0/66	I vs. III NS II vs. III NS I vs. II NS
sTM [ng/ml]	2.6 2.02/4.9	2.36 1.74/3.08	1.97 1,52/2.28	I vs. III p< 0.02 II vs. III NS I vs. II NS
Protein C [%]	134.95+/-25.2	127.41+/-24.04	121.3+/-16.31	I vs. III NS II vs. III NS I vs. II NS
Protein S [%]	115.94 111.84/123.25	112.14 109.74/120.14	108.53 87/113/74	I vs. III p<0.003 II vs. III NS I vs. II NS

Abbreviations: NS- not significant, M- Mean, SD- Standard Deviation, Me- Median Q1- Lower quartile, Q3- Upper quartile, HRT- Hormone replacement therapy, sTM- soluble thrombomodulin

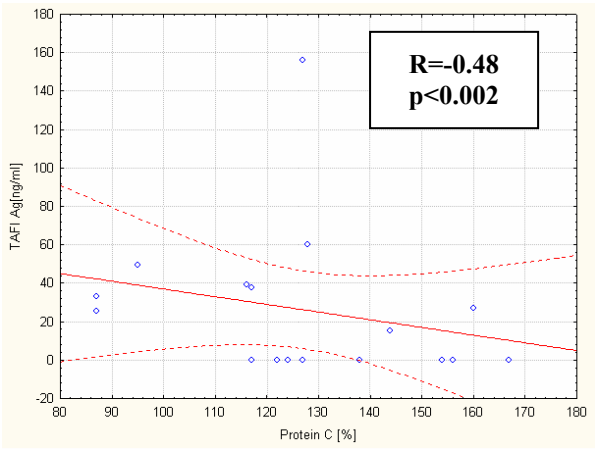

Fig. 1. *Correlation between TAFI: Ag and activity of protein C*

DISCUSSION

Taking HRT and the influence of the route for administration of HRT is still a matter of discussion. However, we observed many differences in correlation between the kind of route for administration of HRT and the reaction to haemostatic process.

Taking oral HRT is related to the first transition effect through the liver. The first transition effect results in a decrease of concentration of taken estadiol because a part of estradiol is resolved into a form with lower activity such as estrone. The estrone/estradiol ratio could increase even 5:1 in women taking oral HRT, whereas this ratio is 1:1 in women taking transdermal HRT [10, 15]. The transdermal HRT is more physiological in term of similarity to a natural route of administration of sex hormones than oral HRT.

Transdermal HRT does not exert a harmful influence on the hepatocytes [16]. A hormone, which was taken, reaches the target organ avoiding the way of the portal circulation. Avoiding portal circulation allows for a decrease in daily doses of medicines [10, 17]. Lower doses of transdermal HRT are suitable to alleviate the symptoms of menopause [18].

Our study showed that in women who took oral HRT, the concentration of sTM and protein S activity were significantly higher in comparison to the control group.

Thrombomodulin, von Willebrand factor and t-PA belong to the same protein group. This group includes markers of vascular endothelial stimulation or markers of vascular endothelial damage [19]. There are tests providing assessment of TM glycoprotein concentration in the blood. When the endothelial cells are stimulated or activated, TM is detached. Then it is present in blood as soluble thrombomodulin. The concentration of sTM in the blood dramatically increases during diabetes mellitus type 1 and 2 and also in patients with atherosclerosis with vast vascular endothelial damage, in patient with pulmonary thromboembolism, ischemic heart disease, disseminated intravascular coagulation (DIC), acute kidney and liver failure, malaria and other inflammatory diseases [19, 20]. As pointed out previously, high sTM concentration is one of the vascular endothelial damage markers in the above mentioned disorders [20].

The increase of sTM concentration in women taking oral HRT in our study proves that a large

amount of estrogens could increase activation process in vascular endothelial cells, which causes detachment of membrane thrombomodulin receptor fragment and increase of soluble TM fraction in the blood.

