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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

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ORIGINAL PAPER

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Polymorphic variants in the *DLX1* gene and the risk of non-syndromic cleft lip with or without cleft palate

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ABSTRACT

Introduction. Non-syndromic cleft lip with or without cleft palate (NSCL/P) is a common developmental anomaly, which etiology is complex and not completely elucidated. Therefore, the aim of the present study was to evaluate whether common polymorphisms in the distal-less homeobox gene 1 (*DLX1*) may contribute to the risk of orofacial clefts in the Polish population.

Materials and Methods. Five single nucleotide variants were genotyped using high-resolution melting curve analysis in a group of 278 patients with NSCL/P and properly matched controls (n = 574).

Results. Statistical analysis revealed that two variants located in the 3' untranslated region of DLX1, rs788172 and rs788173, were associated with a decreased risk of NSCL/P (p_{trend} = 0.041 and p_{trend} = 0.025, respectively). The allelic frequencies for these polymorphisms were significantly lower in patients compared to healthy individuals (p = 0.040 and p = 0.024, respectively). However, all these results did not remain statistically significant after applying the Bonferroni correction for multiple comparisons. The results of single-marker analysis for *DLX1* were confirmed by haplotype analysis. The best evidence of the haplotype association with the risk of NSCL/P was observed for the T-G-G haplotype consisting of rs1047889, rs788172 and rs788173 major alleles. This high risk haplotype was more frequent among cases than controls (p = 0.013, $p_{corrected}$ = 0.026).

Conclusions. We found evidence for the association between *DLX1* gene variants and the risk of NSCL/P in the Polish population. To confirm our preliminary findings further, larger sample size studies are required.

Keywords: NSCL/P; *DLX1*; polymorphism; haplotype.

Introduction

Non-syndromic cleft lip with or without cleft palate (NSCL/P, OMIM %1195130) is one of the most common craniofacial abnormalities. The frequency of NSCL/P varies widely across different regions of the world and depends on racial and ethnic background, and socioeconomic status [1, 2]. The birth prevalence of this malformation in European-derived populations is about 1/1000 live births [3]. Orofacial clefts represents a significant public health burden since their treatment requires long-term and multidisciplinary management strategies. In addition, patients with this developmental anomaly may have a higher incidence of psychological disorders and cancer [4, 5]. Zhu *et al.* have shown that also parents having children with orofacial clefts have an increased risk of developing lymphomas or leukemia [6].

The etiology of NSCL/P is complex and multifactorial, involving the integration of a number of genetic and environmental risk factors [1, 7]. In addition, there are reports showing that maternal genetic and nutritional factors can also be implicated in the pathogenesis of this birth anomaly [8]. Despite a number of molecular and epidemiological studies with different study designs, a definitive statement about causes of NSCL/P still remains obscure. The candidate genes and chromosomal loci identified so far can explain only a fraction of the genetic component of orofacial clefts. The most consistent findings across studies include the association of polymorphic variants of the *IRF6* gene (OMIM *607199) and 8q24.21 locus with the increased risk of NSCL/P [1]. It has been assessed that *IRF6* gene variants may be responsible for about 12% of the genetic contribution to NSCL/P at the population level [9]. In the Polish population, the functional promoter *IRF6* polymorphism (rs642961) and the 8q24.21 gene desert variant (rs987525) have been shown to be associated with an almost two-fold increase in the risk of this developmental defect [10].

One of the approaches to search for new candidate genes in the etiology of NSCL/P is the analysis of phenotypes manifest in mice carrying null mutations (knock-out mice) of genes expressed during craniofacial development [11]. It has been shown that *Dlx1/Dlx2* double homozygous null mice have fully penetrant cleft of the secondary palate and agenesis of upper molar teeth [12, 13]. The cleft palate in these mutants is the result of reduced mesenchymal cell proliferation at the initial stages of palatogenesis and severely deficient growth of the posterior palate [13]. Furthermore, mice lacking both *Dlx1* and *Dlx2* die at birth with multiple defects including abnormal development of forebrain [14]. The *Dlx1* and *Dlx2* belong to a highly conserved family of distal-less homeobox genes encoding homeodomain transcription factors, which play crucial roles in various aspects of embryogenesis [15]. It is worth noting that a polymorphic variant located in the 3' untranslated region (3' UTR) of the human *DLX1* gene (OMIM *600029) has been found to be associated with the risk of NSCLP in the Brazilian population [16]. Therefore the aim of the present study was to evaluate whether common nucleotide variants in the *DLX1* locus may contribute to the risk of orofacial clefts also in the Polish population. Five known single nucleotide polymorphisms (SNPs) were selected and analyzed in a group of patients with NSCL/P and properly matched control samples.

Materials and Methods

Study population

The study included 278 patients (57% males) with a diagnosis of NSCL/P recruited from the Department of Pediatrics at the Institute of Mother and Child in Warsaw, the Department of Plastic Surgery at the Specialist Medical Center in Polanica Zdroj and from the Department and Clinic of Dental Surgery at the Poznan University of Medical Sciences. The patient group comprised 238 (86%) individuals with non-syndromic cleft lip with cleft palate (CLP) and 40 (14%) individuals with non-syndromic cleft lip only (CL). Patients with cleft palate only (CPO) were excluded from the study prior to genotyping. Case eligibility was ascertained by clinicians using detailed diagnostic information from medical records. The control group was composed of 574 healthy individuals (50% males) with no family history of clefting or other congenital birth defects. All study participants were unrelated Caucasians of Polish origin. The study was performed according to the rules of the Ethics Committee of the Poznan University of Medical Sciences, Poland. Written and oral consent was obtained from all participants or their legal guardians.

SNP selection and genotyping

SNPs in the DLX1 locus were identified from public databases such as the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the 1000 Genomes Browser (http://browser.1000genomes.org/index.html), and related literature. A final set of five SNPs was selected based on functional significance, gene-linkage disequilibrium (LD) patterns and association with NSCL/P in previous association studies. LD patterns and the structure of haplotype blocks across the DLX1 locus were determined using genotype data from the HapMap database (http://hapmap.ncbi.nlm.nih.gov/) and the Haploview 4.0 software (http://www.broad.mit.edu/mpg/haploview/). Characteristics of those SNPs that were finally selected are presented in **Table 1** and **Figure 1**. Genomic DNA for molecular analyses was isolated from peripheral blood lymphocytes by a standard salt-out extraction procedure. Genotyping was conducted by high-resolution melting curve analysis (HRM) on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany).

Quality control was ensured by including 10% of the samples as duplicates. Samples that failed genotyping were removed from statistical calculations.

Table 1. Characteristics of polymorphisms genotyped in the *DLX1* locus

rs no.	Location[a]	Alleles[b]	SNP function[c]	MAF[d]
rs1047889	chr2:172946527	C / T	N/A (upstream)	0.47
rs788172	chr2:172953438	A / G	UTR-3	0.35
rs788173	chr2:172953460	A / G	UTR-3	0.35
rs10186317	chr2:172955011	A / G	N/A (downstream)	0.31
rs13390848	chr2:172957643	G / T	N/A (downstream)	0.16

[a] NCBI build 37 / hg19.
[b] Underline denotes the minor allele (based on whole sample).
[c] According to the Single Nucleotide Polymorphism database (dbSNP).
[d] MAF, minor allele frequency calculated from the control samples.

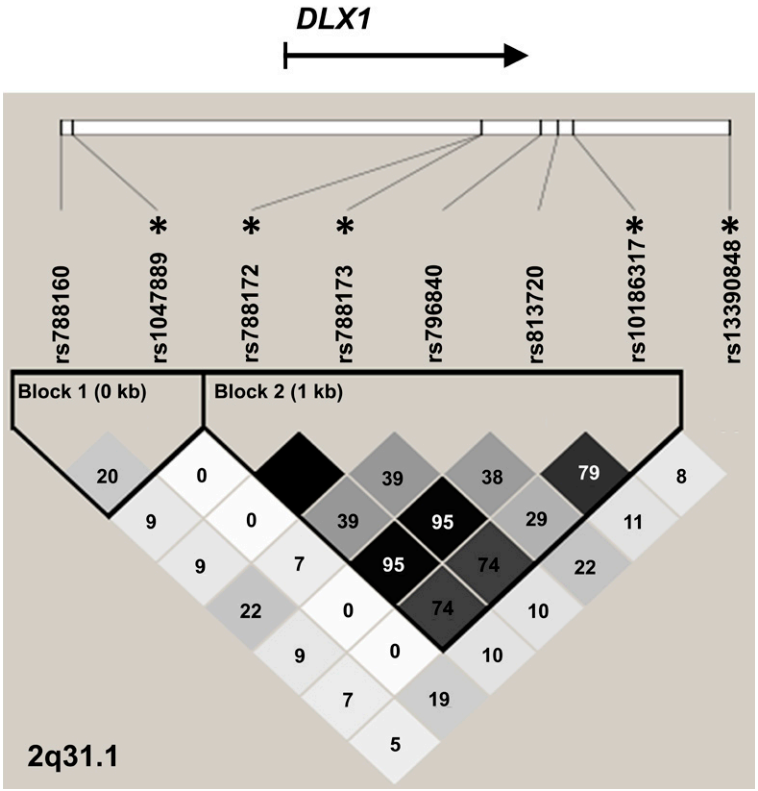

Figure 1. The Linkage Disequilibrium (LD) plot of HapMap SNPs within the DLX1 locus. The plot was generated using the genotype data from HapMap CEU samples and the Haploview 4.0 software (Broad Institute, Cambridge, MA). The examined SNPs are marked with an asterisk (*). The numbers in the squares denote r2 values expressed as a percentage of maximal value (1.0). Square without number corresponds to r2 = 1.0. A black-to-white gradient shows highest (1.0) to lowest (0.0) r2

Statistical analysis

Each SNP was tested for deviation from the Hardy-Weinberg equilibrium (HWE) in both patients and controls using the chi-square (χ^2) test. The differences in allele and genotype frequencies between cases and controls were determined using standard χ^2 or Fisher exact tests. SNPs were tested for association with NSCL/P using the Cochran-Armitage trend test. Odds Ratios (ORs) with 95% Confidence Intervals (95%CIs) were used to assess the strength of the association. The dominant and recessive models were analyzed. The Bonferroni correction was applied to account for multiple comparisons, and the p-values < 0.01 (0.05/5 SNPs) were interpreted as statistically significant. Statistical calculations were performed for the overall phenotype NSCL/P and the NSCLP and NSCL subgroups. The haplotype-based association analysis using a sliding window approach was conducted using Haploview 4.0 software. Significant p values were corrected using the 1,000-fold permutation test.

Results

Single-marker association analysis

None of the tested polymorphisms showed significant deviation from HWE in either NSCL/P patients or healthy individuals (p > 0.05). In controls, the minor allele frequency (MAF) for analyzed nucleotide variants was at least 16%. Statistical analysis revealed that two 3'UTR variants, rs788172 and rs788173, were associated with the risk of NSCL/P (p_{trend} = 0.041 and p_{trend} = 0.025, respectively; **Table 2**). The allelic frequencies of these polymorphisms were significantly lower in affected individuals compared to controls (p = 0.040 and p = 0.024, respectively). The rs788172 and rs788173 were in perfect LD with each other: r^2 = 1.00 and D' = 1.00 (**Figure 1**). Under an assumption of a dominant inheritance, the calculated ORs for these variants were 0.77 (95%CI: 0.579 – 1.030, p = 0.079) and 0.753 (0.564 – 1.005, p = 0.053), respectively. Statistical analysis conducted in the NSCLP subgroup of patients revealed similar results, with the most significant asso-

Table 2. Association of *DLX1* polymorphisms with the risk of NSCL/P

		Genotype distributionb								
		MAF					Dominant modelc		Recessive modeld	
rs no.	Allelesa	Cases	Controls	ptrend	pallelic	pgeno	OR (95% CI)	p-value	OR (95% CI)	p-value
rs1047889	C / T	46 / 143 / 87	129 / 287 / 158	0.055	0.058	0.122	0.825 (0.603–1.129)	0.229	0.690 (0.475–1.000)	0.050
		0.43	0.47							
rs788172	A / G	25 / 116 / 135	73 / 257 / 244	0.041	0.040	0.123	0.772 (0.579–1.030)	0.079	0.684 (0.423–1.104)	0.118
		0.30	0.35							
rs788173	A / G	24 / 115 / 136	73 / 257 / 243	0.025	0.024	0.080	0.753 (0.564–1.005)	0.053	0.655 (0.403–1.064)	0.086
		0.30	0.35							
rs10186317	A / G	22 / 105 / 143	59 / 234 / 279	0.191	0.183	0.423	0.846 (0.633–1.130)	0.257	0.771 (0.462–1.288)	0.320
		0.28	0.31							
rs13390848	G / T	12 / 78 / 188	16 / 149 / 409	0.188	0.180	0.369	1.187 (0.871–1.617)	0.278	1.573 (0.734–3.374)	0.241
		0.18	0.16							

The p-values < 0.010 (0.05 / 5 SNPs) were interpreted as statistically significant.
[a] Underline denotes the minor allele (based on whole sample).
[b] The order of genotypes: dd / Dd / DD (d is the minor allele).
[c] Dominant model: dd + Dd vs DD (d is the minor allele).
[d] Recessive model: dd vs Dd + DD (d is the minor allele).
MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

Table 3. Association of *DLX1* polymorphisms with the risk of NSCLP and NSCL

				ORdominant[b]	ORrecessive[c]
SNP	Alleles[a]	Subgroup	ptrend	(95%CI)	(95%CI)
rs1047889	C / T	ALL	0.055	0.825 (0.603–1.129)	0.690 (0.475–1.000)
		NSCLP	0.069	0.848 (0.609–1.181)	0.662 (0.444–0.986)
		NSCL	0.390	0.705 (0.359–1.386)	0.862 (0.388–1.918)
rs788172	A / G	ALL	0.041	0.772 (0.579–1.030)	0.684 (0.423–1.104)
		NSCLP	0.054	0.764 (0.564–1.037)	0.706 (0.427–1.167)
		NSCL	0.357	0.817 (0.430–1.554)	0.557 (0.167–1.852)
rs788173	A / G	ALL	0.025	0.753 (0.564–1.005)	0.655 (0.403–1.064)
		NSCLP	0.032	0.743 (0.548–1.007)	0.672 (0.403–1.121)
		NSCL	0.351	0.814 (0.428–1.547)	0.555 (0.167–1.848)
rs10186317	A / G	ALL	0.191	0.846 (0.633–1.130)	0.771 (0.462–1.288)
		NSCLP	0.188	0.843 (0.620–1.145)	0.738 (0.425–1.282)
		NSCL	0.711	0.862 (0.453–1.637)	0.966 (0.332–2.810)
rs13390848	G / T	ALL	0.188	1.187 (0.871–1.617)	1.573 (0.734–3.374)
		NSCLP	0.087	1.255 (0.908–1.736)	1.852 (0.862–3.977)
		NSCL	0.439	0.826 (0.395–1.729)	0.418 (0.025–7.097)

The p-values < 0.010 (0.05 / 5 SNPs) were interpreted as statistically significant.
[a] Underline denotes the minor allele (based on whole sample).
[b] Dominant model: dd + Dd vs DD (d is the risk allele).
[c] Recessive model: dd vs Dd + DD (d is the risk allele).
OR, Odds Ratio; CI, confidence interval.

ciation with NSCLP found for the rs788173 variant (p_{trend} = 0.032, **Table 3**). However, all the results of single-marker association analysis were not statistically significant after the Bonferroni correction (p > 0.01).

Haplotype analysis

Haplotype analysis of nucleotide variants in the *DLX1* locus revealed 2- and 3-marker haplotypes associated with the risk of NSCL/P (**Table 4**). These results, consistent with results of single-marker analysis, remained statistically significant even after applying the permutation-based correction (p < 0.05). The best evidence of the haplotype association with the risk of NSCL/P was observed for the T-G-G haplotype consisting of rs1047889, rs788172 and rs788173 major alleles. This high risk haplotype was more frequent among cases than controls (p = 0.013, $p_{corrected}$ = 0.026).

Discussion

The *Dlx* family of homeobox genes is crucial for embryonic development in both invertebrates and vertebrates [16, 17]. In mammals, there are six *Dlx* genes arranged

Table 4. Haplotype analysis of SNPs genotyped in the *DLX1* locus

Polymorphisms	Haplotypes	Frequency	Case, Control Ratios	Chi square	p-value	pcorrected-value[a]
2-marker window						
rs1047889_rs788172	**T-G**	**0.362**	**0.402, 0.343**	**5.690**	**0.017**	**0.032**
	C-G	0.303	0.297, 0.306	0.147	0.701	0.975
	T-A	0.179	0.172, 0.182	0.277	0.599	0.918
	C-A	0.156	0.129, 0.169	4.492	0.034	0.069
rs788172_rs788173	**G-G**	**0.665**	**0.701, 0.648**	**4.656**	**0.031**	**0.039**
	A-A	**0.335**	**0.299, 0.352**	**4.656**	**0.031**	**0.039**
rs788173_rs10186317	G-A	0.661	0.697, 0.644	4.601	0.032	0.080
	A-G	0.292	0.265, 0.304	2.745	0.098	0.307
	A-A	0.042	0.030, 0.048	2.827	0.093	0.297
rs10186317_rs13390848	A-G	0.536	0.540, 0.534	0.041	0.839	0.980
	G-G	0.298	0.276, 0.308	1.796	0.180	0.368
	A-T	0.166	0.184, 0.158	1.886	0.170	0.361
3-marker window						
rs1047889_rs788172_rs788173	**T-G-G**	**0.362**	**0.403, 0.342**	**6.118**	**0.013**	**0.026**
	C-G-G	0.304	0.298, 0.307	0.137	0.711	0.978
	T-A-A	0.179	0.171, 0.183	0.341	0.560	0.918
	C-A-A	0.156	0.128, 0.169	4.804	0.028	0.060
rs788172_rs788173_rs10186317	G-G-A	0.661	0.695, 0.645	4.273	0.039	0.111
	A-A-G	0.293	0.269, 0.304	2.270	0.132	0.310
	A-A-A	0.042	0.030, 0.048	2.754	0.097	0.254
rs788173_rs10186317_rs13390848	G-A-G	0.495	0.512, 0.487	0.987	0.321	0.824
	A-G-G	0.292	0.266, 0.304	2.709	0.100	0.348
	G-A-T	0.166	0.184, 0.158	1.886	0.170	0.458
	A-A-G	0.042	0.030, 0.048	2.791	0.095	0.346
4-marker window						
rs1047889_rs788172_rs788173_rs10186317	T-G-G-A	0.362	0.403, 0.342	6.026	0.014	0.062
	C-G-G-A	0.300	0.295, 0.303	0.110	0.740	1.000
	T-A-A-G	0.153	0.153, 0.153	0.000	0.992	1.000
	C-A-A-G	0.139	0.115, 0.151	4.061	0.044	0.129
	T-A-A-A	0.025	0.017, 0.029	2.392	0.122	0.472
	C-A-A-A	0.017	0.014, 0.018	0.465	0.496	0.965
rs788172_rs788173_rs10186317_rs13390848	G-G-A-G	0.495	0.511, 0.488	0.786	0.375	0.848
	A-A-G-G	0.293	0.269, 0.304	2.270	0.132	0.418
	G-G-A-T	0.166	0.185, 0.157	2.066	0.151	0.457
	A-A-A-G	0.042	0.030, 0.048	2.754	0.097	0.338

Statistically significant results are highlighted in bold font.
[a] p value calculated using permutation test and a total of 1,000 permutations.

as three sets of linked gene pairs: *Dlx2* and *Dlx1*, *Dlx5* and *Dlx6*, and *Dlx3* and *Dlx7* [18]. All *Dlx* genes encode DNA-binding regulators that control large number of downstream effector genes. They are implicated in patterning and development of the brain, craniofacial structures and the axial and appendicular skeleton [15, 17]. Mouse model studies showed that *Dlx1* and *Dlx2* activities are critical for the initial outgrowth of the palatal shelves in a region-specific manner, and are essential for the normal expression of other significant regulators of palate development [13]. Therefore the purpose of this study was to evaluate the association between common nucleotide variants in the *DLX1* locus and the risk of orofacial clefts in the Polish population. Molecular analyses were conducted in patients with non-syndromic forms of cleft lip with cleft palate and cleft lip only. Patients with cleft palate only were excluded from the study due to distinct etiology of this subtype of oral clefts [19].