Protein S is a single-chain glycoprotein synthesized in hepatocytes, megakariocytes and vascular endothelial cells in the presence of vitamin K. Its main function is catalysis of factor Va and factor VIIIa inactivation using active protein C. Insufficiency of protein S could cause thromboembolic complications.

Gilbert et al. showed that protein S activity increases during menopause [21]. The analysis of recent data shows that using HRT causes different activation level of protein S. Caine et al. observed a significant decrease of protein S activity in patients taking HRT which contained horse estrogens of natural origin – *conjugate estrogens in a dose of* 0.625 mg/day [22]. Similarly, Chen et al showed a decreased protein S activity in women taking oral estrogen-progestagen therapy consisting of estradiol valerianate in a dose of 2 mg/day and cyprotherone acetate in a dose of 1 mg/day, and in women using transdermal HRT consisting of estradiol in a dose of 2 mg/day and medroxyprogesterone acetate in a dose of 5 mg/day [23]. Hoibraaten et al. compared protein S activity in the group of women taking HRT consisting of 17β-estradiol in a dose of 2 mg/day and nortesteron acetate in a dose of 1 mg/ day to the control group. They observed increase of protein S activity in women taking HRT [24]. Gilbert et al observed no differences in protein S activity among women taking HRT [21]. Kroon et al studied women taking transdermal HRT consisting of 17β-estradiol and oral medroxyprogesterone acetate in a dose of 2.5 mg/day. They did not observe any statistically significant differences in protein S activity [25]. Decrease of protein S activity was observed only when the dose of estrogens was too high [21]. Decrease of protein S activity after 4-months of hormone therapy was associated with increase of estradiol concentration [21].

It seems that high levels of estrogens in oral HRT are a stimulating factor among liver proteins and protein S synthesis. Increase of protein S activity in women after menopause, which was observed by us and other authors (Hoibraaten E. et al), could be a factor decreasing a risk of thromboembolic complications [24].

Furthermore, we obtained a negative correlation between TAFI: Ag concentration between protein C activity.

TAFI and protein C activation is regulated by thrombin-thrombomodulin complex on vascular endothelial cellular membrane. This mechanism shows an important role of the mentioned inhibitors in keeping the balance between coagulation and fibrinolysis [26].

TAFI does not block protein C activity, because both transformations (TAFI and protein C) can occur simultaneously without distorting their physiological function [5]. Physiological inhibition of TAFI activity occurs with protein C participation, which deactivates factors Va and VIIIa. That leads to suppression of coagulation and a decrease of thrombin generation. Also decrease in thrombin quantity does not stimulate TAFI synthesis [3]. Active protein C improves fibrinolysis inhibition through TAFI induction. On the other hand, it also could stimulate fibrinolytic activity through suppressing TAFI activity [26]. Pathological decrease of TAFI activity could be correlated to bleeding. However, overactivity of TAFI could tend to thrombosis [3, 5].

Active protein C improves inhibition of fibrinolysis through TAFIa induction. On the other hand, it can stimulate fibrinolysis activity through inhibition of TAFI activity [26]. TAFI and protein C activity are competitively added to thrombin-thrombomodulin complex. However, even activation of both substances depends on the structure and thrombomodulin properties. The site of TAFI and protein C binding could overlap. The fourth domain – EGF affects protein C, but it does not influence TAFI. The third domain – EGF is indispensable for binding TAFI, but it is unnecessary for protein C activation. The removal of third domain – EGF makes it impossible for TAFI to block protein C activation. If the third domain – EGF is missing, TAFI will not be able to bind or get activated by thrombin -TM complex. Decrease of protein C concentration in plasma could cause thrombosis [27, 28].

Moreover, changes in TAFI levels or defects in structure of thrombomodulin could selectively weaken activation of inhibitors (TAFI or protein C). That could impair the balance between anticoagulation and antifibrinlolytic tract and cause bleeding or tendency to thrombosis [27, 28].