Statistical analysis of genotyping results revealed that two nucleotide variants located in the 3'UTR of the *DLX1* gene were associated with the risk of NSCL/P in a tested group of patients. The data showed that rs788172 and rs788173 minor alleles are more frequent among controls than among affected individuals. We found that variant allele carriers at rs788172 and rs788173 have a 1.3-fold decreased risk of NSCL/P. However, all these results were not statistically significant after applying the correction for multiple comparisons. In order to adjust for multiple testing, we employed the Bonferroni correction, which is the most conservative approach to control for false positives and may lead to the underestimation of weak and moderate genetic effects [20]. It is worth noting that the results of single-marker analysis for *DLX1* were confirmed by haplotype analysis. We found that 2- and 3-marker haplotypes containing the rs788172 and rs788173 variants are associated with the risk of NSCL/P. These results remained statistically significant even after applying the permutation-based correction. Similar results have been obtained by Saboia *et al.* in the Brazilian population, where the rs788173 polymorphism was associated with the risk of NSCLP subphenotype [16]. They have demonstrated that the A allele of this 3'UTR variant is undertransmitted (protective allele) from heterozygote parents to their affected offspring [16]. In the Brazilian study, however, only one marker in the *DLX1* gene was tested for association with orofacial clefts [16]. Interestingly, the common alleles of *DLX1* rs788172 and rs788173 were shown to be significantly correlated with autism [21].

There is an evidence that *Dlx1* and *Dlx2* regulate the initiation of palatogenesis through promoting proliferation of mesenchyme in the posterior palate independently of Sonic hedgehog (Shh) signaling [13]. The loss of *Dlx1* and *Dlx2* function results in down-regulation of a signaling loop involving Shh, Bone morphogenetic protein 4 (Bmp4) and Fibroblast growth factor 10 (Fgf10), which is important for cell proliferation in the epithelium of the middle palate around E13.5 [13]. In addition *Dlx1* and *Dlx2* activity is required for normal expression of several transcription factor genes, including *Lhx6*, *Barx1*, *Sim2*, *Osr1* and *Osr2*, which mutations result in defects of palate growth and morphogenesis [13, 22–24]. In humans, mutations and polymorphisms of *BMP4* (OMIM *112262) and *FGF10* (OMIM *602115) have been found to increase the risk orofacial clefts in various populations [25–28]. It has been suggested that the FGF signaling pathway may contribute to as many as 3 to 5% of NSCLP [25].

The present study has certain limitations including (a) the number of selected nucleotide variants that do not cover the *DLX1* gene fully and extensively, (b) the lack of information about functional relevance of tested SNPs (c) the relatively small number of available NSCL/P patients and healthy individuals and (d) the lack of association analysis of polymorphisms in the *DLX2* gene (OMIM *126255), which is located head-to-head with *DLX1* on chromosome 2q32. It has been demonstrated that *Dlx1* and *Dlx2* have functionally redundant roles during the embryonic development. The *Dlx1/Dlx2* double mutant mouse embryos had a cleft of the secondary palate, also seen in 80% of the *Dlx2-/-* embryos, while only 10% of *Dlx1-/-* embryos had a mild cleft palate [12]. In addition, further studies should focus not only on analysis of common variants in *DLX1* and *DLX2*, but also on identification of rare variants and etiological mutations of these genes in patients with orofacial clefts. Recently, a novel single nucleotide deletion in the *DLX4* gene was described in a family with bilateral CL/P and minor dysmorphic features [29]. In addition, craniofacial abnormalities, including cleft palate, are common clinical features observed in patients with 2q31.1 microdeletions encompassing both *DLX1* and *DLX2* [30, 31].

In conclusion, our case-control study contributes to a better understanding of the role of genetic factors in the etiology of orofacial clefts. We found evidence for the association between *DLX1* gene variants and the risk of NSCL/P in the Polish population. To confirm our findings further, larger sample size studies in different populations are required.

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Conflict of interest statement
The authors declare no conflict of interest.

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Evaluation of nutritional habits and the body mass index (bmi) of students of the University of the Third Age at the Koszalin University of Technology

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ABSTRACT

Introduction. Nutrition plays an important role in the elderly stage of life. A proper proportion of the individual nutritional ingredients in a diet may positively impact the ageing body. This positive influence consists in slowing down the undesired and unfavourable physiological alterations leading inevitably to the general weakness of the body.
Aim. The aim of the study was to evaluate the nutritional habits and body mass indexes (BMI) of students of the University of the Third Age at the Koszalin University of Technology.
Materials and Methods. A total of 189 people (34 men and 155 women) took part in the study in 2014, which involved a diagnostic survey and an evaluation of the nutritional state based on BMI.
Results. Approximately 3/4 of the respondents were found to be overweight or obese. A large majority of them viewed their health status as good. The majority (70%) declared regularly eating 3–4 meals. Half of the respondents admitted eating snacks between meals. The majority of the respondents did not control the amount of calories consumed, but they reduced the consumption of cholesterol. About 3/4 of the respondents ate wholemeal bread and spread butter over it. They mainly used all-purpose oil for frying. About half of the respondents ate unprocessed fruit and vegetables every day and only 1/3 of them consumed milk and dairy products every day. About 3/4 of the respondents ate fish once a week. About 1/3 of the males and nearly half of the females ate vitamin and/or mineral supplements. About 3/4 of the respondents performed some physical exercise several times a week. Male and female respondents reported eating snacks between meals to a different extent. It was also found that the educational background significantly differentiated the amount of fruit and vegetables consumed as well as the time of the last meal of the day. The place of residence also significantly differentiates the number of meals and the extent to which the consumption of cholesterol is reduced. The respondents' financial situation was also found to differentiate the form in which fruit are eaten.
Conclusions. The findings suggest increased control of the amount of calories consumed, increased consumption of fruit and vegetables, dairy products and fish as well as the need for nutritional education.

Keywords: evaluation of nutritional habits; elderly people; BMI.

Introduction

The disadvantageous demographic situation which was caused by a smaller number of births and a longer average life expectancy is the reason for the inevitable ageing of society [1]. As a result, health care providers are showing an increased interest in that population group [2]. The elderly are a heterogeneous group due to their health status [3, 4]. Some of them, with good physical and intellectual capacities, prevent their own social

exclusion by creating associations for seniors. These persons undertake social, cultural or intellectual activities in such groups. The Universities of the Third Age are one of such organizations providing educational services and satisfying the psychosocial and health needs of students [5]. However, the advancing physiological changes as a person becomes older are the reason for physical and mental problems [6–8, 3]. The physical problems include, for instance, a decreasing resistance of the body to chronic diseases. The mental problems are mainly advancing neurodegenerative diseases leading to isolation and social exclusion of persons suffering from them. The lifestyle has a significant impact on health. It includes, for instance, physical activity and nutrition [9, 10]. However, it should be borne in mind that advancing physiological changes modify body's nutritional needs as the person becomes older [11], whereas improper nutrition leads to the occurrence of the so-called diet-related diseases [12]. The frequently coexistent health problems combined with the poor financial condition of the elderly most often lead to an unbalanced diet and result in deficiencies of certain nutrients [13]. The knowledge the elderly possess about nutrition, which is most frequently out of date since it comes from the time they were young or was obtained from not entirely reliable sources such as glossy magazines or TV advertisements, encourages multi-layer activities aimed at nurturing proper nutrition habits [9, 14]. Those activities must be based on proper research in the scope of the nutritional status and diets in order to enable the prevention of irregularities which may occur [7].

Aim

The aim of the study was to evaluate the nutritional habits and body mass indexes (BMI) of students of the University of the Third Age at the Koszalin University of Technology using a diagnostic survey method and anthropometric measurements.

Materials and Methods

189 people, including 34 men and 155 women, took part in the study. Evaluation of the eating habits among students of the University of the Third Age of the Koszalin University of Technology was based on data obtained from an anonymous original questionnaire using a diagnostic survey conducted in 2014.

The questionnaire comprised 22 questions divided into 4 parts. The first part concerned an evaluation of the socio-economic and health status. The respondents were asked about their educational level, place of residence and to provide a self-evaluation of their financial status and health status. The second part of the questionnaire concerned general eating habits. The respondents were asked about the number of meals eaten during the day, eating snacks between meals, the regularity of eating the meals, the time of the last meal and how well they controlled the amount of calories and cholesterol consumed. The third part concerned the frequency of consumption of specific groups of products. The respondents were asked about the type of bread they eat, the type of fat used to spread on bread, the type of fat used for frying, the frequency of eating fruit and vegetables and the form in which they are eaten, the frequency of consuming milk and dairy products and the frequency and form of eating fish. Part Four of the questionnaire concerned dietary supplements and a self-assessment of the level of physical activity. The respondents were asked to declare the type of supplementation used and to self-assess the level of their physical activities. The results were expressed as absolute figures – the number of respondent's specific behaviours, and in relative indexes – the percentage of respondents (%) who declared a specific behaviour. Filling in the questionnaire was preceded by a detailed discussion of the way in which the answers should be given, and the person responsible for the study was present all the time in the room to answer any questions. The body mass index (BMI) of the group of students was determined using the basic anthropometric parameters (body height and weight). The data was used to calculate Quetelet's Body Mass Index: [body weight (kg)]:[body height B-v (m^2)]. The body height and weight were measured on a certified medical scale with a height meter and the waist circumference measured with a tailor's measuring tape. The anthropometric measurements were conducted in a student outpatient clinic in accordance with the rules adopted in anthropometry [15]. The BMI was interpreted according to the WHO guidelines [16]. The statistical significance of the differences of the anthropometric parameters evaluated in the study was assessed with the U Mann-Whitney test. The relationship between the sex, education level and self-assessment of the financial situation and general nutritional habits of the respondents as well as the frequency and form of eating specific groups of food was determined by an x^2 test [17]. The levels of significance of $\alpha = 0.05$ and $\alpha = 0.01$ were adopted throughout.

Results

The data for the average age, waistline circumference, height, body weight and body mass index (BMI) of the students are given in **Table 1**. A detailed analysis of the waistline circumference and the BMI of the students are

presented in **Table 2**. As the data indicates, the average age of the respondents was about 67 years. The average height was 1.63 m, and the average body weight was approx. 70 kg. In analysing the data by gender, it is clear that the men were taller and heavier than the women. When the waistline measurements were analysed in a detailed manner, it was shown that approximately 40% of the assessed students were at risk of metabolic derangements and complications. The statistical analysis revealed that height, weight and waist circuference significantly distinguished the males from the females ($p < 0.05$). The BMI values, when analysed specifically, demonstrated that the problem of quantitative malnourishment almost did not affect this group of people, although it was found that about 63% of the students were overweight or obese. Considering the gender, it was shown that obesity and overweight were more common among the men than the women.

As **Table 3** shows, the group of men and women under evaluation were mostly well-educated city resi-

Table 1. Characteristics of the tested students

Parameters	Men n = 34					Women n = 155					p
	$\overline{X}$	SD	Min.	Max.	Med.	$\overline{X}$	SD	Min.	Max.	Med.	
Age (years)	69.44	6.03	55.00	81.00	70.00	66.70	4.95	56.00	81.00	66.00	$p > 0.05$
Body height (m)	1.72	0.07	1.56	1.88	1.72	1.61	0.05	1.5	1.76	1.61	$p < 0.05$
Body weight (kg)	80.00	9.8	59.00	100.00	81.00	68.15	10.20	43.00	98.00	68.00	$p < 0.05$
Waist circuference (cm)	93.38	9.56	74.00	110.00	92.00	83.35	11.12	60.00	120.00	80.00	$p < 0.05$
BMI (kg/m^2)	26.96	3.32	22.23	35.25	26.15	26.07	3.64	18.13	36.89	25.71	$p > 0.05$

Table 2. A detailed analysis of the waistline circumference and the BMI of the students

	Men n = 34		Women n = 155	
	n	%	n	%
Waist circuference ⩾ 80*/ ⩾ 94**	14	41.18	58	37.42
BMI (< 18.5)	-	-	1	0.64
BMI (18.5–24.99)	10	29.52	59	38.06
BMI (25–29.99)	17	50.00	70	45.16
BMI (30–34.99)	6	17.54	22	14.19
BMI (35–39.99)	1	2.94	3	1.95
BMI (> 40)	-	-	-	-

* for women; ** for men

Table 3. Socio-economic and health status

	Men		Women	
Education	n	%	n	%
Basic	1	2.94	2	1.29
Vocational	2	5.88	4	2.58
Secondary	14	41.18	92	59.35
Higher	17	50.00	57	36.78
Place of residence				
City	32	94.12	148	95.48
Village	2	5.88	7	4.52
Financial position				
Bad	3	8.82	2	1.29
Average	11	32.36	92	59.35
Good	17	50.00	59	38.07
Very good	3	8.82	2	1.29
The health status by self-assessment				
Bad	11	32.36	19	12.26
Good	21	61.76	130	83.87
Very good	2	5.88	6	3.87

Table 4. General feeding behavior

	Men		Women	
Number of meals	n	%	n	%
< 3	-	-	5	3.23
3–4	28	82.35	112	72.25
5 >	6	17.65	38	24.52
Eating between the meals				
Yes	10	29.41	84	54.19
No	24	70.59	71	45.81
Regularity of meals				
Yes	27	79.41	113	72.90
No	7	20.59	42	27.10
Time of last meal (hours)				
18–20	27	79.41	125	80.64
20–22	7	20.59	27	17.42
22–24	-	-	3	1.94
Control over energetic value				
Yes	16	47.06	56	36.13
No	18	52.94	98	63.87
Limiting cholesterol				
Yes	31	91.18	122	78.71
No	3	8.82	33	21.29

dents. Men saw their financial situation as better than women did, while women saw their health status as better than men. An evaluation of the general eating habits has showed (**Table 4**) that they were mostly correct. Only five women did not eat enough meals during the day. It was also worrying that most women ate snacks between meals and that the majority of men and women did not control the amount of calories consumed. The statistical analysis showed that the place of residence significantly affected the statistical number of consumed meals and the limitation of cholesterol intake ($p < 0.05$), sex significantly affected eating snacks between meals ($p < 0.05$) and education significantly affected the time of the last meal of the day ($p < 0.05$).

An assessment of the frequency of consumption of specific groups of foods (**Table 5**) showed that about 3/4 of men and women ate wholemeal bread and they usually spread butter on it. About 3/4 of the respondents declared that they used all-purpose (rapeseed) oil for frying. Only a little more than half of the respondents ate vegetables every day, which was slightly better than their consumption of fruit. However, women declared that they ate fruit every day more often than men. It was also shown that education significantly affected the statistical frequency of consumption of vegetables ($p < 0.05$). Less than 40% of men and women ate milk and dairy products every day. However, the frequency of eating fish was the lowest. The majority of both men and women ate fish once a week. The population under study took vitamin and mineral supplements and about

Table 5. Frequency of consumption of specific groups of products

	Men		Women	
Often bread eating	n	%	n	%
Wholemeal	26	76.47	113	72.90
Ordinary	7	20.59	11	7.10
Don't eat bread	1	2.94	31	20.00
Spreading bakery products				
Butter	17	50.00	112	72.26
Margarine	10	29.41	33	21.29
Don't spread backery	7	20.59	10	6.45
Frying dishes				
Universal oil	25	73.53	106	68.39
Olive oil	8	23.53	42	27.10
Lard	1	2.94	7	4.52
Frequency of eating vegetables				
Every day	18	52.94	81	52.26
A few times per week	15	44.12	67	43.23
Once a week	1	2.94	7	4.52
Never	-	-	-	-
Type of vegetables consumed				
Raw	23	67.65	103	66.45
Prepared	11	32.35	52	33.55
Frequency of eating fruits				
Every day	20	58.82	107	69.03
A few times per week	13	38.24	43	27.74
Once a week	1	2.94	4	2.58
Never	-	-	1	0.65
Type of fruits consumed				
Raw	33	97.06	139	89.68
Prepared	1	2.94	16	10.32
Frequency of eating milk and milk products				
Every day	13	38.24	56	36.13
A few times per week	8	23.53	56	36.13
Once a week	11	32.35	26	16.77
Never	2	5.88	17	10.97
Frequency of eating fish				
Every day	1	2.94	3	1.94
A few times per week	10	29.41	21	13.55
Once a week	21	61.76	120	77.42
Never	2	5.88	11	7.10
Type of fish consumed				
Fatty	21	61.76	84	54.19
Low fat	11	32.35	60	38.71

3/4 of both men and women declared physical activity at least several times a week (**Table 6**).

Discussion

The nutritional habits of elderly people need to be constantly monitored in order to maintain their health. Evaluation of eating habits helps to carry out effective actions to prevent chronic diseases and, in consequence, to improve the quality of life [18].

Table 6. Assessment supplementation and physical activity level

	Men		Women	
Type of supplementation	n	%	n	%
Vitamins and Minerals	12	35.29	73	47.09
Omega-3 fatty acid	9	26.47	38	24.51
Other	2	5.88	9	5.80
Physical activity				
Every day	16	47.06	47	30.32
A few times per week	10	29.41	62	40.00
Once a week	5	14.71	25	16.13
Never	3	8.82	21	13.55

Undernourishment in elderly people results in progressive deterioration of their health and lowering of their quality of life [19]. The anthropometric measurements showed suspected undernourishment in only one female student. A study conducted by Krajewska-Pędzik et al. [20] among female students of the University of the Third Age in Szczecin did not reveal any undernourishment in that group of respondents. However, according to many reports, the rate of undernourishment in elderly people ranges from 5% to 10% [21]. Excessive fatty tissue in elderly people results mainly in hyperlipidaemia, arterial hypertension and type II diabetes [22]. An increase in the waist circumference is positively correlated with nutritional diseases. The risk of metabolic complication increases above 80 cm in women and above 94 cm in men [23]. This study has shown that a waist circumference which indicates a risk of metabolic complications was observed in 38% of the study population. The BMI is also positively correlated with potential nutritional diseases. A study conducted by Krajewska-Pędzik et al. [20] among female students of the University of the Third Age in Szczecin showed – like in students of the UTA at the Koszalin University of Technology – that the BMI was normal in only 1/3 of all students. Obesity in a considerable portion of the elderly population has also been confirmed by Stawarska et al. [24].