CONCLUSION

1. The occurrence of increased concentration of sTM in women taking oral HRT is most likely an effect

of high-dose of estrogen on activation and stimulation of endothelium.

2. High dose of estrogen probably has an impact on increased protein S hepatic synthesis and secretion into blood in women taking oral HRT.

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CASE REPORT / OPIS PRZYPADKU

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FIBROCEMENTOMA OF THE RIGHT MAXILLA – A CASE REPORT

FIBROCEMENTOMA PRAWEJ SZCZĘKI – OPIS PRZYPADKU

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S u m m a r y

Fibrocementoma of the maxilla is a rare pathology. It can be of odontogenic or nonodontogenic origin. The etiology is unknown. The age of onset of the disease was most frequently recorded during the second and fourth decades of life with definite female predilection. As possible etiologic factors, trauma and carious teeth were described. The disease is generally asymptomatic and is discovered incidentally. Sometimes pain, swelling, paresthesia, tooth displacement and facial deformity are observed. The recurrence after surgery is uncommon.

S t r e s z c z e n i e

Fibrocementoma szczęki jest niezmiernie rzadko występującą patologią jako przyczyna zębopochodna lub nie związana z tkanką zębową. Etiologia schorzenie jest niejasna, a częstość zachorowań jest najwyższa w drugiej i czwartej dekadzie życia z przewagą kobiet. Jako czynniki patologiczne rozwoju schorzenia przyjmuje się przebyte urazy lub zaawansowaną próchnicę zębów. Przebieg choroby w większości przypadków jest bezobjawowy, a rozpoznanie przypadkowe. W niektórych przypadkach obserwuje się występowanie dolegliwości bólowych, obrzęków, parestezji, przemieszczenia zębów czy też deformacji twarzy. Leczenie chirurgiczne jest metodą z wyboru, a nawroty obserwowane są sporadycznie.

Key words: fibrocementoma, maxilla, diagnosis, treatment
Słowa kluczowe: fibrocementoma, szczęka, diagnostyka, leczenie

INTRODUCTION

Some cystic and cyst-like lesions of a mandible or maxilla are mainly oval, radiolucent or radioopaque and sometimes well demarcated [1]. Such pathology can be of odontogenic or nonodontogenic origin [1, 2]. Cementoma is a benign disease that mostly involves the mandibular anterior teeth and it can be also found rarely in the maxillary molars and premolars [1, 2, 3, 4]. In the literature, the cementoma is described as: periapical osteofibroma, cementifying fibroma, fibrocementoma, periapical fibrous dysplasia or odontogenic fibroma [1, 2, 5]. The diagnosis of cementifying fibroma is done when a tooth is associated with fibrous lesion containing ‘cementum’.

If the lesion does not contain a tooth, it is postulated that cementum is originated from osteoid tissue and it should be diagnosed as ossifying fibroma [2, 5]. Lesions of the jaw with features of cemento-ossifying fibroma and some mixed ones with patterns of fibrous dysplasia have been referred to fibro-osseous lesions of periodontal ligament origin [3, 4, 6]. The etiology of cementoma is unknown [2, 3, 5]. The age of onset of the disease was most frequently recorded during the second and fourth decades of life with definite female predilection [4, 6]. As the possible etiologic factors, trauma and carious teeth were described [1, 2, 3]. In many cases, in the early stages, the process is asymptomatic, sometimes such lesions are discovered incidentally [1, 2, 4]. The most common symptoms could be pain with or without swelling [1, 2, 5], sometimes paresthesias and tooth displacement or mobility are described [1, 2, 4, 5, 7]. The clinical and radiographic features of the lesion help to establish a differential diagnosis, although histopathology examination is mandatory to accurately identify the disease. The treatment of choice is surgery [1, 3, 4].

In this paper, authors present an unusual case of young woman with symptomatic fibrocementoma of the right maxilla.