The number of meals declared by about 3/4 of students of the UTA at the Koszalin University of Technology indicates correct behaviour which is consistent with recommendations [18]. A study conducted by Krajewska-Pędzik et al. [20] showed that about 90% of female students of the UTA in Szczecin had more than three meals a day. For eating snacks between meals, the study found that men were more disciplined than women. For regularity of eating and the time of the last meal, this habit was correct in a considerable percent of the respondents. A majority of respondents also declared that they controlled the amount of cholesterol consumed. However, a number of studies of the eating habits of elderly people have indicated that an excessive amount of cholesterol is eaten in Poland [25, 26]. In addition, the current study has also shown that respondents should pay more attention to controlling the amount of calories consumed. Appropriate control of the supply of calories may reduce problems with excessive body weight, thereby considerably improving the quality of life [27]. More frequent consumption of wholemeal bread than white bread in the study population indicates that many valuable nutrients are supplied, including dietary fibre, which is particularly beneficial to health [28]. However, a deficit of dietary fibre is frequently observed in the diets of elderly people. This tendency has been confirmed in the studies conducted by Ilow et al. [29] and Stawarska et al. [30]. A study conducted by Różańska et al. [31] showed that the dietary fibre consumed by a selected population of elderly people met only 15% of the daily demand. Despite declarations of controlling the amount of cholesterol consumed, the majority of the respondents chose to spread butter on bread. Only about 1/4 of the respondents declared eating a margarine whose fatty acid profile was much more beneficial and which did not contain cholesterol [32]. The majority of the respondents used an all-purpose (rapeseed) oil for frying, which was characterised by lower oxidative stability, thereby exposing themselves to harmful products of adverse chemical transformations [33]. Vegetables were consumed every day by about half of the respondents, although fruit consumption was slightly higher. Since these products are a source of many bioactive components (antioxidants as well as vitamins and minerals) they should be the main component of an elderly person's diet [34]. A study conducted by Krajewska-Pędzik et al. [20] showed that about 3/4 of students of the UTA in Szczecin ate fruit and vegetables every day. The low consumption of milk and dairy products is worrying. Although production of lactase at an elderly age

decreases, dairy products should be eaten regularly by elderly people. Apart from containing valuable proteins, they are an important source of calcium. Studies of eating habits have shown an adverse tendency in the consumption of this group of products in elderly people. Only 44% reported the daily consumption of milk in the years 1989–2004 [35]. A study conducted by Krajewska-Pędzik et al. [20] showed that only 58% of them declared drinking milk and eating dairy products every day. A study conducted by Myszkowska-Ryciak et al. [36] among students of the UTA in Warsaw also found a low level of calcium consumption. Markiewicz et al. [37] showed that the amount of calcium supplied with the diet did not cover the recommended standards. The low level of fish consumption among students of the UTA at the Koszalin University of Technology reflected the general trend in Poland. Although it is recommended that they should be eaten 1–2 times a week as a source of valuable proteins, fatty acids with beneficial biological properties (n-3) and many vitamins, including vitamin D and minerals, studies have shown that 80% of Poles eat fish once a week or less often [38, 12, 39]. Nearly half of the study population took supplements, especially minerals and vitamins. Kałuża et al. [40] found that elderly people commonly take vitamin and/or mineral supplements. A study conducted by Tokarz et al. [41] on a group of 86 people aged 60–90 years showed that the respondents did not consume most minerals at a safe level. Considering that the absorption rate of nutrients decreases with age, which causes development or exacerbation of many diseases, such supplementation seems justified [42]. Regular exercise by elderly people reduces levels of diabetes, osteoporosis, atherosclerosis, arterial hypertension and brain diseases, such as Alzheimer's disease [43]. It can also significantly delay or reduce the effects of ageing of skeletal muscles [44]. This is why the fact that about 3/4 respondents declared performing some physical exercise at least several times a week should be seen as positive.

Conclusions

1. The high obesity rate among the students of the UTA indicates that it is necessary to take actions to further control of the amount of calories consumed.
2. It is recommended that the consumption frequency of vegetables, fruit, dairy products and fish should be increased in order to eliminate the risk of a deficit of certain nutrients.
3. Education regarding healthy eating should be provided to eliminate nutritional errors.

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Conflict of interest statement
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Mental and social well-being versus physical disability – the diagnosis of the problems

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ABSTRACT

Introduction. Health is most frequently defined as full well-being in the biological, psychological and social sense. A disorder of a person's functioning in any of these spheres triggers changes in the remaining ones. Such destabilization may result in losing the ability to cope with everyday situations.
Aim. The objective of the study was to recognize the self-assessment of the mental well-being, along with the selected elements of the social situation, of young, physically impaired persons living in the countryside.
Materials and Methods. The research was conducted on the population of physically disabled individuals – with legally granted disability class, living in the countryside in the Wielkopolskie province, aged 18–45 years. The applied technique was an interview based on an original questionnaire with 96 questions.
Results. Over a half of the respondents confirmed that their disability affects their mental state. They often suffer from depressed mood, feeling of loneliness, feeling of being a "burden" for others. As many as 41.7% of respondents admitted suicidal thoughts.
Conclusions. The situation of disabled people living in the countryside is particularly difficult. Financial problems, unemployment, limited access to health care negatively influence the mental well-being of young persons, often leading to a depressed mood. In consequence, they often experience suicidal thoughts.

Keywords: mental well-being; social well-being; physical fitness; physical disability.

Introduction

The perception of health affects a person's well-being and their activities of daily life and also shapes their pro-health behavior. Health as a person's both potential and a quality is a disposition enabling them to adaptively function in a specific environment. It is perceived as a developmental-functional category and a resource conditioning comprehensive human development and enabling them to face current challenges. Health is a positive value that should be aimed at. If it occupies a high position in a person's or a social group's hierarchy of values, it is an essential decision-making element in human life, enabling individuals to choose behaviors convergent with their life style [1].

The concept of physical health refers to the body, the biological functioning of the organism and its particular systems. As far as mental health is concerned, it consists of two components. One of them, mental health, is defined as the ability to clearly and coherently think, learn and implement one's intellectual potential. The other component, emotional health, refers to the ability to control one's emotions and to express them in a way adequate for the situation. Moreover, it is also the capability of coping with difficulties [2]. Dynamic balance and health potential are the essence of both these areas. Balance reflects normal relations between spheres of a person's functioning, so disturbing any of these spheres will disrupt also the remaining

ones. A condition of obtaining this balance is the health resource adequate for a person and his/her environment [3]. Nevertheless, it should be remembered that different environments (e.g. urban and rural) are characterized by different opportunities, requirements and barriers to functioning [4, 5].

Aim

The general objective of the study was to recognize the self-assessment of the mental well-being, as well as selected elements of the social situation of young, physically disabled people living in the countryside. Particular attention was paid to the consequences of the disability, suicidal thoughts, problems with the acceptance of one's own impairment, as well as the opportunities of obtaining help.

Material and the methods

The research was conducted on the population of physically disabled persons living in the countryside in the Wielkopolskie province.

By means of the stratification method, Kluczbork district and two communes: Koźminek i Lisków, were selected for the research. The main selection criteria were: age 18–45 years, legally confirmed disability, residence in Koźminek or Lisków commune, as well as the agreement for the participation in the research. Before the research, the documentation provided by the District Family Assistance Center in Kalisz was analyzed, with a view to select persons with physical disability who meet the earlier accepted criteria. After the analysis of the collected information, a group of 245 subjects was formed. The participation in the research was voluntary and anonymous. Before the research respondents filled out a form of conscious agreement. Out of the selected group 64 individuals did not meet the criterion of the type of disability (they were mentally disabled), and 6 did not agree to take part, giving the reason of the lack of time. Finally, 175 persons were included in the research, which accounts for 96.7% of all the subjects selected for the research – in accordance with the criteria.

The theoretical foundation of the study and the selection of the research method was the functional definition of disability – International Classification of Functioning (ICF), or International Classification of Impairments, Disabilities and Handicaps (ICIDH) -2 [6, 7].

The research was conducted in 2009. The interview technique was used, on the basis of an original tool – a questionnaire consisting of 96 questions.

A pilot study was carried out on a group of 20 disabled persons. It turned out that the respondents had problems with answering some of the open questions. Adequate corrections were made.

The obtained information was subject to statistical analysis. All the calculations were conducted by means of Statistica 8.0 program made by StatXact. In the nominal scale the groups were analyzed with the help the chi square test $\chi 2$ (chi2). In the case of tables larger than 2x2 and the occurrence of observed zero numbers, the Fisher-Freeman-Halton test (FFHT) was applied. 5% non sequitur was accepted. The tests were analyzed at the significance level $\alpha = 0.05$.

Research results

The demographic – social characteristics

Among the 175 disabled countryside residents who participated in the research, men accounted for 46.1% and women – for 53.9%. The disabled persons in the age group 40–45 years were most numerously represented. Disabled subjects accounted for 11.2% of all the residents of the researched area.

By far the largest group among the studied impaired individuals were married persons, 107 individuals in total, i.e. 61.1%; there were 60 bachelors and maidens (34.3%), 7 divorced persons (4.0%) and 1 widower (0.6%). Among the unmarried persons, there was the highest number of the youngest respondents in the age groups 18–24 and 25–29 years (56.6%). Divorced persons belonged only to the age group 40–45 years. Also in this group there was the only widower.

The majority of the respondents had elementary education (59%), the second biggest group had vocational education (25%). 24 persons declared secondary education (14%), and 3 (2%) respondents – university education.

In the majority of cases (97.7%) the respondents did not live on their own and did not run their households singlehandedly. 41.1% of the research participants did not have children.

The most frequently raised problems were those difficult to solve. Among others, these were: financial difficulties, no apartment or poor living conditions, illness or disability of other family members, unemployment, conflicts (however, 67% of respondents defined the relations in their families as "good"), alcohol abuse in the family, lack of assistive devices.

Financial difficulties (for 60% of respondents the per capita income was not higher than 500 zlotys, depending on: gender – men earned more than women 2 = 8.581938, p = 0.0136, education – the

higher the education, the higher the declared income FFH p = 0.0001, as well as the cause of the disability – persons with the inborn impairment had the lowest earnings $\chi 2 = 21.33457$, p = 0.00002) – did not motivate respondents to try to increase their income by taking a job. In the majority of cases, disabled persons had sources of income other than work – pensions, allowances. The above mentioned decision was related to: age ($\chi 2 = 19.29$, df = 8, p = 0.0133), education (TFFH p = 0.0148), the cause of disability ($\chi 2 = 13.63$, df = 4, p = 0.0085) and the age at which the impairment occurred ($\chi 2 = 16.098$, df = 4, p = 0.0028). In general, work was the source of income for subjects from the age group 25–29 years, with the university or secondary education. Illness and the older age of becoming disabled were the factors inclining respondents towards income forms other than work. It was also observed that for almost half of the respondents (49.7%), the disabled person's individual income was the only source of income for the whole household. It should be noted, however, that due to disability 74 persons (42.3%) were forced to resign from work and 29 respondents (16.6%) had to change a job.

The characteristics of the disability

In the researched population there were individuals with inborn (13.1%) and acquired – diseases, accidents (86.9%) – physical dysfunctions. The most frequent causes of the acquired disability were the diseases of the musculoskeletal system (21.7%), circulatory system (13.7%) and nervous system (9.7%). The musculoskeletal diseases were three times more common for women than men. However, circulatory diseases were the more frequent cause of disability for men. As for the degree of disability, the largest group of respondents had moderate degree of disability (48.5%), the second largest was the group with the high degree (26.9%) and the smallest group – those with the low degree of disability (24.6%). The most common age of occurrence of impairment was over 30 years (46.9%). One third of the male respondents and a half of the female ones also suffered from a chronic disease, apart from their disability.

Mental well-being vs. selected factors affecting the acceptance of disability

Disability exerts a negative influence on a person's mental well-being. Disability, as a difficult experience,

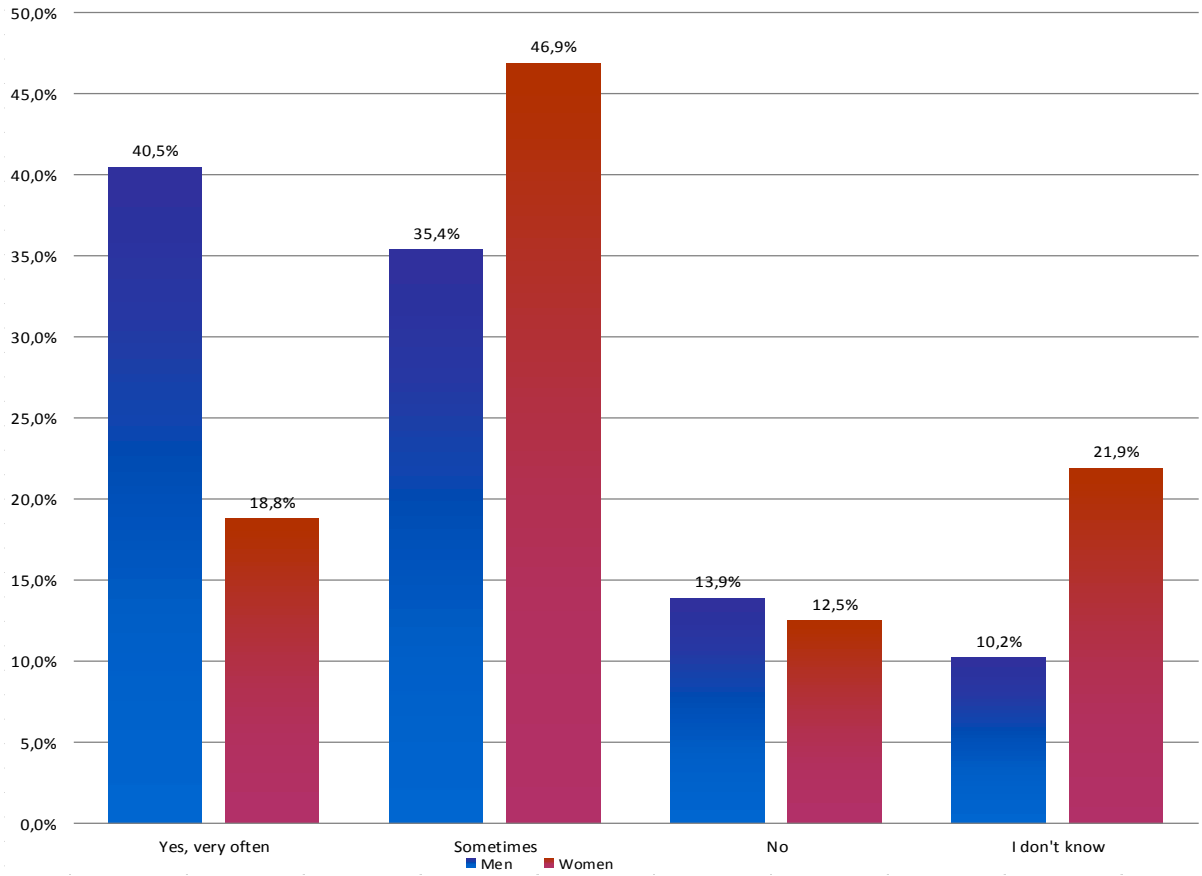

Figure 1. The influence of disability on the mental state of respondents – depending on the gender

may lead to an emotional collapse, anxiety and loneliness. Over a half of the studied disabled people confirmed that their disability affected their mental state. (cf. **Figure 1**).

The influence of disability on the mental state was similar for men and women ($p = 0.1369$). The respondents were also asked about the occurrence of such states as: loneliness, being a burden for others, feeling of loss, and lack of sense of life; they could also describe their feelings within the option: "other". The respondents stated that they saw their disability as a burden for others (25.1%) and 16.6% of the respondents had a feeling of lost life. The research results also showed that 13.1% of the disabled persons had a permanent feeling of loneliness. The "other" category (it meant: "mental discomfort", "isolation", helplessness", "lack of communication with the family", "debility", "powerlessness", "irritability", "lack of self-confidence", "lack of independence", "lack of trust" and "lack of understanding") was chosen most frequently (36% of respondents). Among men, the highest proportion of respondents marked the answers: "other" (36.7%) and "everything is lost" (27.8%). As for women, the highest percentage selected the answers: "other" and "being a burden for others" (35.4% and 34.3%, respectively).

It also turned out that in a large number of cases, the depressed mood was accompanied by suicidal thoughts. As many as 41.7% of respondents admitted to having them. The frequency of their occurrence was the same for men and women, regardless of biological age, the age of becoming impaired, marital status, or education ($p > 0.05$). The application of the test for independence $\chi 2$ allowed the authors to find out that there was a dependency between the cause of impairment and the occurrence of suicidal thoughts (**Figure 2**).

Suicidal thoughts were the most frequent for persons with post-traumatic disabilities.

Although respondents indicated that their periods of depressed mood were temporary, 9.7% of them stated that they had never accepted their disability.

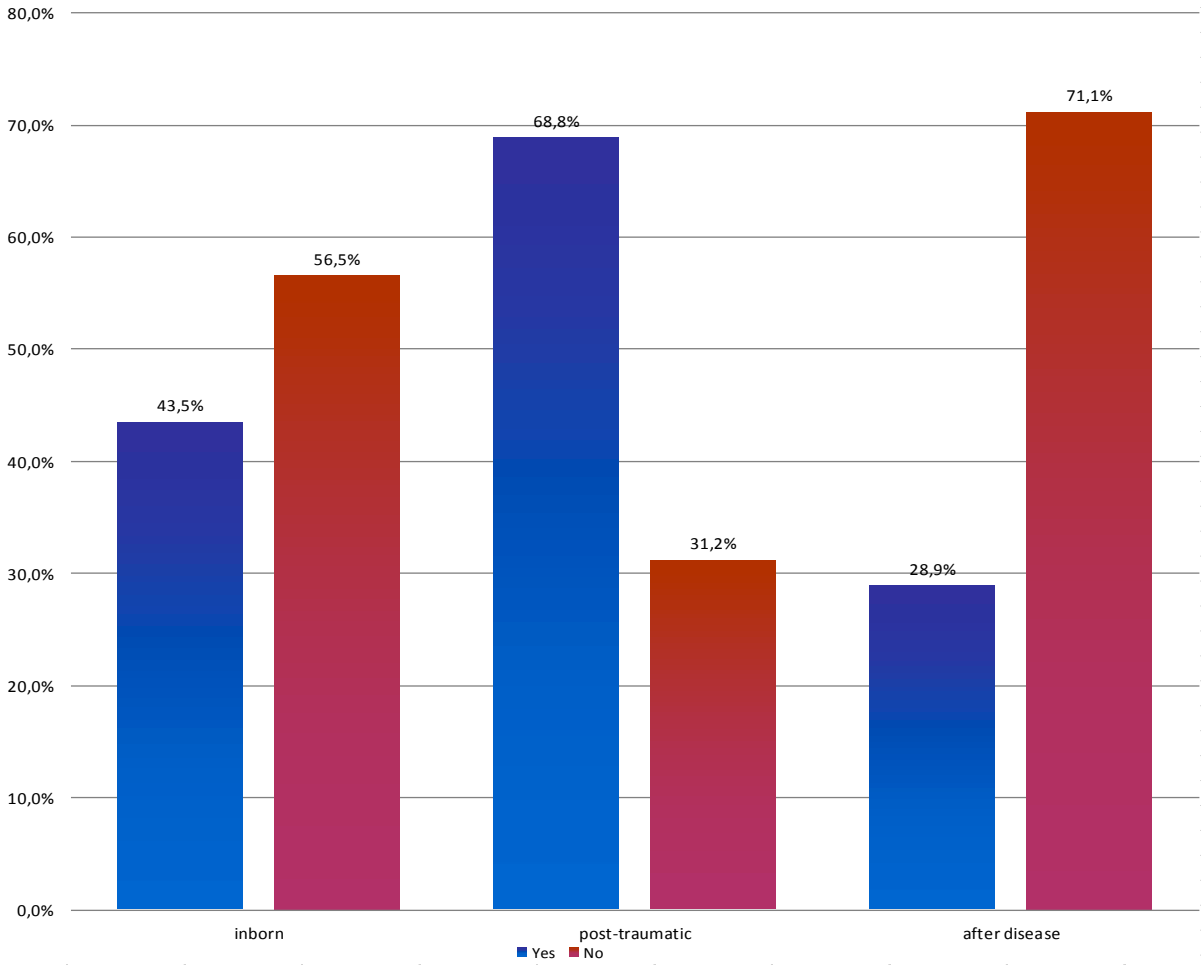

Figure 2. The cause of disability versus the frequency of occurrence of suicidal thoughts

The statistical analysis showed that, among the studied factors, only education influenced the degree of acceptance of a person's own disability (**Table 1**).