CASE REPORT

A 35 year-old woman was admitted to the Department of Otolaryngology with severe headache on the right side. She localized the pain generally around the right maxilla with its extension to the right orbit, as well as temporal and occipital region. The patient also complained of nasal blockage on the right side without any discharge. Those symptoms had begun 2 years ago. Seventeen years ago, she had a car accident with fracture of right femur and head trauma without bone fractures. On admission, the patient was afebrile, without swelling of the cheeks and with no tenderness reported. On examination, the teeth of the alveolar ridge on the right side were tender to palpation and an absence of 6th premolar tooth was noted. Nasal examination showed deviated septum to the right side – diagnosed as septum spur. The laboratory data was in normal range. A CT scans showed a mixed opacity of radiolucent and radioopaque material in the maxillary sinus. In the right nasal cavity, at the level of the middle turbinate a ‘tooth like’ element was presented. This ‘tooth like’ structure was blocked between the lateral nasal wall and the nasal spur (Fig. 1a and Fig. 1b). An endoscopic procedure was performed to take a biopsy for histopathology. In the middle meatus, a root of tooth was found. It was blocked between the septum spur and middle turbinate as it was shown on CT. The root was removed and the spur was dissected (Fig 2). Through the medial antrostomy the biopsy of hard bony like tissue was taken. Histopathologic examination revealed a bony tissue material. Three weeks after the surgery, as the symptoms still persisted, the patient was prepared for external surgery procedure. A Caldwell-Luc procedure was done at the right side. The maxillary sinus was opacified with bony like very hard material and some fibrous tissue. The cavity of the sinus was reconstructed with drilling to the normal looking bone of the maxillary walls. Below the orbital floor a small cavity was recognized with mucous material which was completely removed. Then, a wide passage to the nose under the inferior turbinate was created. The mucoperiosteal flap was reapproximated and closed. The healing was good and the patient was discharged after seven days. The histopathology examination showed fragments of hard partially osseous material. Histological examination revealed a dens stroma composed of spindle cells (fibroblasts) with abundant collagen deposits. Within such stroma multiple psammoma-like bodies were present. The microscopic picture was consistent with fibrocementoma (Fig. 3).

At present, 7 months after the surgery the patient is free of symptoms and disease.

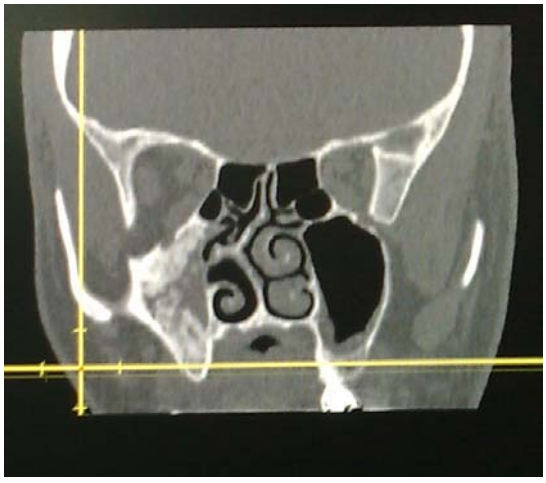

Fig 1a. *CT scan: mixed opacity of radiolucent and radioopaque material in the maxillary sinus*

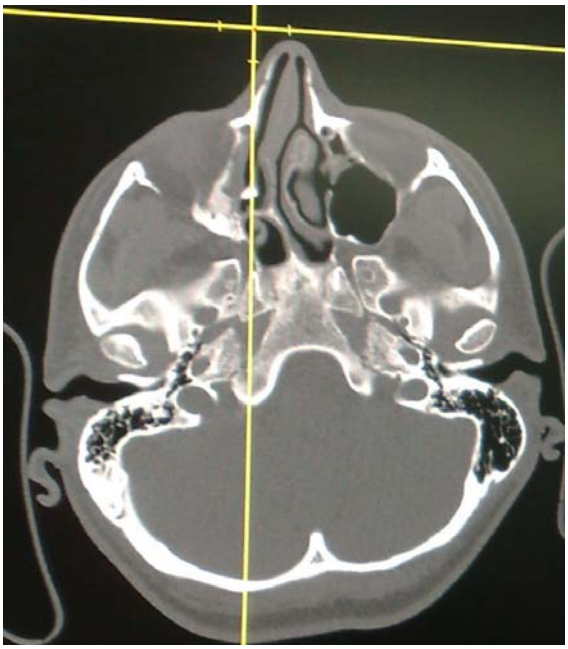

Fig 1b. *CT scan: A "tooth like" element in the right nasal cavity at the level of the middle turbinate*

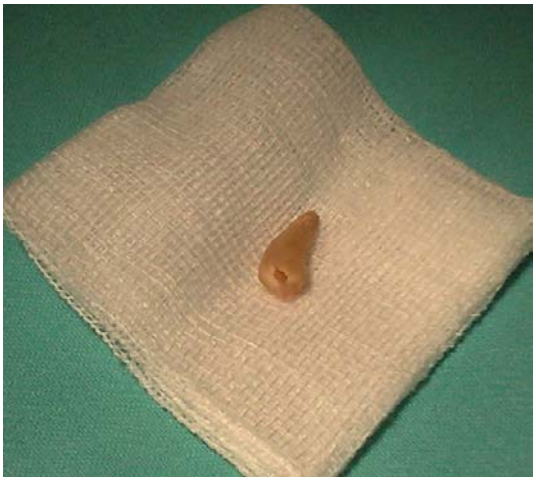

Fig. 2. *A root of the 6th premolar tooth*

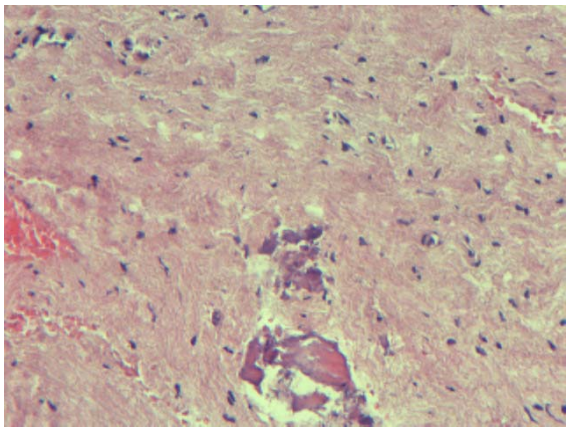

Fig. 3. *Histological examination : a dens stroma composed by spindle cells (fibroblasts) with abundant collagen deposits and multiple psammoma-like bodies presented in the stroma. The microscopic picture was consistent with fibrocementoma.(H&E 20X)*

DISCUSSION

The cementoma is a benign disease that very rarely involves the maxilla. Its etiology is unknown, but trauma is described as a very possible initiatory of the process [2]. Normally the tooth is in the conjunction with the lesion, and bone is not invaded. The disease is mostly referred to malpositioned of unerupted teeth [2, 5]. In the maxilla the molars and premolars teeth are predominant. In our case, the lesion occupied the maxillary cavity, but no evidence of tooth root was found in the maxillary. We assume that here the trauma experienced several years earlier probably indicated the process and unerupted 6th premolar tooth was the origin of it. For several years the disease was absolutely asymptomatic. Enlargement of pathological tissue inside the maxilla could have changed the position of the tooth root into a direction of natural sinus ostium and prolapsed it into the nose. On the other hand, the root, in unknown way or by the trauma, could have separated from the tumor and migrated with natural mucous flow to the ostium and forward to the nose. When the maxillary cavity was filled with the tumor and the nasal space was narrowed by the tooth root, the disease became symptomatic. The histopathologic report proved the etiology of fibrocementoma. Histologically ossifying fibroma, cemento-ossifying fibroma and fibrous dysplasia share common features [2, 5]. The progress of the disease could be divided in to three stages according to the clinical and radiographic appearance. The first stage is characterized by the 'osteolytic' process where there is a bone destruction and proliferation of fibrous connective tissue (appearing as radiolucent on radiographs). The second stage is the cementoblastic (calcifying) stage with radiolucent area with radioopaque objects (a deposition of cementum in the fibrous mass) leading to the third and final 'mature inactive' stage called hypercalcified stage. In the last stage, cementum can completely replace the fibrous tissue. The progression through these stages is rather slow and mostly asymptomatic [2, 4, 5, 6]. During osteoblastic activity, the lesion is composed of fibrous tissue in varying degrees of cellularity and it can look like fibrous dysplasia, especially in the first two stages [2, 6]. In differentiated diagnosis the presence of calcified spherules in the mass of the lesion is the most typical for fibrous dysplasia [3]. The diagnosis of cemento-ossifying fibroma is that a tooth must be associated with a fibrous lesion containing 'cementum'