Respondents with the university education more often than others could not come to terms with what had happened to them (p = 0.0027). The majority of the research participants (81.7%) also stated that although sometimes it was hard for them to accept the reality, they loved themselves as they were. It should be underlined that as many as 18.3% of the respondents did not accept their disability.

All the respondents reported the occurrence of situations which negatively affected their mood. They used the following effective methods to minimize the effects of this discomfort: pain-relieving medicines – 88.6% of men and 67.7% of women (gender did not have an influence on the frequency of taking painkillers, p = 0.3468), alcohol (men more often than women, p = 0.0074, as well as persons with post-traumatic disabilities, p = 00001) and cigarettes (smoking men definitely outnumbered smoking women, p = 0.0000).

The assessment of the employment situation

As many as 47.2% of all the respondents evaluated their situation on the labor market as bad (48.1% men and 46.9% women). The opinions varied in relation to the age of the disabled people (TFFH p = 0.0330). In the group of respondents negatively assessing their situation on the labor market the majority belonged to the oldest age group of 40–45 years (58.5%), and the lowest number – to the 25–29 years age group (28.6%). Furthermore, the highest proportion of both the disabled participants with elementary education (p = 0.0001) and those who became impaired after the age of 30 years (p = 0.0140) negatively assessed their employment situation. However, the research did not show any relationship between the evaluation of the employment situation and the marital status (p = 0.610), or the cause of disability (p = 0.2040).

Social life and support

The social well-being was researched from the view point of the social life, as well as the disabled person's chance of obtaining support. In the vast majority of cases the

Table 1. The acceptance of one's own disability in relations to: age, education, marital status, the cause of disability and the age of acquiring it

The studied variable		Acceptance of one's own disability						Statistical analysis
		Yes		No		Total		
		N	%	N	%	N	%	
Age [years]	18–24	16	76.2	5	23.8	21	100.0	$\chi^2 = 2.37$ df = 4 p = 0.6212
	25–29	23	82.1	5	17.9	28	100.0	
	30–34	23	79.3	6	20.7	29	100.0	
	35–39	29	90.6	3	9.4	32	100,0	
	40–45	52	80.0	13	20.0	65	100.0	
	Total	143	81.7	32	18.3	175	100.0	
Education	elementary	85	81.7	19	18.3	104	100.0	TFFH p = 0.0027
	vocational	38	86.4	6	13.6	44	100.0	
	secondary	20	83,3	9	16.7	24	100.0	
	university	0	0.0	3	100.0	3	100.0	
	Total	143	81.7	32	18.3	175	100.0	
Marital status	single	46	76.7	14	23.3	60	100.0	TFFH p = 0.4685
	married	91	85.1	16	14.9	107	100.0	
	divorced	5	71.4	2	28.6	7	100.0	
	widowed	1	100.0	0	0.0	1	100.0	
	Total	143	81.7	32	18.3	175	100.0	
Cause of disability	inborn	17	73.9	6	26.1	23	100.0	$\chi^2 = 1.73$ df = 2 p = 0.4208
	accident	38	79.2	10	20.8	48	100.0	
	disease	88	84.6	16	15.4	104	100.0	
	Total	143	81.7	32	18.3	175	100.0	
Age of acquiring disability [years]	up to 18	24	80.0	6	20.0	30	100.0	$\chi^2 = 1.44$ df = 2 p = 0.4857
	19–30	49	77.8	14	22.2	63	100.0	
	over 30	70	85.4	12	14.6	82	100.0	
	Total	143	81.7	32	18.3	175	100.0	

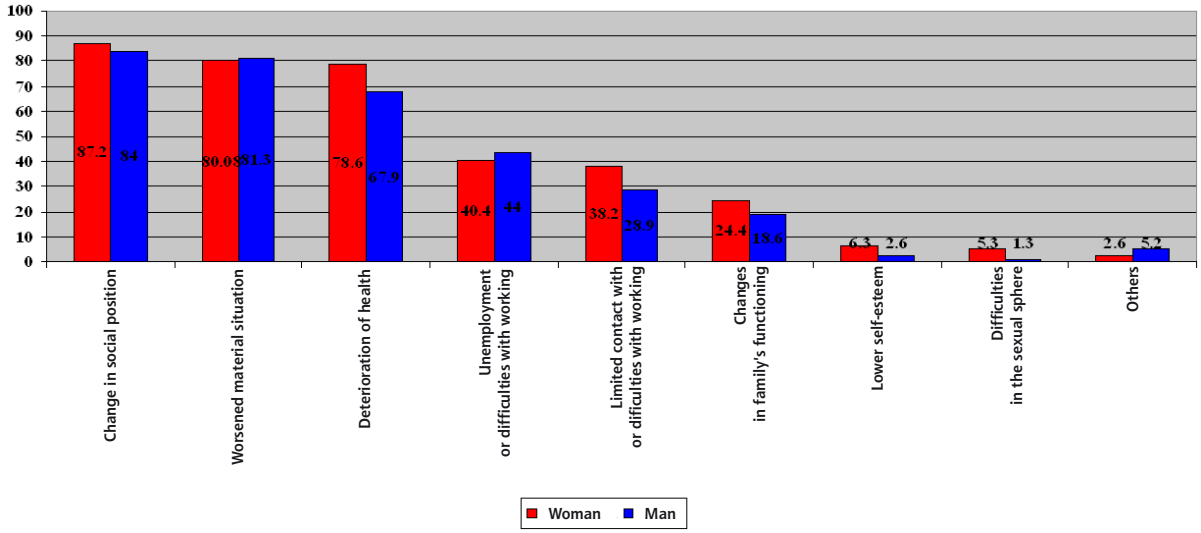

Figure 3. Consequences of disabilities. The results do not sum up to 100% because respondents could choose more than one answer

respondents declared that they had friends and acquaintances with whom they were constantly in touch. For 69% of participants, friends and acquaintances were mainly healthy people, for 9.9% of respondents – mainly disabled persons, whereas for the remaining 21% the proportions were approximately the same. Two sources of support were distinguished – individual and institutional. As far as the individual sources were concerned, the most common one were family members 54.2%. As for the institutional sources, these were: the Communal Social Welfare Center – 15.4% and organizations for disabled persons – 7.4%. It needs to be emphasized that 41% of the respondents claimed that there are no institutions or organizations which they can turn to for help. There was a statistically significant relationship between the assessed level of the offered support and the respondents' age $p = 0.0071$. The level of support was negatively evaluated only by the oldest age group of 40–45 years (9.5%). Also the cause of the impairment affected this evaluation ($p = 0.0007$). Persons with the inborn disability far more frequently (43.5%) positively assessed the level of the received help than those whose disability was caused by an accident (8.3%), or disease (8.7%). Also single persons more positively evaluated such help than the married ones ($p = 0.0027$). However in the light of the research there was no relationship between the evaluation of the received support and the gender ($p = 0.2136$), or the education ($p = 0.1515$) of the respondents.

The consequences of disability in the respondents' opinion

The majority of respondents indicated a high frequency of consequences of their disability. Only 6 persons declared a lack of them (cf. **Figure 3**).

The definitely most frequently selected answer was the change of the social role and position. Only slightly less often mentioned ones were: worsening of the material situation and the deterioration of health.

Discussion

Diseases and accidents were the main causes of disabilities in the studied population. This conclusion is also was reflected in other publications [8–15]. Regardless of the cause of disability, for many years the emphasis has been placed on the optimal functioning of people – especially those with certain somatic or mental limitations. Research focuses mainly on the self-assessment of the state of health, as an important and independent predictor of the state of health, recommended by WHO [16, 17]. In relation to the subjective assessment of health, there are various consequences of disability. [18, 19] They refer to many areas of human life and, among others, research conducted by A. Ostrowska and B. Szczepankowska [20] confirmed the material marginalization of the disabled. According to D. Gorajewska [21], another factor which negatively affects their well-being is the lack of chances for the professional development. This view is shared by Stelcer [21], in whose opinion the difficult material situation, as a consequence of impairment, not only lowers the standard of living, but also leads to difficulties in performing social roles. These theses are reflected in the results of conducted research.

As many as 75.9% of disabled men and 65.7% of disabled women admitted that their mental health had deteriorated (despite their families' support). It may result from the process of disordered adjustment to the

reality. If the quality of life with disability is high, a person is capable of accepting the situation sooner, as they have a high self-esteem and the ability to achieve their life objectives. However, the accomplishment of the sense of security requires support from both the closest family and institutions. Research shows that social support is a prerequisite of disabled persons' effective activity and their achievement of life satisfaction. [23] In J. Kirenko's opinion, the successful adjustment of a disabled person depends on the question whether or not they accept their own dysfunction. It does not only mean coming to terms with one's own disability, but also the dynamic acceptance of the whole state, through the modification of their approach to themselves and the environment and, in consequence, developing skills and habits facilitating their change of lifestyle. [24] The lack of acceptance of somatic differences often leads to the disorder of the mental well-being, which is confirmed by the research. In addition, respondents emphasized the feeling of loneliness, being a burden for others and losing a sense of life. These statements have a special dimension in the light of the suicidal thoughts experienced by disabled people, which was confirmed by 41.7% of respondents. Other authors' research also shows that at least in the case of certain types of impairment, the suicide rate is considerably higher than for the population at large. The disabled individuals studied by the authors of this work were looking for solutions to the above mentioned problems, but since living in the rural areas is characterized by limited access to professional support, their activities often amounted to pharmaceuticals, alcohol and cigarettes. According to J. Kirenko [26, 27], unfortunately, there are many barriers and harmful stereotypes dividing the world of the healthy people and the impaired ones. Because of the lack of the social acceptance of being different, these people live in the fear of rejection. According to T. Lake [28], a big number of the disabled live in loneliness or „a certain isolation from others". The researched disabled inhabitants of the rural areas emphasize that their dysfunctions limit their activity and isolate them from the world. Research conducted by experts indicate that loneliness is one of the most significant factors influencing health [29, 30].

The main guidelines of the social policy towards disabled people aim at fighting all acts of discrimination and at organizing a state without barriers and without social exclusion. The society should "learn disability" in the sense of understanding the significance of the problem and undertaking attempts of introducing rational changes. It is essential, because disability infringes on individual's precious values: health and psychophysical efficiency, also makes it difficult or impossible to perform certain social roles. Nevertheless, as A. Hulek [31] said, „there is no disability which has taken more than left". These words should be a motto of the integration of disabled persons, aiming at eliminating barriers to their functioning, supporting their development and enabling them to achieve their life objectives. Unfortunately, at present functioning of these people, especially those living in the countryside, is hampered by such obstacles as: lack of acceptance of a different person, still common (also among the disabled themselves), conviction about the uselessness of disabled people, insufficient financial resources, as well as the lack of complex institutional solutions.

Conclusions

Over half of the respondents confirmed that their disability influences their mental condition, which is manifested by a feeling of loneliness, being a burden for others and lack of a sense of life.

The major consequences of disabilities are: the change of social role and position, worsened material and health situation, unemployment or difficulties with performing a job.

As many as 41.7% of the respondents declared the occurrence of suicidal thoughts. Such thoughts were more frequently experienced by persons with post-traumatic impairments than with the inborn ones.

Although all the respondents feel the burden of their disability, the majority of them – 81.7% accepted it. Persons with the university education have the greatest problems in accepting it.

The respondents reported that the main source of support is their family. The institutional support is much more limited.

The proposal resulting from the research is the need to immediately introduce measures to help disabled individuals, in order to improve both their social situation (professional development, better access to public institutions) and their mental well-being (self-acceptance, elimination of suicidal thoughts).

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Parameters of dermatomal somatosensory evoked potentials in normal conditions and patients with clinical symptoms of low back pain

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ABSTRACT

Introduction. Dermatomal somatosensory evoked potentials (DSEP) are used to assess the function of afferent pathway following electrical stimulation of the skin around selected dermatomes of single spinal roots.
Aim. The aim of the study was to characterize the parameters of DSEP latencies for assessment of L5 and S1 nerve root transmission in healthy people taking into account the important diagnostic difference between the right and left side and the impact of height, age and sex on values of DSEP latencies.
Materials and Methods. DSEP tests were performed in the control group of 30 healthy volunteers and in the patients with low back pain radiating to one of the lower extremities for comparison. Disc-root conflict was confirmed in MRI studies. Clinical examination included assessment of muscles strength innervated from the L5 and S1 roots and the sensory perception from L5 and S1 dermatomes. In all patients straight leg raising (SLR) and Schober's tests have been performed. To assess the severity of pain, the visual analogue scale (VAS) was used. The clinical and neurophysiological evaluation of patients was performed before and after 4 weeks of the physiotherapeutic exercises selected for this study.
Results. Results indicated statistically significant relationship between the DSEP N33, P40, N50 components latencies and the height. The gender and age did not affect DSEP latencies. Values of DSEP latencies in the first and second periods of observations were normal and no sensory and motor disturbances have been observed in patients. DSEP test showed the high conformity with the results of clinical studies.
Conclusions. DSEP diagnostic determines well the subjective sensation changes in patients suffering from low back pain. Kinesiotherapy treatment of patients with low back pain without neurological deficits seems to be the appropriate therapeutic method.

Keywords: dermatomal somatosensory evoked potentials; low back pain.

Introduction

Dermatomal somatosensory evoked potentials (DSEP) are used to assess the function of afferent pathway by electrical stimulation of the skin around the selected dermatomes of single spinal roots, that triggers synchronous wave stimulation transmitted along the peripheral nerve trunk, posterior column-medial lemniscus pathway of the spinal cord, thalamocortical tract and to the appropriate fields of contralateral somatosensory cortex. The most useful parameters in the evaluation of DSEP are latencies and interlatencies between the individual components of the somatosensory response. The amplitude and shape of the response are less important [1].

Dermatomal somatosensory evoked potentials was a technique introduced in the early eighties [2]. DSEP method is effective in the diagnosis of the consequences of inflammation to sensory spinal roots, tumors of the cauda equina and radiculopathy. It involves stimulation of the skin areas innervated by individual roots.

As innervation of individual roots (dermatome) partially overlap each other, the areas on the skin have been determined in which the overlapping is minimized. Dermatomal fields are excited by electrical stimuli with appropriate parameters, and the responses are recorded from the dermal surface of the skull of the cortical representation associated with the sensory innervation. Responses are averaged and represent the negative or positive waves with reference to the isoelectric line. Latency response, i.e. the time of onset of each wave after stimulus application and their amplitude are recorded and compared. The P40 wave is the most characteristic and constant component of DSEP. Its absence, increased latency or significant difference in latency between left and right side lead to the conclusion of root damage [1–7].

Aim

The aim of the study was to characterize the parameters of DSEP latencies for assessment of L5 and S1 nerve root transmission in healthy people taking into account the important diagnostic difference between the right and left side and the impact of height, age and sex on values of DSEP latencies. A preliminary comparison of selected parameters of DSEP in patients with unilateral sciatica to values obtained in healthy volunteers have also been performed as well as the comparative analysis of results from clinical trials and DSEP studies in the group of patients before and after the specially designed conservative treatment.

Materials and Methods

Subjects

The control group consisted of 30 healthy volunteers, including 25 women and 5 men aged from 22 to 57 years (mean 26.1 ± 7.2) and height from 158 to 191 cm (mean 171.6 ± 7.4). The aim of examination in this group was to ascertain the normative values of latency of each dermatomal somatosensory evoked potentials. The obtained results are presented in **Table 1**.

The control group included patients who had never reported pain in the lumbosacral spine or there were only sporadic episodes, which did not last longer than four weeks. The pain sensation was limited only to the lumbosacral segments without radiation to the lower extremities. Prior to the test, a thorough medical history has been collected from each volunteer with a focus on potential contraindications for the examination. Each volunteer was informed about the purpose of the study and signed the informed consent form, according to the valid questionnaire in the Department of Pathophysiology of Locomotor Organs, University of Medical Sciences in Poznań. Group of patients were those with pain syndrome at lumbosacral spine with pain radiation to one of the lower extremities in the disc-root conflict, documented with magnetic resonance imaging (MRI). The study group consisted of 5 patients (4 women and 1 man), aged 24 to 47 years (mean 38 ± 10.4) and height of 160 to 183 cm (mean 170.2 ± 8.4).

Table 1. Reference values of recorded DSEP components latencies (ms) in a group of healthy volunteers after stimulation of nerves in right and left extremities. Values refer to results calculated following stimulation on both sides (N = 60)

		N33	P40	N50	P60
L5	Mean	39.8	47.8	58.4	71.0
S1		41.8	49.5	59.4	71.6
L5	Median	39.3	47.2	58.1	70.7
S1		41.5	49.2	59.2	71.5
L5	SD	3.7	4.0	4.3	5.6
S1		3.7	3.3	4.0	5.0

Abbreviations: N33, P40, N50, P60 – DSEP components; L5, S1 – sensory dermatomes

Before performing the study, a medical history has been collected and physical examination has been performed. Each patient was informed about the study and its progress and gave a written consent to the study. Inclusion criteria for patients were pain syndrome in lumbosacral region of the spine with pain radiating to one of the lower extremities in disc-root conflict on the L5 or S1 level, documented with magnetic resonance imaging (MRI), no other contraindications for examination. Exclusion criteria were a state after implantation of the pacemaker, cochlear implant, insulin pump and other electronics devices used for therapeutic purposes in an individual, no MRI of lumbosacral region confirming the diagnosis, symptom duration of less than 4 weeks, history of trauma or surgery of the spine, diabetes, polyneuropathy, history of injuries and fractures of the lower extermities.

Instruments

Clinical evaluation

In past medical history we collected information on the current episode of pain, i.e. since when and how long the pain lasts, what is the pain like and how often it appears during the day. Additionally, we have asked for coexisting diseases in accordance with the exclusion of patients from the study.

Examination consisted of assessing the strength of muscles innervated from the L5, S1 root, sensory disturbances from L5 and S1 dermatome, patellar tendon

Table 2. Characteristics of the study group including elements of a clinical study before and after rehabilitation treatment

Clinical test	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	I	II	I	II	I	II	I	II	I	II
Schober test	14	15	15	15	13,5	15	15	15	12	15
	8	8	8	8	8	8	8	8	8	8
SLR	+ 30°	–	+ 30°	–	+ 40°	–	–	–	+ 40°	–
VAS	6	0	6	0	10	5	5	1	10	3
Sensory test	N	N	N	N	N	N	N	N	N	N
Pain radiation	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
Left	YES		YES		YES		YES			
Right									YES	

Abbreviations: I, II – First and second examinations, N-normal, + positive, - negative

and Achilles tendon reflex testing. In all patients the straight leg raising test (SLR) and Schober's test have been performed. To assess the severity of pain the visual analogue scale (VAS) was used. Characteristics of the study group including elements of a clinical study before and after rehabilitation treatment are presented in **Table 2**.

Kinesiotherapy program

Patients performed exercises therapy towards low-back pain for 4 weeks. In the recommendations, they were to systematically perform 10 repetitions of each exercise, every day for 4 weeks (patients were supposed to perform 10 repetitions of each exercises, every day for 4 weeks' time). In the case of pain appearing the patient should adjust the number of repetitions to their (his/her) abilities and always remembered about breathing exercises (take a deep breath in through a nose, exhale through your mouth). A set of exercises has been explained to each patient individually. A set of exercises was as follows:

- Exercise lying on their backs
  - Lying down with legs bent, hands under the lumbar region of spine. Approximating the navel to the spine ("press the navel to spine") with a stand for 5 s
  - Lifting the head and shoulders from the ground. Both legs bent at hips and knees. Hands pushing knees. Legs at a standstill. Hold for 5 s
  - Lifting the head and shoulders from the ground. The left hand pushes the right knee. Hold for 5 s, than swap.
  - Raising the pelvis to a height of 10–15 cm. Arms along the body, withstand at 3s
- Exercise front lying (folded blanket / towel under the abdomen)
  - Lifting the head up-looking ahead. Withstand 3 s
  - Lifting the right upper extremity and left lower extremity. Withstand 3 s, than swap
- Exercise in kneeling (bottom to heels stretch)
  - Bend forwards and rest your forehead on the floor with the arms stretched in front of you.
- Exercise in kneeling
  - "Cat-camel" exercise (10 repetitions)
  - The "Bird-Dog" exercise. Simultaneous raising of right upper extremity and the left lower extremity. Withstand 5 s, than swap.
- Breathing exercises
  - Upright kneeling position
    - » Inhale – raising arm up and elongating the spine
    - » Exhale – lower arm sideways down

A set of exercises has been explained to each patient individually. For the first time, patients performed exercises under the supervision of a physiotherapist.

DSEP test

Cutaneous areas of L5, S1 sensory roots were stimulated in both lower limbs with the electrical impulses of 0.2 ms duration, frequency of 3.3 Hz and intensity 3 times higher than the sensory threshold determined individually for each subject. Stimulating electrode was located at a distance of 4 cm from the base of the fifth finger, on the outer edge of the foot for S1 root, 3 cm from the base of the big toe and the second toe on the dorsal surface of the foot for L5 root. Silver, the cup-shaped recording electrodes were placed in the following location: active electrode in position 2 cm at the rear of Cz, the reference electrode in the position Fpz in accordance with the international system 10–20. Grounding electrode was located on the side of the neck. DSEP test was performed according to the method described by Rakowicz et al [3]. Responses have been analyzed after averaging of up to 500 waveforms, twice, in order to verify the reproducibil-

ity of responses. Analyzed parameters were latencies of N33, P40, N50 and P60 components and the latency difference between the right and left side. Tests have been performed in Department of Pathophysiology of Locomotor Organs University of Medical Sciences in Poznan in the Wiktor Dega Orthopaedics and Rehabilitation Hospital using an integrated diagnostic system KeyPoint (Medtronic A/S, Skovlunde, Denmark).

The clinical examination and DSEP test in patients were performed twice, before and after the period of regular exercises specially designed for this study.

The implementation of the research was approved by the Bioethics Committee of Poznan University of Medical Sciences (Resolution No. 496/15). All personal data of patients have been kept confidential.

Statistical analysis

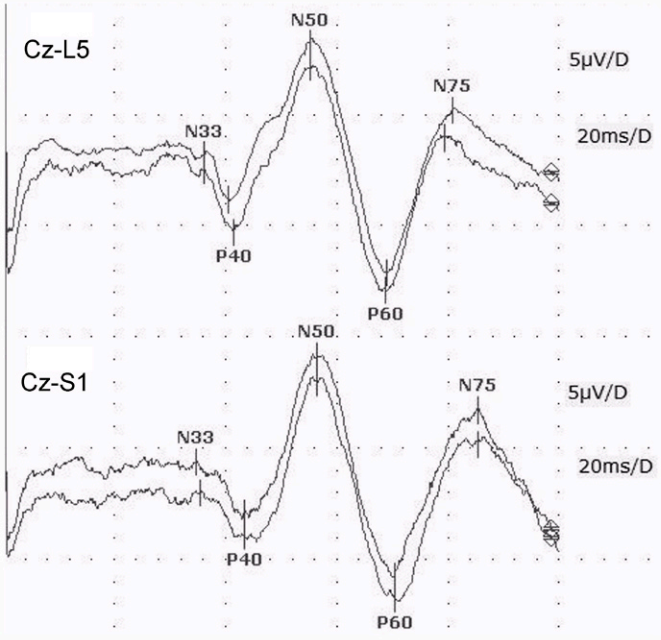
For statistical analysis of the results of the latencies recordings of each DSEP components dependence on the sex, age and height nonparametric Mann-Whitney U test and Spearman rank correlation coefficient were used. A statistically significant difference in the latency of each DSEP wave on the right and left side of the respective root in the control group was calculated by paired Student t test. Test results with significance level of $P \leqslant 0.05$ were considered statistically significant. Calculations were performed using STATISTICA v. 10 StatSoft. Quantitative variables were expressed as the mean, median and standard deviation.

Results

All the selected locations of stimulation allowed for the recordings of well formed, repeatable, of similar shape dermatomal potentials. N75 latencies due to the difficulties in the correct determination of the latency of the component and its low stability when comparing the two consecutive curve potentials were not analysed (**Figure 1**).

The latency normative values of each DSEP component for L5 and S1 roots are presented in **Table 1**.

Table 3 shows the diagnostically significant differences in latency of each DSEP component between the right and left side. The number of results shown in this table was N = 60, because it was calculated as



Recording site	Component	Latency L (ms)	Latency R (ms)	L/R diff (ms)
Cz-L3	N33	22.7	28.2	5.5
	P40	27.9	31.7	3.8
	N50	39.7	40.3	0.60
	P60	52.7	51.5	1.20
	N75	63.1	63.2	0.10
Cz-L4	N33	29.7	31.5	1.80
	P40	33.7	38.1	4.4
	N50	46.7	48.7	2.0
	P60	59.8	60.4	0.60
	N75	73.2	73.5	0.30
Cz-L5	N33	36.2	36.3	0.10
	P40	41.5	40.9	0.60
	N50	55.2	56.5	1.30
	P60	68.9	71.2	2.3
	N75	79.5	83.4	3.9
Cz-S1	N33	35.5	31.4	4.1
	P40	43.4	47.2	3.8
	N50	56.6	55.7	0.90
	P60	70.4	70.4	0
	N75	85.5	82.1	3.4

Figure 1. Examples of dermatomal evoked potentials recorded after stimulation of the L5 and S1 sensory areas on the right and left side in a healthy 28 years old women, with the values of each DSEP peak latency for the right and the left side

Table 3. Reference values (mean ± 2SD) of latencies (ms) for particular DSEP components recorded in healthy volunteers (N = 60). Statistical significant differences in recorded latencies following stimulation of nerves on right and left extremities at $P \leqslant 0.05$ are marked with asterisks

	N33	Right vs left difference	P40	Right vs left difference	N50	Right vs left difference	P60	Right vs left difference
L5	39.8 ± 7.4	1.1*	47.8 ± 8	1.0*	58.4 ± 8.6	0.8	71.1 ± 11.2	0
S1	41.8 ± 7.6	0.2	49.5 ± 6.6	0.8	59.5 ± 8	0.3	71.7 ± 10	0.3

Abbreviations: N33, P40, N50, P60 – DSEP components; L5, S1 – sensory dermatomes

the average value of the latency of each DSEP wave obtained in a group of 30 healthy volunteers together from the right and left lower extremity.

A statistically significant difference between the values of N33 and P40 latencies in recordings following the stimulation of both legs from the L5 root dermatome have been found. Diagnostically important it proved to be the difference latency equal to or greater than 1 ms.

There was also performed an additional analysis of the dependence of the latency of each dermatomal evoked potentials components in the control group (N = 30) from gender, height and age. The results are summarized in **Tables 4, 5** and **6**.

The values showed in Tables 4 and 5 indicate no statistically significant differences, which meant that the gender and age did not affect the results of DSEP parameters.

The results summarized in **Table 6** indicate the statistically significant dependence of the latencies of N33, P40, N50 components of dermatomal evoked potentials from height. It is a positive relationship. There was no statistically significant correlation of P60 component latency from height of a subject.

Table 2 shows the characteristics of the patients including data from medical history and clinical examination in the first and second period of observation, i.e. before and 4 weeks after the introduced treatment.

Table 4. Correlation results between DSEP latencies and gender in the control group of healthy volunteers (N = 30)

	Rank sum Women	Rank sum Men	U	Z	P-value	Z adjusted	P-value	Women	Men	P-value*
N33 dexter	381.5	114.5	56.5	-0.900	0.368	-0.9	0.36	25	5	0.364
N33 sinister	380.5	115.5	55.5	-0.950	0.342	-0.95	0.34	25	5	0.338
P40 dexter	402	94	73	0.0750	0.940	0.0	0.94	25	5	0.94
P40 sinister	393.5	102.5	68.5	-0.300	0.764	-0.3	0.76	25	5	0.751
N50 dexter	377	119	52	-1.125	0.260	-1.12	0.26	25	5	0.268
N50 sinister	375	121	50	-1.225	0.220	-1.22	0.22	25	5	0.227
P60 dexter	408.5	87.5	66.5	0.400	0.689	0.4	0.68	25	5	0.678
P60 sinister	408	88	67	0.375	0.707	0.37	0.70	25	5	0.714

Abbreviation: *calculated with Mann-Whitney U test, a P⩽ 0.05 was accepted as significant

Table 5. Correlation results between DSEP latencies and age in the control group

	Number of participants (N)	rS	t (N-2)	P-value
Age vs N33 dexter	30	0.203612	1.119943	0.271928
Age vs N33 sinister	30	0.280641	1.574575	0.126202
Age vs P40 dexter	30	0.202828	1.115449	0.273817
Age vs P40 sinister	30	0.314620	1.784923	0.084739
Age vs N50 dexter	30	0.261212	1.457266	0.155787
Age vs N50 sinister	30	0.326986	1.863302	0.072576
Age vs P60 dexter	30	0.174023	0.951665	0.349132
Age vs P 60 sinister	30	0.170638	0.932592	0.358731

Abbreviations: r_S - Spearman's rank correlation coefficient, a P⩽ 0.05 was accepted as significant

Table 6. Correlation results between DSEP latencies and height in the control group of healthy volunteers

	Number of participants (N)	rS	t (N-2)	P-value
Height vs N33 dexter	30	0.4287	2.5559	**0.0160**
Height vs N33 sinister	30	0.6565	4.6869	**0.0000**
Height vs P40 dexter	30	0.3679	2.1306	**0.0417**
Height vs P40 sinister	30	0.5172	3.2544	**0.0028**
Height vs N50 dexter	30	0.4363	2.6117	**0.0141**
Height vs N50 sinister	30	0.6547	4.6645	**0.0000**
Height vs P60 dexter	30	0.0652	0.3520	0.7273
Height vs P60 sinister	30	0.2325	1.2876	0.2080

Abbreviations: r_S - Spearman's rank correlation coefficient, a P⩽ 0.05 was accepted as significant

The patient group consisted of 5 people with low back pain and one-sided sciatica. Four patients reported a pain with radiation to the left lower extremity and one to the right lower extremity. The physical examination of all patients showed no sensory disturbances within the dermatomal areas of skin innervated by L5 and S1 nerve roots, sensory perception was comparable to the asymptomatic side. Also, the patellar tendon and Achilles tendon reflexes and strength of tibialis anterior muscle, extensor digitorum brevis muscle and gastrocnemius group muscles on the symptomatic side were correct. Every of the patients stood on toes and heels. Three patients had the left L5 disc-root conflict, one person the right S1 and one person left S1 disc-root conflict detected in MRI imaging. Radiation of pain in all patients was consistent with the results of MRI. The SLR test result during the first assessment was positive in 4 patients from the study group. Only one result was negative. Straight leg raise was regarded as positive to the angle of 60° of hip flexion [8]. However, in the second study, every individual test result was negative (**Table 2**). A comparison study of pain intensity VAS scale in subjects revealed, that the therapy resulted in a substantial reduction from the mean value of 7.4 to 1.8, as it is shown in **Figure 2**.

Comparison of Schober's test results of subjects showed that the therapy improved the range of motion in the lumbar region of spine. The extension range remained unchanged and fitted in the standard, while the range of flexion increased from the 13.9 cm to the value of 15 cm, which is shown in **Figure 3**.

The latencies of N33, P40, N50, P60 components to the standard values obtained in healthy volunteers of the control group were compared. Both in the first and in the second study, all patients latencies values of DSEP components were within the range of normative values (**Table 7**). For the correct N33, P40, N50, P60 latencies values, the average value ± 2.0 SD was taken. The difference between the latencies of DSEP recorded from the symptomatic (**Table 8**) and asymptom-

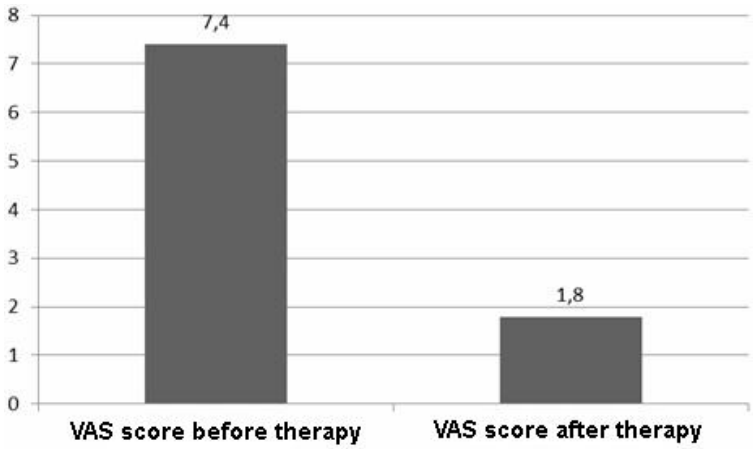


Figure 2. Results of pain intensity assessment in VAS scale before and after therapy in the patients group

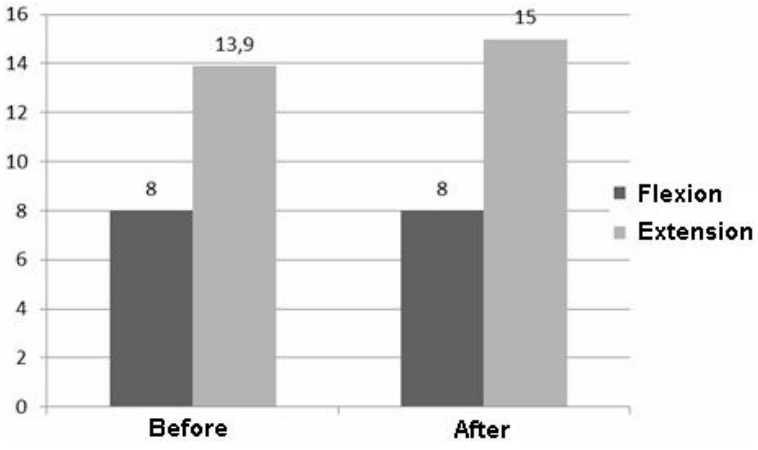


Figure 3. Results of range of motion assessment of the lumbar spine using the Schober's test

Table 7. The values of DSEP latency (ms) in patients group (N = 5)

		N33		P40		N50		P60	
		Test I	Test II	Test I	Test II	Test I	Test II	Test I	Test II
L5	Mean	41	40.9	49.3	48.7	60	59	72.9	71.5
S1		41.6	42	49.3	49	60.1	57.7	71.4	69.3
L5	Median	38.9	39	47.3	47.5	59.6	58.3	70.5	69.9
S1		40.7	40.3	47.4	46.9	57.9	57	71.3	67.6
L5	SD	4.2	4.1	4.8	4.2	3.5	4.2	7.7	3.7
S1		4.8	4	3.9	3.7	4.1	3.7	5.2	5.4

Table 8. The values of DSEP latency on symptomatic side of patients group (N = 5)

		N33		P40		N50		P60	
		Test I	Test II	Test I	Test II	Test I	Test II	Test I	Test II
L5	Mean	39.2	41.2	48.3	48	59.3	57.2	71.1	67.7
S1		43.9	41.9	51.8	50.1	61.2	54.1	72.6	73.3
L5	Median	38.9	39.3	45.8	46.6	58.3	56.4	71.3	67.6
S1		43.9	41.9	51.8	50.1	61.2	54.1	72.6	73.3
L5	SD	2.9	3.6	4.7	3.4	3.9	3.7	4.3	2.6
S1		7.6	5.7	8.5	9.1	5.5	5.7	8.8	6

Table 9. The values of DSEP latency on asymptomatic side of patients group (N = 5)

		N33		P40		N50		P60	
		Test I	Test II	Test I	Test II	Test I	Test II	Test I	Test II
L5	Mean	41	39.9	49	48	59.2	57	74	71.6
S1		41.6	42.3	48.2	47.5	50.3	49.4	55.5	55.1
L5	Median	39.4	39.9	39.4	46.6	57.6	57.5	71.3	67.3
S1		41.6	42.3	48.2	47.5	50.3	49.4	55.5	55.1
L5	SD	4.7	0.7	2.9	3.4	3.1	4.2	7.8	9.4
S1		5.7	7.4	13.6	13.6	6.8	5.3	13.6	15.2

atic side (**Table 9**) was also analyzed in the group of patients. The values were compared to the diagnostically relevant differences in latency values calculated in healthy volunteers which are shown in **Table 3**. There was no recorded the diagnostically significant increase of DSEP latency in the symptomatic side compared to the asymptomatic side. Due to the very small number of patients (N = 5), differences of DSEP component latency in the first and in the second study were not subjected to the statistical analysis.

Discussion

The normal values of N33, P40, N50, P60 latencies were found to be comparable to those published in other studies [3, 9–11].

Similar to the presented studies we also did not record the statistically significant correlation between the age or gender of a patient and the value of DSEP wave latency, especially P40 latency, which is the most easily detectable DSEP component [3, 10].

In studies of Albeck et al [12], in 40% of patients with disc herniation, the DSEP study was incorrect and only in 15% of patients the results were consistent with the level of damage confirmed by CT scans. It therefore can be concluded that the examination should be considered as a supplemental test in neurophysiological diagnostics, especially in patients with symptoms of sensory disturbances.

Many authors emphasize the importance of dermatomal somatosensory evoked potentials in the diagnosis of patients with lumbosacral discopathy [13–17]. Sitzoglou and his colleagues [14] also draw the attention to the noninvasiveness of this technique. Dumitru et al [15] and Florczak et al [16] in their studies evaluated the N33 and P40 components latencies and DSEP amplitudes. According to the other authors [3,16], the most common DSEP abnormality observed in patients with sciatica and damage to the lumbosacral spinal nerves is a prolongation of DSEP latency. These authors also highlighted the importance of diagnostic P40 latency difference between the symptomatic and asymptomatic side. In our study N33, P40, N50 and P60 wave latency of DSEP parameters were assessed. DSEP amplitudes have not been analyzed, because dur-

ing the test in a control group of healthy volunteers the attention was drawn to the significant difference in DSEP amplitudes in people of the same sex and of the same age which is consistent with studies of Katifi and co-workers [10].

The results presented in this study showed in all patients that DSEP latency was correct, although in a clinical study in those patients the numbness from the L5 or S1 dermatome and neurological deficits from relevant muscles of lower extremity have not been recorded. In study of Florczak et al [16], the increase of P40 wave latency parameter had coincided with impaired sensory sensation in the clinical trial. Also in Wasilewska and Kotowicz study [2] who evaluated the patients with lumbo-sacral discopathy in whom in a DSEP study a prolonged latency of each wave has been observed, were characterized by the presence of lower extermity neurological deficits. As it was presented in our study, in patients group the DSEP test showed a high conformity with the results of a clinical study despite the presence of disc-root conflict showed in the MRI results.

Quante et al [18] presented in their studies a new method of neurophysiological, dermatomal laser-evoked potentials, used to evaluate the root impulses transmission in early monosegmental radiculopathies.