and/or psammoma bodies. Because in this case the lesion was without a tooth, it is supposed that cementum derived from osteoid tissue, and the diagnosis would be an ossifying fibroma [2, 7, 8]. Jaffe recognized two types of cementomas with microscopic examination: the first called fibrocementoma, where the basic spindle cell stroma overshadows the cement substance, and the, so called, sclerosing cementoma in which the cementum is abundant so that the fibrous matrix can be completely submerged [9].

CONCLUSIONS

The benign fibro-cemento-osseous lesions of jaws are very rare. They often occur in women in the second to fourth decades of life. Trauma is considered an inaction of the process. The disease is generally asymptomatic and discovered incidentally. Sometimes pain, swelling, paresthesia, tooth displacement and facial deformity are observed. Proper classification of lesions of jaws requires good correlative analysis of clinical symptoms, radiographic scans and microscopic histopathology features. Surgery should always be individualized to localization and extent of the disease. The recurrence after surgery is uncommon.

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CASE REPORT / PRACA KAZUISTYCZNA

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SEBACEOUS LYMPHADENOMA OF THE PAROTID GLAND

GRUCZOLAK ŁOJOWY LIMFATYCZNY ŚLINIANKI PRZYUSZNEJ

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Summary

Introduction. Sebaceous lymphadenoma of the parotid gland is a very rare tumor which can mostly occur in the elderly. It is a benign tumor with no recurrence after surgery treatment, but in some situation it can transform to a sebaceous lymphadenocarcinoma.

Case report. We report a case of a 51-year-old woman with a sebaceous lymphadenoma of the left parotid gland. The patient had an ultrasound scan of parotids and fine-needle aspiration biopsy (FNAC) of the tumor done. The dimensions of the tumor were 23 x 17 x of 17 mm and images suggested a benign tumor, possibly tumor mixtus, also sustained by FNAC.

Discussion. Sebaceous lymphadenomas are encapsulated, well circumscribed benign tumors of variably sized and shaped nests of sebaceous cells. The origin of sebaceous differentiation in salivary glands is unknown. It can be a metaplastic process secondary to ductal obstruction or congenital in origin.

Summary. Sebaceous lymphadenomas are very rare benign tumors occurring mostly in parotid glands. The tumors are encapsulated, well circumscribed, but a detailed histopathology examination is mandatory to avoid misdiagnosis.

Streszczenie

Wstęp. Gruczolak łojowy limfatyczny ślinianki przyusznej jest bardzo rzadkim guzem, występującym najczęściej w podeszłym wieku. Jest to nowotwór łagodny, bez wznowy po leczeniu operacyjnym, ale w niektórych przypadkach ulegający transformacji złośliwej w gruczolakoraka łojowego limfatycznego.