Therapeutic treatment for lumbosacral region pain episode is quite complex. Lack of appropriate treatment regimen, the duration of the disease process and neglect in the sphere of prevention and lack of ergonomics are the reasons for this phenomenon. The treatment should be focused on improving the range of motion of lumbosacral segment and strengthening the back muscles, which are a kind of stabilizing corset [19–22]. This study evaluated the efficacy of physiotherapy treatment in the cases of lumbosacral pain. According to the previous descriptions [20, 21], the efficacy of physiotherapy in such cases may reach 80%. Indeed, only properly selected physiotherapy is able to improve the health status of the patient. In all patients after four weeks kinesiotherapy, the range of motion in lumbo-sacral region and the reduction of pain intensity had improved, although the study was conducted on a small number of patients. Święcicka and Święcicki conducted a study on a group of 190 patients [20] and Suszynski et al [21] on a group of 40 patients.

According to the statement of Lisiński et al [22], the kinesiotherapy is the primary method of treatment for back pain, while the electrotherapy is only complementary to the proceeded physiotherapy. In this study, patients during the study period were treated only with kinesiotherapy, which proved to be fully effective method and allowed to obtain the satisfactory therapeutic results.

Functional tests are a valuable complement to the diagnosis of low-back pain. Positive tests indicate damage or irritation of neuromuscular structures, which can cause the pain radiating to the lower extremity. Well-conducted tests complement the diagnostic data and are necessary to determine the need for further imaging tests. For the evaluation of nerve root components the SLR test was used. The sensitivity and specificity of the SLR test was presented in the analysis conducted using the MEDLINE and EMBASE databases. We found that the SLR test showed a high sensitivity – 91%, but low specificity – 26% [23]. In the first conducted clinical study, four patients showed positive scores of SLR tests. Only in one case test result was negative, while the disc root conflict was confirmed on the basis of the result of MRI in all patients.

According to several authors, in the diagnosis of back pain the important element of the study is to evaluate the sensation disturbance [1, 20, 24]. In our clinical trial, in the patients group no sensory disturbances have been observed. Clinical trial results in this study are compatible with the results of the DSEP study, because also in the DSEP examination there have been no patients with diagnostically significant prolongation of DSEP latency.

According to Depa et al [25], in the subjective sensation of pain it is also important to assess an efficacy of physiotherapy. In this study we used VAS for assessment of pain intensity as a tool evaluating the effectiveness of applied kinesiotherapy. The results of this study show the importance of rehabilitation in patients with low back pain. The use of appropriate diagnostic methods together with complex therapeutic treatment determines meeting the expectations of the people suffering from back pain, which is also consistent with studies of other authors [20–22].

Conclusion

DSEP study is a simple, noninvasive method for evaluating nerve conduction of L5, S1 dorsal nerve roots neural transmission. DSEP examination seems to be a good diagnostic tool determining the subjective pain in patients suffering from low back pain. Kinesiotherapy treatment of patients with low back pain without neurological deficits seems to be the appropriate therapeutic method.

Taking into account the results of presented study, the future studies should be extended to a group of

patients including those in whom a clinical study concludes lower extremities neurological deficits.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Bisphenol A modifies human spermatozoa motility *in vitro*

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ABSTRACT

Introduction. The decrease of men's sperm quality was reported to be related to exposure to xenoestrogens. Bisphenol-A (BPA) is a synthetic xenoestrogen commonly present in our environment, for instance in food containers.
Aim. The aim of this study was to investigate the influence of BPA on human spermatozoa motility.
Materials and Methods. The effects on spermatozoa of BPA at final concentrations of 10^{-10}, 10^{-8} and 10^{-6} mol/L were studied regarding to the following phenomena: (1) evaluation of sperm motility using computer-aided sperm analysis system providing four parameters: velocity straight linear VSL, cross beat frequency CBF, lateral head displacement LHD and homogeneity of progressive movement velocity HPMV, (2) spermatozoa vitality (propidium iodide staining), (3) phosphatidylserine membrane translocation (staining with annexin V conjugated with fluorescein) and (4) kinetics of intracellular free calcium ions changes (using Fluo-3).
Results. BPA caused a transient, significant increase of VSL and HPMV at 15 minutes after stimulation. One hour incubation of spermatozoa with BPA did not alter cells vitality nor stimulated phosphatidylserine membrane translocation, for all three concentrations. BPA in the final concentration of 10^{-6} mol/L initiated a rapid (observed after a few seconds), and transient (resolving after a few minutes) increase of intracellular free calcium ions concentration.
Conclusions. Human spermatozoa can be considered target cells for BPA. BPA significantly modified spermatozoa motility. BPA affected spermatozoa involving free calcium ions as second messenger.

Keywords: bisphenol A; human spermatozoa; sperm cells motility.

Introduction

Male infertility contributes to 50% of all infertility cases. Risk factors for male infertility include: varicocele, aging, sexually transmitted diseases, lifestyle factors and long-term or intensive exposure to certain types of toxins, chemicals and medications [1]. Estrogen-like and endocrine disrupting (EDs) chemicals that alter the normal function of hormones, such as phthalates, organochlorines and bisphenol A are of particular potential concerns. When xenoestrogens enter the body they increase the total amount of estrogen resulting in a phenomenon called estrogen dominance. Xenoestrogens are not biodegradable therefore, they are stored in fat cells [2, 3].

Several studies have reported the association of exposure to phthalates and bisphenol A with impaired semen quality [4, 5]. Bisphenol A (BPA; 4,4'-(propane-2,2-diyl) diphenol) belongs to the group of diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups. BPA are non-persistent EDs mainly used as plasticizers, which are widely present in foods. BPA is used in food containers (bottles, microwave ovenware, and linings for canned foods and beverages) but also in non-food items, including epoxy-resin based paints,

PVC medical devices, thermal paper and parts of electronic devices [2, 6, 7].

Estrogenic effects are mediated by two types of intracellular receptors: estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2). BPA is mainly considered as an ESR1 and ESR2 agonist and in that manner has significant impact on human cells biology. Previous research revealed that it can also act as antagonist of the androgen receptor or as agonist of the aryl hydrocarbon receptor and other nuclear receptors [8, 9].

The review of available literature demonstrates that mature sperm cells are target cells for estrogen action. ESR1 and ESR2 presence was confirmed in spermatozoa [10, 11]. It is pointed out that estrogens may influence both the capacitation and acrosomal reaction [12, 13]. BPA is present in woman's reproductive tracts. It's concentration within the follicular fluid ranges from 1.0 ng/mL to 2.0 ng/mL [14]. It is assumed that BPA may affect the biology of mature sperm cells in woman's reproductive tract and thus influence the fertilization process [12].

Motility is the basic parameter that enables sperm cells to fertilize the egg cell. Motility parameters change throughout capacitation in process called hiperactivation and influence their biological quality [15]. The aim of the study was to investigate whether BPA influences sperm motility under *in vitro* conditions.

Materials and Methods

Preparation of human spermatozoa

Semen samples obtained from 20 normozoospermic men were analyzed according to WHO criteria 2010 [22]. 3–5 days period of sexual abstinence was required prior to obtaining the material. High motility sperm cells were isolated with the use of the swim-up technique [23]. Ham's F-10 medium served as sperm cell extender.

Isolated cells were incubated with BFA in final concentrations of 10^{-10}, 10^{-8} and 10^{-6} M. Spermatozoa motility was noted at 5, 10, 15, 30 and 60 minutes after exposure to BPA. Spermatozoa incubated in Ham's F-10 medium were used as controls. Spermatozoa vitality and phosphatidylserine membrane translocation were assessed at 60 minutes after exposure to BPA.

Assessment of sperm cells motility

Sperm cells motility was analyzed in human spermatozoa (1×10^5 sperm cells/mL) suspended in Hams F-10 medium using a computer-assisted spermatozoa motility analysis system. Ten microliters of spermatozoa suspension was spotted onto a Cell Vision chamber slide, producing a specimen with uniform thickness. Images were collected at a frequency of 60 frames per second using a Pixel-Link camera. Motility in the microscopic specimen was assessed in a minimum of 10 different fields for each case, yielding a minimum of 700 analyzed sperm cells. Acquisition time was 2.08 s; the analyzed area was 640 × 470 μm; and the resolving power was 0.86 points. All measurements were made at a constant, controlled temperature of 24°C.

The following spermatozoa motility parameters were analyzed: (1) velocity straight linear (VSL), (2) cross-beat frequency (CBF), (3) lateral head displacement (LHD) and (4) homogeneity of progressive movement velocity (HPMV). These parameters were calculated as follows:

1. VSL was calculated matching the sperm path with two sectors (the method involves n sectors where n-1 is the period of observation in seconds). The sectors were obtained minimizing total sum, evaluated for all sectors, of squared distances from the sector to sperm mass center. The idea was to avoid erratic evaluation of VSL for colliding sperms, often found for higher sperm concentrations.
2. CBF was evaluated using Fourier series calculated on basis of distances from sperm mass centers to corresponding sectors (see VSL calculation).
3. LHD was calculated as standard deviation of distances from sperm mass center to corresponding sectors (see VSL calculation).
4. HPMV was evaluated as standard deviation of distances from predicted sperms orthogonal cast on corresponding (see VSL calculation) and sperm mass center cast. This parameter describes vibration of sperm observed at velocity VSL along sperm pathway.

Assessment of sperm cells vitality and phosphatidylserine membrane translocation

To determine phosphatidylserine membrane translocation (PST) from the inner to the outer layer of the plasma membrane, the annexin-V labeled with fluorescein (AnV-FLUOS) (Molecular Diagnostics, Darmstadt, Germany) was used. Simultaneously, to distinguish between viable and dead spermatozoa the propidium iodide (PI) staining was used, in the final concentration 0.125 μg/L (Sigma-Aldrich, St. Louis, MO). Double staining was conducted according to manufacturer's recommendations.

Flow cytometry

The fluorescence signals of labeled spermatozoa were analyzed by flow cytometer FACSCalibur (Becton–

Dickinson, USA). 10 000 cells were examined for each experiment. The fluorescence of An-V-FLUOS and PI was excited by argon laser (488 nm) and emission of An-V-FLUOS was measured in the FL1 channel (515–545 nm), while the red fluorescence of PI was detected in the FL3 channel (650 nm). All data was collected and analyzed using CellQuest Pro software (v.5.2.1) (Becton–Dickinson).

Changes in intracellular free calcium ions level

Fluo-3 (Molecular Probes; Ex/Em = 488/526 nm) was used to study changes in free calcium ions level in human sperm cells. Spermatozoa (1 x 10^6 cells/mL) were incubated with 4 μM Fluo-3 for 45 min at 37°C according to the manufacturer's protocol. For confocal microscopy, spermatozoa were immobilized in 1% (w/v) agarose and then treated with BPA. Microscopic images were used for gating single sperm cells in which fluorescence changes were recorded. Forty images were collected (every 10s) and used to study the kinetics of intracellular free calcium ions changes. Spermatozoa were observed using LSM 510 confocal microscope (Zeiss, Jena, Germany) equipped with a Plan Apochromat 63x/1.4 Oil DIC objective. Sperm cells incubated in Ham's F-10 medium were used as a control for fluorescence intensity changes. Changes of Ca^{2+} level were examined throughout 400 seconds after exposure to BPA, every 10 seconds. Sperm cells vitality was checked with saturated potassium chloride solution.

Statistical analysis

Each variable was tested using the Shapiro-Wilk W-test for normality. Homogeneity of variance was assessed with Levene's test. Since the distribution of the variables was normal and the values were homogeneous in variance, all statistical analyses were performed using parametric tests. The analysis was made using Statistica 10 software (StatSoft Inc., Tulsa, OK, USA). Data were presented as mean ± SD and considered statistically significant at $P < 0.05$.

Ethics Statement

The study protocol was approved by the Institutional Review Board of the Poznan University of Medical Sciences (No 119/09). All the involved patients provided written informed consent.

Results

Control group motility

Marked heterogeneity of human sperm cells motility parameters was observed (**Table 1**). No significant parameters change ($P > 0.05$) was observed during 60 minutes observation.

Table 1. Control group motility parameters

	Mean	Minimum	Maximum	SD
VSL [μm/s]	13.6	2.6	56.7	11.7
CBF [Hz]	7.6	0.5	30.0	7.5
LHD [μm]	0.6	0.2	3.5	0.5
HPMV [μm/s]	1.1	0.2	8.2	0.7

VSL – velocity straight linear, CBF – cross-beat frequency LHD – lateral head displacement, HPMV – homogeneity of progressive movement velocity, SD – standard deviations

Impact of bisfenol A on spermatozoa motility

Velocity Straight Linear

Spermatozoa stimulated with BPA in final concentration of 10^{-10} mol/L and 10^{-8} mol/L revealed transient, statistically significant increase of VSL at 15 minutes after stimulation. The VSL values at 30 and 60 minutes after stimulation did not significantly differ from controls. Spermatozoa stimulated with BPA in final concentration of 10^{-6} mol/L revealed statistically significant ($P < 0.05$) increase of VSL both at 15 minutes and at 30 minutes after stimulation (**Figure 1A**).

Cross-Beat Frequency

No significant CBF changes were observed for all BPA concentrations or times after BPA stimulation (**Figure 1B**).

Lateral Head Displacement

No significant LHD changes were observed for all BPA concentrations or times after BPA stimulation (**Figure 1C**).

Homogeneity of Progressive Movement Velocity

Spermatozoa stimulated with BPA in final concentration of 10^{-10} mol/L and 10^{-8} mol/L revealed transient, statistically significant increase of HPMV at 15 minutes after stimulation. The VSL values at 30 and 60 minutes after stimulation did not significantly differ from controls ($P > 0.05$).

Spermatozoa stimulated with BPA in final concentration of 10^{-6} mol/L revealed statistically significant ($P < 0.05$) increase of VSL both at 15 minutes and at 30 minutes after stimulation (**Figure 1D**).

Effects of bisphenol-A on sperm vitality and phosphatidylserine membrane translocation

Flow cytometry analyses identified four fractions of spermatozoa: (1) An-V^-/PI^- viable sperm without PST, (2) An-V^+/PI^- viable sperm with PST, (3) An-V^-/PI^+ dead sperm without PST and (4) An-V^+/PI^+ dead sperm with PST (Figure 2). The sperm cells percentage of each frac-

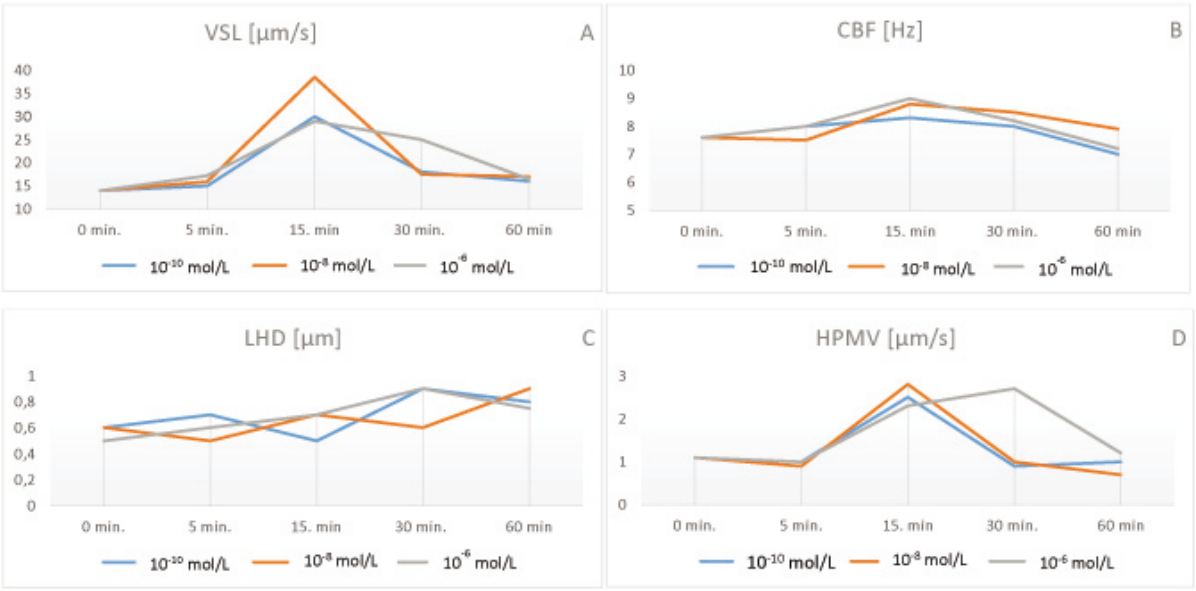

Figure 1. Changes of spermatozoa motility parameters after BPA stimulation. (A) VSL – velocity straight linear, (B) CBF – cross-beat frequency, (C) LHD – lateral head displacement, (D) HPMV – homogeneity of progressive movement velocity

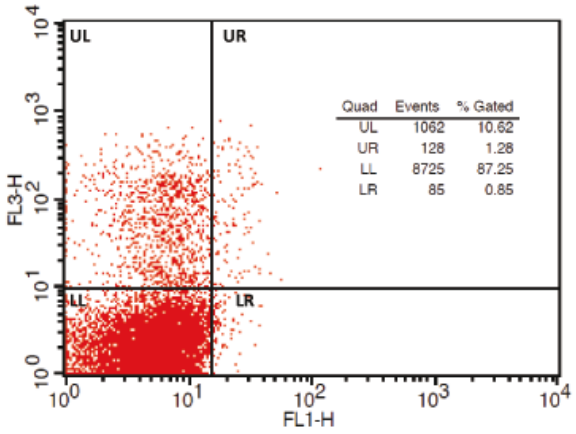

Figure 2. Flow cytometry analysis of spermatozoa vitality and phosphatidylserine membrane translocation in swim-up selective cells – representative graph. UL (upper left quadrant) – positive propidium iodide cells, UR (upper right quadrant) – positive propidium iodide and annexinV cells, LL (lower left quadrant) – non stained cells; LR (lower right quadrant) – cells positive only to annexin V. FL1-H – fluorescence channel 515 – 545 nm, FL3-H – fluorescence channel > 650 nm

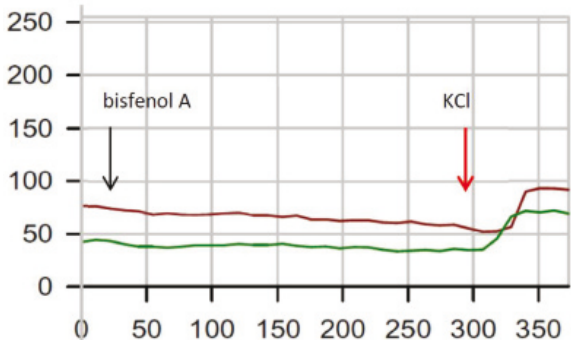

Figure 3. Kinetics of free intracellular calcium ions change in human spermatozoa stimulated with BPA at 10^{-10} mol/L final concentration. Arrows indicate administration of BPA and KCl

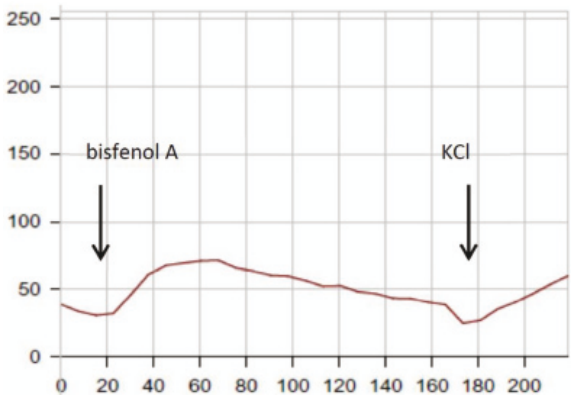

Figure 4. Kinetics of free intracellular calcium ions change in human spermatozoa stimulated with BPA at 10^{-6} mol/L final concentration. Arrows indicate administration of BPA and KCl

tion was 81.2 ± 6.1%, 0.4 ± 0.3%, 15.9 ± 5.5% and 2.4 ± 1.4%, respectively. It did not change after 60 minutes incubation with BPA, for all concentrations used.