Opis przypadku. Przedstawiamy przypadek 51-letniej kobiety z gruczolakiem łojowym limfatycznym lewej ślinianki przyusznej. U pacjentki wykonano badanie ultrasonograficzne oraz biopsję aspiracyjną cienkoigłową (BAC) guza. Wymiary guza 23 x 17 x 17 mm oraz wyniki badań obrazowych sugerowały nowotwór łagodny, prawdopodobnie tumor mixtus, potwierdzony również w BAC.

Dyskusja. Gruczolaki łojowe limfatyczne to guzy otorebkowane, dobrze odgraniczone, zmienne pod względem wielkości i kształtu. Etiologia nie jest do końca wyjaśniona. Może być związana z procesem metaplazji wtórnym do niedrożności przewodów lub z defektem genetycznym.

Podsumowanie. Gruczolaki łojowe limfatyczne to bardzo rzadkie, łagodne guzy występujące najczęściej w śliniance przyusznej. Są otorebkowane, dobrze odgraniczone. Aby uniknąć błędnej diagnozy konieczne jest wykonanie badania histopatologicznego.

Key words: sebaceous lymphadenoma, parotid gland, diagnosis , treatment
Słowa kluczowe: gruczolak łojowy limfatyczny, ślinianka przyuszna, diagnostyka, leczenie

INTRODUCTION

Sebaceous glands are normally present in the parotid or submandibular glands, but primary sebaceous tumors of the salivary glands are very rare.[1,2] The sebaceous gland tumors were first described in the submandibular gland and in the parotid gland.[3] It is difficult to confirm the salivary origin of sebaceous tumors in minor salivary glands.[4,5,6] Sebaceous differentiation has been reported in 11% and 28% of normal parotid glands, and in 6% of normal submandibular glands.[2] This type of tumor very rarely occurs in the sublingual gland.[2,7] Sebaceous lymphadenoma of the parotid gland was reported in less than 0,2% of salivary gland neoplasms.[1] In the present paper, we report a case of 51-year-old woman with a sebaceous lymphadenoma of the left parotid gland.

CASE REPORT

A 51-year-old woman (No. of medical record 00317/09), was admitted to the Department of Otolaryngology Collegium Medicum in Bydgoszcz for surgical treatment of a tumor of the left parotid gland. The tumor has been noticed about 1 year before the patient visited a clinician and diagnosis was given 3 months before present admission to our clinic. The lesion started to expand and it became painful. The patient had an ultrasound scan of parotids done and fine-needle aspiration biopsy (FNAC) of the tumor of the left parotid gland before admission to the clinic was also performed. The ultrasonography showed solid tumor with smooth outline in the bottom pole of the left parotid gland hypoechogenic. The dimensions were 23 x 17 x of 17 mm. The images suggested a benign tumor, possibly tumor mixtus. Apart from that, salivary glands on both sides were normoechogenic and without visible focal changes.

The FNAC examination no. 3490 revealed numerous, benign lymphocytes and single benign elements of the salivary gland. Patient's previous medical history revealed the right ovariotomy because of the cyst (1979) and surgery for the carpal tunnel syndrome on both sides (2007). Physical examination of the left parotid gland disclosed a tumor 2x2 cm in diameter. It was located in lower pole with smooth contours and limited mobility, and with slight tenderness during palpation. The skin above the tumor was unchanged and moveable. The other parts of the head and neck examination were normal, and there was no evidence of cervical lymphoadenopathy. Both facial nerves were functionally intact. Results of the laboratory tests were in normal range values. The patient was prepared for the surgical removal of the lesion. A left superficial parotidectomy was performed without any further complications. Intraoperative histopathology showed a lymphocytic inflammatory process (no. 2153). Postoperative histopathological report no. 2181 showed a tumor size 2.5 x 1.8 x 1 cm with diagnosis of : Lymphadenoma sebaceum.