Changes in intracellular free calcium ions level

In spermatozoa isolated with swim-up technique the highest concentration of intracellular free calcium ions was observed within the midpiece and distal part of head. Stimulation with 10^{-10} mol/L or 10^{-8} mol/L BPA did not change intracellular free calcium ions level (**Figure 3**). Stimulation with 10^{-6} mol/L caused a rapid,

transient increase of intracellular free calcium level. The reaction was observed at 10 seconds after stimulation and lasted a few minutes (**Figure 4**).

Discussion

Bisphenol A is the synthetic compound that exhibits activity similar to 17β-estradiol (E2). Due to its physicochemical properties it is extensively used in the manufacture of wide variety of common consumer goods. The safety of BPA is often disputed. Therefore, its usage in production process has to be regulated according to applicable law [6, 7].

Sperm cells are potential target cells for estrogens and xenoestrogens, which may alter their biological function. It is established that exogenous 17β-estradiol stimulates hamster's sperm cells motility. Jin et al. indicated that different concentrations of 17β-estradiol caused significant increase of all spermatozoa motility parameters, excluding the linear index in the group of animals treated with high doses of E2. The research also revealed that active immunization to E2 decreased significantly the sperm cells motility [16].

Available literature demonstrates that also xenoestrogens, including BPA, may influence semen quality and its parameters such as sperm motility and velocity [17, 18].

Rat sperm cells motility decreased in relation to the diet containing health hazardous xenoestrogens such as dicofol and mixture of dieldryn, endosulfan, dicofol, dichlorvos and permethrin. There were no changes in motility after administration of above mentioned compounds separately [19].

It was revealed that genistein and 4-tert-octylphenol affect capacitation and acrosome reaction of boar spermatozoa. It turned out that genistein acts similarly to estrogen while, in comparison to 4-tert-octylphenol, its stimulative effect on both above mentioned processes is stronger [20].

The correlation between increased level of BPA in urine and decreased semen quality was also proven in men exposed to BPA in work environment. BPA exposure resulted in decreased sperm cells count, concentration, motility and vitality [21].

Described research data is not consistent with results obtained in our study. For all the BPA doses we observed transient increase of both, velocity straight linear and homogeneity of progressive movement velocity parameters, which occurred 15 minutes after stimulation. We also observed increase of these parameters at 30 minutes after stimulation with the highest concentration of BPA (10^{-6} mol/L). The lateral head displacement and cross-beat frequency values did not change significantly with regard to either BPA dose or time of incubation. In presented research model sperm cells were incubated in estrogen free medium, thus the only compound able to mimic estrogen action was BPA. Therefore, it can be assumed that bisphenol-A stimulates spermatozoa motility in a transient way and the reaction is dose dependent.

Probably, BPA uses endogenous estrogen signaling pathway and similarly to 17β-estradiol modulates sperm motility. This may be related to its effects on sperm mitochondrial potential, and thus, the production of ATP. It seems probable because of the proven midpiece localization of estrogen receptors in human sperm. This region of sperm cell is also characteristic for exclusive mitochondrial occurrence and mitochondria are supposedly target organelles for estrogen action [10, 22, 23].

There are few studies examining the effects of estrogens and xenoestrogens on vitality and apoptosis of mature sperm. In the conducted studies we analyzed whether bisphenol-A affects the vitality and the process of phosphatidylserine membrane translocation, which is the marker of apoptosis. There were no significant changes in viable sperm cells percentage and the percentage of spermatozoa presenting phosphatidylserine membrane translocation, irrespectively to the applied doses [24–26].

The level of calcium ions, which is one of important factors controlling the process of capacitation and acrosome reaction, was also assessed in the present study. Unlike somatic cells, spermatozoa have much less buffering capacity of Ca^{2+} in its organelles. That is why the proper calcium homeostasis is maintained through the system of calcium channels and pumps. Calcium ions storage takes place mainly in mitochondria, acrosome and posterior part of the head [27, 28]. Stimulation with 17β-estradiol causes transient increase of intracellular free calcium ions level in sperm cells [29]. Our research revealed that stimulation with bisphenol-A at final concentrations 10^{-10} mol/L and 10^{-8} mol/L induced the increase of calcium ion levels only in single cells while the concentration 10^{-6} mol/L caused rapid, transient, significant increase of intracellular free Ca^{2+} level in the whole population of observed cells.

In conclusion, our study along with literature findings have shown that human spermatozoa are target cells for BPA. BPA significantly modified spermatozoa motility. BPA affected spermatozoa involving free calcium ions as second messenger.

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Conflict of interest statement
The authors declare no conflict of interest.

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Students of Poznan University of Medical Sciences are not enough prepared to provide high quality basic life support

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ABSTRACT

Introduction. In case of sudden cardiac arrest (SCA) quick recognition and provision of immediate high quality cardiopulmonary resuscitation (CPR) increases chance of survival. It has been proven that Poles' knowledge about the basic life support is insufficient. Alumni of medical universities are expected to have practical skills and extensive knowledge to provide appropriate help to SCA victim.

Materials and Methods. This was a descriptive, cross-sectional study. We invited all Polish-language students of Poznan University of Medical Sciences (PUMS). 434 people took part in the survey. The research tool was online questionnaire containing 20 questions.

Results. Most of correct answers was given by students of Medical Faculty I, then Faculty of Health Sciences, Medical Faculty II and Faculty of Pharmacy (median of correct answers as follows: 9.48, 8.86, 7.90, 6.93). The biggest problem our students had with questions about: time of interruptions in chest compressions, depth of compressions and duration of a single breath (respectively: 27.63%, 36.53%, 38.64% of correct answers). 42.56% of students would initiate CPR if they saw agonal breaths. 34.50% percent believed that they are properly prepared for CPR while 60.05% said that the number of first aid course hours is insufficient. We found statistically significant relationship between number of critical mistakes and faculty of study ($p = 0.00003$, contingency factor = 0.2282).

Conclusions. The knowledge of PUMS students about CPR is inadequate. It is suggested to evaluate the number of hours dedicated to CPR classes. We should pay attention to identifying agonal breaths and all the criteria of CPR quality.

Keywords: cardiopulmonary resuscitation; knowledge; students.

Introduction

The data concerning Poles' mortality collected by the Central Bureau of Statistics had showed that leading cause of death in Poland are cardiovascular diseases which constitute 46% of all deaths. The sudden cardiac arrest (SCA) that is a result of dysfunction of the cardiovascular system is a direct cause of death [1]. Early SCA recognition and implementation of cardiopulmonary resuscitation (CPR) to the victim triples the chances of survival and reduces the risk of complications connected with the central nervous system (CNS) caused by hypoxia. Existing Polish regulations impose on each bystander obligatory provision of first aid (FA) to any person in life threatening situation. These regulation are included in both The State Traffic Code and the Act of State Medical Rescue. Currently FA trainings are widely available to citizens. Previous studies have reported that most extensive knowledge have young people living in large cities and having a higher education [2]. However, several observations have indicated a lack of knowledge about CPR among Polish society [3, 4]. Special expectations the society directs towards

healthcare providers as well as students of medical universities. This means in particular that these people should be able to adequately respond, quickly and effectively act, when life threatening situation appears. European Resuscitation Council (ERC) in the "Guidelines for Resuscitation 2010" presented basic life support (BLS) algorithm and described its correct execution, such as assessment of patient's condition, opening the airway, rescue breaths, chest compressions and use an automated external defibrillator (AED). Clear criteria for chest compressions quality, which are the most important part of CPR have also been defined. The updated guidelines from 2015 upheld these recommendations. The discussed indicators of quality are: rate and depth of compression, correct hands position and full recoil after each compression. Rescuers should switch every two minutes to avoid fatigue. It has been proven that the failure to meet these criteria causes low quality actions which entail inadequate perfusion and thus reduces the chances of survival. Chest compression should be given immediately. Beginning CPR in more than four minutes of the onset of SCA is associated with a poor outcome [5]. The Poznan University of Medical Sciences (PUMS) educates students in four faculties: Medical I (MF I), Medical II (MF II), Faculty of Pharmacy (FPh) and Faculty of Health Sciences (FHS). The training leads to BSc, MSc, MD, DDS and Pharm. D. degrees. The number of hours provided for education in helping people in life-threatening condition varies depending on the department and faculty. During classes, students gain knowledge and skills for dealing with emergencies, tailored to the level of future professional competence. Each training program regardless of the level of advancement includes a recognition of SCA and providing BLS. The central thesis of this paper is to examine the level of knowledge of students in the field of CPR and to compare that level in individual faculties. Part of the aim of this project is to answer to the question whether students feel adequately prepared to provide resuscitation. The authors believe that results of this study can improve the education in this subject.

Materials and Methods

The survey was conducted among 434 PUMS students in December 2015. The research tool was author's internet questionnaires addressed to students of Polish-language faculties. Five questionnaires have not been included for further analysis because of the lack of information. The results were elaborated in Statistica version 12 GB (analysis of statistical significance using Chi2 test, with level of alpha = 0.05). Charts and tables were prepared in Microsoft Office Excel 2007 (v12.0). For critical mistakes made by the respondents we considered wrong answer to at least one of three following questions. The first concerned the taking or not CPR to the victim when rescuer is in doubt about presence of normal breathing. The second – an indication of actions to be taken as a priority to the victim, who presents agonal gasps. While the third concerned the correct way to open the airway.

The cohort was diverse in terms of gender, faculty, field and year of study and the fact of being trained or no being trained in FA during academic education. Among 434 students participating in the study, most of the respondents were women (68.43%). The group of men was significantly lower (31.57%). The largest group of individuals were students of the MF I (39.16%) next students of the FHS (38.93%), MF II (11.19%) and the FPh (10.72%). According to field of study the largest group consisted of medicine students (39.16%), next paramedic students (11.19%) and nursing students (11.19%). During the research, most respondents were studying on the first (28.90 %) and second (28.90%), year. The vast majority (88.11%) said that they had already been trained in FA during education on PUMS before felt out the questionnaire.

Results

The surveyed were asked the question, whether they think that they are well prepared to perform CPR if necessary. 34.50% stated that they are not adequately qualified. There was no statistical relationship between the feeling of being well prepared to responding and PUMS faculties ($p = 0.18704$). The detailed answers broken down into faculties are compared in **Figure 1**.

The questionnaire contained 14 questions that evaluated the knowledge. Among all respondents, the median of correct answers was 9 points (14.45%), while the most common result was obtained at 8 points (16.32%). 75% of students obtained a score of 11 points or less.

There has also been analyzed the number of correct answers, depending on the student's participation in a FA course, during education at PUMS. Average points scored among the people who haven't been trained was 6.96. The most common result obtained was 8 points (27.45%), while 25% of respondents in this group received a score 8 points or higher. On the other hand, students who participated in the FA course, had the most frequently score of 9 points (16.18%) and the average score was 9.03 points. 75% of respondents in this category achieved the result of 7 points or more.

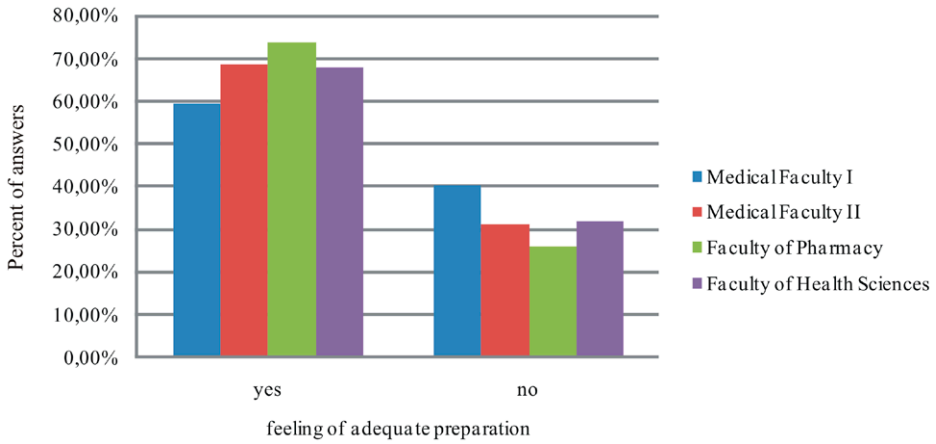

Figure 1. Answer for question: "Do you feel adequate prepared to provide BLS", depending on faculty

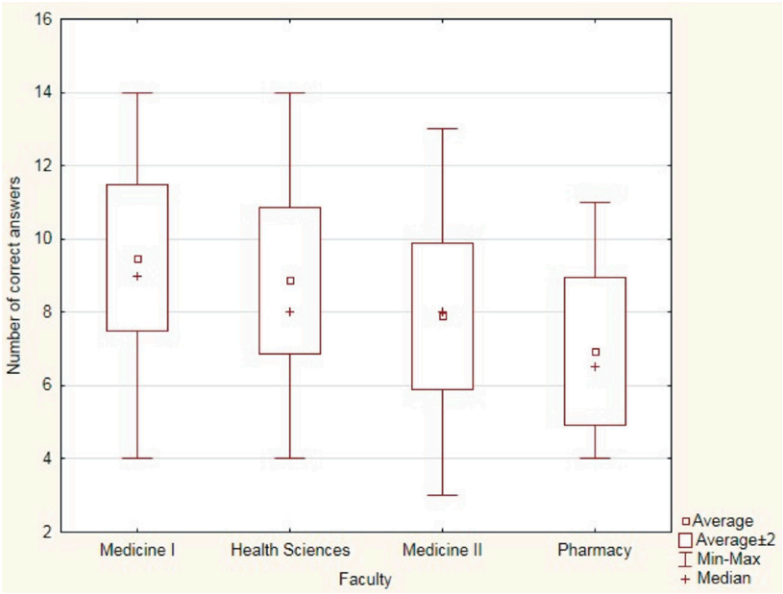

Figure 2. Number of correct answers given by responders, depending on faculty

When comparing number of correct answers depending on faculty, we received the highest average points from students of MF I (9.48 points), next of FHS (8.86 points), MF II (7.90 points) and FPh (6.93 points). Distribution of number of correct answers divided into faculties is presented in **Figure 2**.

In response to question, that concerned the appropriate behavior in case, when the rescuer see the collapsed person presenting agonal gasps, 42.56% of all scholars answered incorrectly that the victim should be placed in recovery position. The majority of incorrect answers was given by FPh students – 65.22%, 49.98% students of MF II, 45.51% students of FHS and 31.55% MF I. The correct answer, which was chest compression, was given most often by students of MF I (53.57%), the least likely by students of FPh (19.57%).

Then the participants were asked about the duration of breathing assessment. This parameter was known to most PUMS students – 85.45% of them pointed to a 10-second assessment. Among the departments the most correct answer was given by students of the MF I (91.62%), then FHS – 83.73%, FM II – 80.85% and FPh – 73.91%.

Another question concerned the situation, when the rescuer is in doubt that the victim is breathing. Most of the students in all faculties correctly pointed out that in such a situation rescuer should begin CPR (85.55% of all individuals). We haven't found a statistically significant difference between number of correct answers and PUMS faculty ($p = 0.09070$).

Respondents were also asked to indicate which emergency number would they prefer, if there it was

a necessary to call for ambulance. In Poland we can seek medical assistance calling either 112 or 999. Most students (67.83%) would choose 999 number to call for help. This trend was most noticeable among students of FHS (80.84%), next by students of FM II (68.75%). Future physicians would choose 999 number in 58.33%, and those studying at the FPh less likely did the same (54.35%). By contrast 112 number was chosen less likely.

In next question we asked where the rescuer should put her or his hands to perform chest compressions during CPR – 54.08% of all students indicated, that in the middle of the sternum, which was incorrect answer. When dividing the results depending on faculties, the appropriate hands position (in the middle of the chest) was indicated most likely by students of FHS – 44.91%, then MF I - 38.69%. In contrast, only two of individuals did not marked correct answer, when asked about chest compression:ventilation ratio.

ERC Guidelines recommend that chest compressions during CPR should have a depth of at least 5 centimeters but not more than 6 cm. Knowledge on this item had 36.53% of all respondents – the majority of correct answers we received from of MF I students(44.05%) and FHS students (37.58%).

Another crucial element of CPR is appropriate rate of chest compressions. This must be at least 100 per minute but no more than 120 per minute. This recommendation was not obvious for 28.26% of FPh students and 58.33% students of MF II, while MF I students pointed the proper answer in 73.65% of cases and the Faculty of Health Sciences in 64.67%. There was no statistical significant difference between knowledge about the correct hands position and faculties ($p = 0.99294$).

The ERC Guidelines also strongly indicate that maximal time, without chest compressions that the rescuer may dedicate to ventilation is 10 seconds. This was known for 27.63% of all PUMS students. The most frequently incorrect answer shown by responders was 5 seconds. (MF I – 59.04%, FHS – 67.07%, MF II – 52.08%, FPh – 63.04%).

On the other hand, information about the best method to open airway had 98.60% of the respondents (in all departments the percentage of correct answers ranged from 95.65% to 99.40%). The proper volume of single rescue breath, which should cause chest rise was also well known to the individuals (92.97% of correct answers). The lowest score was recorded among students of the MF II (85.42%).

However, for question about duration of single rescue breath, only 38.64% of all respondents correctly answered that it should be 1 second. MF I students were less likely wrong (44.64% correct answers), while FPh students were wrong most often – 8.70% appropriate indications.

According to the BLS algorithm, after 30 compressions the rescuer proceed to make two attempts of rescue breaths. Such knowledge showed approx. 50% of students from all faculties. There was no statistically significant difference between knowledge of ventilation in CPR and faculties ($p = 0.99294$).

Then we asked individuals about their reaction to choking when the victim is still loud coughing and asking for help – 68.60% of the students indicated the correct procedure (MF I – 86.90%, MF II – 61.22%, FPh – 58.70%, FHS – 55.09%).

According to the ERC Guidelines to maintain high quality of CPR rescuers should switch roles every 2 minutes to avoid fatigue – this knowledge had 53.72% of the PUMS students. In a similar percentage correct answers were given by MF I (61.31%) and FHS (59.28%), Further, FPh students were the least accurate in this question (19,57%).

Aside from checking technical knowledge students were asked about their opinion about duration of FA classes. 60.05% of those who took part in the survey stated the opinion that the number of hours of FA course is insufficient. Only 30.95% of respondents claimed that the number of hours is adequate. The results obtained from this analysis can be compared in **Figure 3**.

Among all the questions we indicated for three, the most crucial. These questions did not evaluate only the knowledge of quality determinants but primarily the knowledge of elements that have directly impact on victim's survival. 38.93% of all respondents did not made any critical mistakes (FM I – 50.60%, FHS – 37.13%, MF II – 27.08%, FPh – 15.22%). In the other hand, 61,07% made one or more mistakes (FM I – 49.40%, FHS – 62.87%, MF II – 72.92%, FPh – 84.78%). Nearly half of all respondents from the PUMS (48.95%) indicated a wrong answer in one critical question. Two critical mistakes was made by 11.66% of learners. Among the 429 people three critical mistakes were done by only two students – one from FHS and one from FPh. The least critical mistakes were committed by students of FHS, then MF I and MFII. The greatest number of crucial mistakes was reported among FPh students. We found statistically significant difference between number of critical mistakes and faculty ($p = 0.00003$, contingency factor = 0.2282). The differences between faculties are highlighted in **Figure 4**.