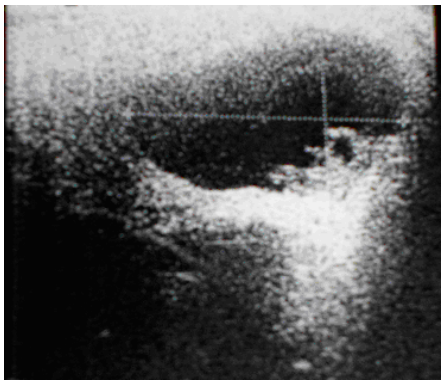

Fig. 1. *The ultrasonography: hypoechogenic, solid tumor smooth outline, dimensions 23 x 17 x of 17 mm of the left parotid gland*

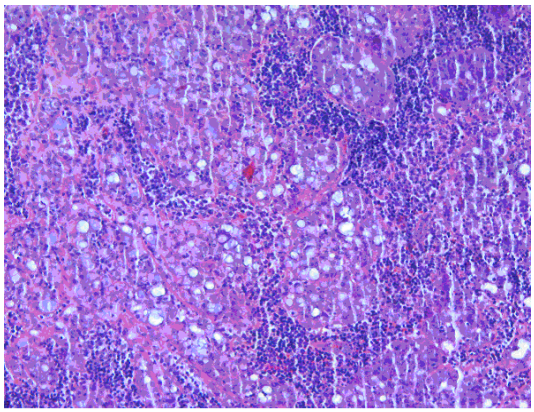

Fig. 2. *Sebaceous lymphadenoma primary objective magnification. Variably sized and shaped nests of sebaceous cells, sebaceous glands and salivary ducts with minimal atypia surrounded by a background of lymphocytes and lymphoid follicles HE 10X*

The patient was discharged home on the 7th day after the operation without complications. A follow-up 5 months later revealed no recurrence.

DISCUSSION

Sebaceous lymphadenomas are encapsulated, well circumscribed benign tumors of variably sized and shaped nests of sebaceous cells. There are sebaceous glands and salivary ducts with minimal atypia surrounded by lymphocytes and lymphoid follicles in background [2, 8]. This type of tumor has no tendency to invade local structures and there are no recurrences after surgery [2]. The origin of sebaceous differentiation in salivary glands is unknown. Sebaceous foci might result from a metaplastic process secondary to ductal obstruction from a tumor or inflammation [2]. Also, it can be congenital in origin, or a naturally developed process stimulated by the same hormonal factors that induce the development of sebaceous glands elsewhere [1, 2]. Most oftumors (90%) are diagnosed in the 6 – 8th decades and nearly all arise in the parotid gland or around it.[1] Since a sebaceous lymphadenoma is a benign tumor, its malignant counterpart is sebaceous lymphadenocarcinoma which is extremely rarely diagnosed [2, 9]. In the later, lesion is composed of areas of sebaceous lymphadenoma with intermixed regions of carcinoma of salivary ducts as a "collision" or incidental tumors [2, 8]. Although there are some reports on such tumors, the origin of sebaceous lymphadenoma and sebaceous lymphadenocarcinoma is controversial. The salivary gland inclusions in lymph nodes seem to be an initiation of the disease [1, 2]. The demonstration of germinal centers, subcapsular margined sinuses, and sebaceous lymphadenoma within adjacent normal lymph node strongly suggests that Warthin tumors, sebaceous lymphadenoma and sebaceous lymphadenocarcinoma all arise in ectopic salivary gland tissue in lymph nodes [2]. Other hypotheses suggested the origin of the sebaceous lymphadenoma as derivation from branchial cleft remnants or from sebaceous gland lymphoid inclusions in periparotideal lymph nodes [1]. Sebaceous lymphadenomas are benign tumors that will not recur if they are adequately excised. The standard treatment in such cases is parotidectomy.

SUMMARY

Sebaceous lymphadenomas are very rare benign tumors mostly occurring in parotid glands. The tumors are encapsulated, well circumscribed, but a detailed histopathology examination is mandatory to avoid misdiagnosis.

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