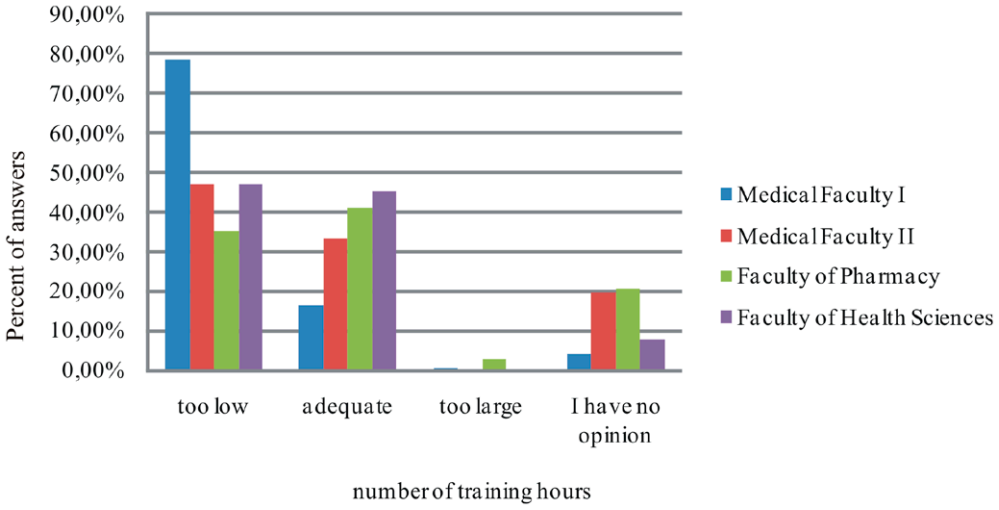

Figure 3. Opinion of students who have completed first aid course at Poznan University of Medical Sciences on the number of training hours, depending on faculty

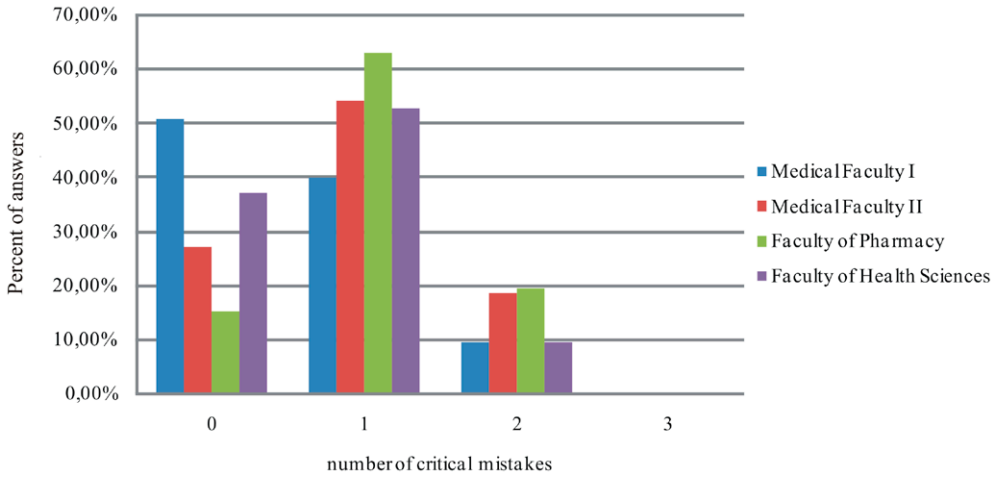

Figure 4. Number of critical mistakes that has been done by responders, depending on faculty

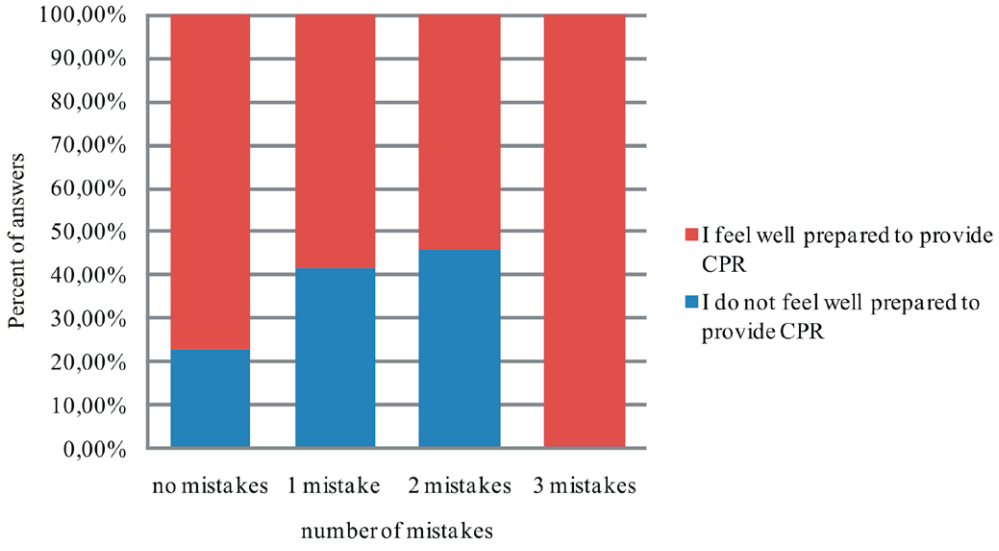

Figure 5. Number of critical mistakes compared with self-confidence

The mistakes were done most commonly to the question about proceeding with casualty that is presenting agonal gasps (57.81% of all incorrect answers). In case of doubt, whether the victim is breathing or not, students gave 14.45% incorrect answers. While the method of opening airway CPR was clear for the vast majority of respondents – reported 1.40% of the errors among all the people who fulfilled to the questionnaire. A statistically significant difference was found between the sense of proper preparation for giving CPR and the number of critical mistakes committed. Those who did not feel confident also did more errors (**Figure 5**). The results obtained from the analysis of the questionnaire are summarized in **Table 1**.

Only slightly more than 1/3 of our students did not made mistake in any question concerning the implementation of interventions that are crucial for patients' survival such as taking CPR if agonal breaths are noticed or doubt about the presence of breath. Insufficient knowledge about agonal breaths that are associated with about 40% cases of SCA can be worrying whether future medics will be able to complete the first link in the chain of survival, which is to recognize life-threatening condition. Only the question about the correct method of opening airway was not an issue. The correct answer was given by 98.60% of the study group. A slightly weaker knowledge of this technique, was presented by researchers from India –

Table 1. Percent of correct answers for given questions

Issue	Percent of correct answers
Compression interruption no longer than 10 seconds	27.63%
Depth of compressions	36.53%
Duration of single rescue breath	38.64%
Appropriate reaction towards victim presenting gasping	42.56%
Correct action after 30 compressions	50.00%
Rescuers' switch time	53.72%
Correct hands position on the chest	54.08%
Rate of compressions	60.43%
Appropriate reaction towards choking preson	68.60%
Appropriate duration of patient's assessment	85.45%
Appropriate reaction, when have doubt on presence of the breath	85.55%
Volume of rescue breath	92.97%
Correct method to open airway	98.60%
Compression-ventilation ratio	100.00%

Discussion

Our study referred to the level of knowledge about CPR among PUMS students. The University educates young people in different fields of medicine. The area of education is very wide. It assumes preparation to work both in occupations in which providing medical help to people with SCA is part of daily work, and those in which life-threatening situations are rare. Clearly, however, the ability to perform CPR should be known to every citizen and alumni of a medical universities are required to perform these tasks with due diligence and in accordance with the latest knowledge. Many studies have shown that the level of knowledge acquired during FA course has decreased over time [6–8]. According to previous, as well as the fact that the Guidelines are periodically updated, CPR courses should be repeated. 81.7% of students knew the correct answer [9]. According to data collected by Pilip et al. [10] knowledge of the issue among Polish firefighters and lifeguards balanced from 57% to 92%. However, such persons were not eligible as students in this study.

Among the determinants of the quality of CPR our responder had the lowest level of the knowledge that concerned the depth of chest compressions (only 36.53% gave the correct answer). A very low awareness of this parameter was also indicated by Alanazi et al. (15.8% correct answers) [11]. In contrast to aforementioned findings, results of Olejniczak et al. can be suprising. They fonund that the correct answer on the depth of chest compressions was granted by 54% of study group (studies have been performed based on the ERC Guidelines 2010) [12].

Burkhardt et al. [13] showed that more than 93% of the group correctly identified the rate of compressions. It is a surprisingly satisfactory result in comparison with our research (60.43%). Skitek et al. [4] also found that percentage of PUMS students that properly defined the rate was 60%. Another sources show the lack of knowledge as well: Alanazi et al. – 63.3% [11], Chew et al. – 55.70% [14] and Owojuyigbe et al. – 85.30% of the students after training [15]. This study considered not only medical and nursing students but also emergency department workers and didn't specify the results for each group. It is interesting due to the fact that the researchers compared the obtained knowledge with the measurements obtained during practical exercises. They highlighted that the knowledge of guidelines has a significant impact on practical CPR skills, at least in terms of rate and hands position. Chemperek et al. [16], showed that only 24.4% of individuals granted the right answer on the rate of chest compressions in adults.

An important and also analyzed in this research aspect of CPR was to determine respondents' knowledge of depth of compressions. Unfortunately, most students (54.08%) answered incorrectly, indicating that correct place is on center of the sternum, not on center of the chest. This result is better than in a similar study conducted by Chemperek et al. [16], where 62.8% of respondents gave wrong answer.

One unanticipated finding was that nearly 100% of our students were able to correctly identify appropriate compressions:ventolatio ratio. This is another very important component of CPR and our result puts our students in a good light compared with students from foreign universities, who gave the correct answer in 58.4%, 72.9% and 97.1% [11, 14, 15], and also other universities in Poland, where the percentage of correct answers to this question was up to 72.6% in studies of Chemperek et al. [16], 85% Olejniczak et al. [12] and 92% of PUMS students in our previous study by Skitek et al. [4].

Comparing the different PUMS faculties we showed that the greatest knowledge had MF I students, then FHS, MF II and FPh. Attention should be drawn to the fact that the number of hours dedicated to BLS education varies considerably depending on the field of study. This number is as follows: paramedic students – 1035, physicians – 155 hours, dentistry – 140 hours, pharmacy and medical analysis – 45 hours, nursing and midwifery – 30 hours.

It does not change the fact that the knowledge of basic life-saving techniques should be sufficient regardless of the field of study. During education MF I students discuss issues several times during following courses: first aid, cardiopulmonary resuscitation, disaster medicine, emergency medicine, advanced medical simulation. We do not find surprisingly the fact of higher than in the other groups level of knowledge. It only serves to emphasize the value of CPR classes that are repeated.

The differences in the percentage of correct answers to individual questions can arise both from a different number of hours dedicated to teaching CPR at various universities and from the time that has elapsed from training to research. But despite their differences, all cited authors agree that the knowledge of students is insufficient.

The vast majority of our students (65.5%) declared that feel properly prepared to provide CPR. At the same time a similar number of individuals felt that the CPR training is too short. Only FPh students pointed out that the training is sufficient – although they gave the least correct answers. In other studies done by Skitek et al., in which PUMS students participated, 68% of students declared good and very good CPR knowledge [4]. In study conducted by Olejniczak et al., only 53% of nursing students concluded that the knowledge they acquired during studies (and after graduation) is sufficient to effectively provide FA [12].

Our research has some limitations. We could not get a comparable number of students in all faculties. The most numerous students were involved in fields of which the graduates frequently have to deal with patients in life-threatening situations. These were the: paramedic, medicine, nursing, dentistry students. It certainly indicates a greater interest in emergency medicine subjects. Therefore, unfortunately, it can be expected that the obtained results might be overstated. We are also aware that the assessment of knowledge may not correlate with CPR skills. It is difficult to assess whether the knowledge of CPR is sufficient, when each of the studied aspects is very important and can affect the victim's chances of survival. We believe that our criteria of "critical errors" and "quality indicators" are a compromise in this difficult assessment.

We are curious to compare our students to other medical universities in Poland. We did not find, however, research conducted in our country, which cross-section profile of the study group and the evaluation criteria would allow to direct comparison with our results and draw the appropriate conclusions. This probably increases the originality of our work.

Conclusions

1. The level of PUMS students' knowledge is insufficient.

2. It is suggested to evaluate or increase the number of hours dedicated to CPR classes. These activities should be carried out in various years of study so that the issues discussed could be systematically repeated
3. We should pay particular attention to identifying agonal breaths and all the criteria of quality CPR.
4. It is recommended to perform more detailed analysis to assess students of various directions.
5. The greatest attention should be given to students of the Faculty of Pharmacy and Medical Faculty II.

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REVIEW PAPER

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Civil liability of doctors and the employment relationship

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ABSTRACT

Article takes the issue of liability of doctors in connection with the provision of health services. The author suggests that this responsibility depends on the type of their employment by the therapeutic entity. So it is regulated by civil law and labor law. Therapeutic entity is as responsible as a company, ie. on the basis of risk. Responsibility of the doctor depends on whether he is employed on the basis of a civil contract, or contract of employment. The author widely discusses implications of different employment contracts. He indicates that the best situation the doctor has as an employee within the meaning of the Labor Code. His liability is in this case reduced to a minimum, i.e. up to 3 – monthly salaries. In the case of civil law contracts, the liability is unlimited. Therefore, the author recommends in the conclusions, that doctors should make contracts of liability insurance.

Keywords: doctor; hospital; employment relationship; patient; damage; liability.

The article is devoted to the civil liability that doctors bear due to provision of health services. This liability is placed primarily in the area of civil law, some of its aspects are also regulated by labor law. Due to the fact that it involves the civil liability of health care entities that employ doctors, especially hospitals, I also pay some attention to this issue at the outset.

The issue of the civil liability of health care entities is systematically gaining importance. This is a result of system and economic changes in the current economic and social reality. Thus, it is worth to briefly indicate the most important issues.

The principles of the civil liability are varied and they depend on its addressee. They are another basis for the responsibility of the hospital, another for a doctor and wider medical staff.

The responsibility of the hospital is associated with the functional aspect of its activities, while the liability of doctor is, among other, related to the employment relationship which connects him with the hospital.

Analyzing the legal aspects of the functioning of hospitals, in consequence of which may arise all sorts of damage, it is necessary to pay attention to the regulations governing enterprise's liability[1]. It is worth remembering that, at present, under the Act of 15 April 2011 on medical treatment[2], the hospital is treated just as an enterprise. According to art. 2 paragraph 1 point 9 of this Act – "the hospital is an enterprise of health care entity in which this entity carries out medical treatment such as hospital services". In point 8 of that paragraph, the enterprise is defined as a set of assets, through which the health care entity performs a specific type of medical activity[3].

The basic regulation in defining the civil liability of the hospital as an enterprise is art. 435 of the Civil

[1] This issue was discussed in detail, i.a., in the following comments to the Civil Code: E. Gniewek (ed.) Kodeks cywilny. Komentarz, Wydawnictwo C.H. Beck, Warszawa 2008; Z. Radwański, A. Olejniczak, Zobowiązania – Część ogólna, Wydawnictwo C.H. Beck, Warszawa 2008.

[2] Dz. U. 2011 Nr 112 poz. 654.

[3] Slightly different an enterprise is defined by art. 55 of the Civil Code. It provides that an enterprise is an organized set of tangible and intangible elements intended for conducting business activity. As shown, the difference consists in taking into account the intangible components and to indicate the goal of economic activity.

Code. This article regulates the liability for damages on the basis of the principle of risk[4]. This is a risk associated with the movement of the enterprise based on forces of nature and as a result of this risk damage may arise. According to the norm contained in it, the liability for damages is borne by the person who runs the enterprise.

At present hospital is classified as an enterprise moved by forces of nature as well[5]. This justifies a conclusion that its civil liability towards patients and third person is formed like liability of a enterprise. Therefore, it is responsible for all the events, as a result of which the damage associated with its movement may arise. It includes a responsibility for damage occurring during and in connection with the provision of health services by medical staff, especially doctors. In addition, random events which can't be predicted. For example, when a patient broke his hand, going to the toilet in the corridor. So, this type of responsibility extends very far. In the certain inevitability of such events, it's very difficult to avoid it.

Taking this fact into consideration, the legislature gave hospital various possibilities of defense against this type of liability for damages. Its basic principles are defined by the article indicated above – 435 of the Civil Code. This article allows for the exclusion of liability for damage caused by the movement of the hospital, if the damage is caused by force majeure, or solely by the fault of the victim or solely by the fault of a third party, for which hospital is not responsible. However, a conditions is to prove that one of the abovementioned situation has occurred. It must be proved by the hospital, because it involves certain legal consequences for the hospital. More specifically, the abolition of civil liability (cf. Art. 232 of the Code of Civil Procedure). However, this is not facile. An observation of judicial practice shows that proving of occurrence of one of evidence mentioned above which causes the exclusion of liability for damages in civil proceedings, often fails. Unless, it includes obvious cases. For example, a patient's inattention who enters the slippery floor in spite of the warning and consequently falls over. Or, the provision of stale foods to the hospital, consumption of which causes poisoning. The paradox lies in the fact that in such cases the compensation disputes generally do not occur.

It should agree with M. Nesterowicz that the damage in hospitals may concern two aspects of their functioning, i.e. "organizational fault" and the doctors' actions of and other medical staff[6].

Analyzing a little more closely this second aspect, it should be noted that, at present, among others, a type of employment relationship, which connects doctors to hospital decides about the civil liability of doctors. Currently, the most common is fact that doctors are employed in public hospitals on the basis of the so-called "contracts" ("kontrakt" in Polish), which are agreements of civil law and employment contracts. In the non-public hospitals, or clinics and private clinics doctors are employed also on the basis of contracts, or beyond them on the basis of contract of mandate or contract involving performance of medical services which is a variation of the previously mentioned contracts.

The importance of the type of employment for liability has clearly increased[7]. So, it is necessary to devote a little more attention to this issue. All the more that, due to the transformation of property relations, the responsibility of doctors from public hospitals is only a narrow fragment of their total liability.

In the past decades, the contract of employment was a common, widely used in practice basis of the employment relationship. A feature that distinguishes this agreement from the so-called civil contracts (contract, contract involving performance) is the specific subject of this contract. In fact, it is work performed personally, under the conditions of subordination, in a place designated by an employer, as well as at his risk[8].

A further reasoning in this regard, because of greater transparency, should be based upon the analysis of an example of the specific case of damage.

A claimant J. N. on September 19, 2008 had an accident on the way to work. As a result, he suffered a right knee meniscus injury. On October 12, the same year, in a public center of orthopedics, a surgical intervention was performed. After surgery, the claimant was discharged from the hospital with the recommendation of walking with elbow crutches. His disability to work

[4] Widely about this principle i.a.: A. Śmieja, Odpowiedzialność za szkody wyrządzone przez ruch przedsiębiorstwa (art. 435 k.c.), Prace naukowe Uniwersytetu Ekonomicznego we Wrocławiu – Research Papers of Wrocław University of Economics, nr 372 • 2014.

[5] Cf. art. 2 ust. 1 pkt 9 Ustawy z dnia z dnia 15 kwietnia 2011 r. o działalności leczniczej.

[6] M. Nestorowicz, Prawo medyczne, Toruń 2007, p. 334.

[7] Widely about this i.a.: P. Stępniak, Prawne aspekty odpowiedzialności cywilnej zakładu opieki zdrowotnej oraz jego personelu (in:) Sprawne zarządzanie zakładem opieki zdrowotnej (eds.) M. Głowacka, J, Galicki, Poznań 2010.

[8] Cf. Judgment of SN of 18 June 1998, I PKN 191/98, OSP 1999, nr 10, poz. 184; also: Kodeks pracy. Komentarz (ed.) B. Wagner, Gdańsk 2004, p. 35.

was determined until December 3, 2008. However, after this date, the claimant has continued to stay on medical sick leave. As a consequence, he was dismissed on April 26, 2009.

On June 22, 2009 he was admitted again to the center of orthopedics, where arthroscopic surgery and removal of loose body was performed. Three months later, he was examined by magnetic resonance imaging. This examination found an increased signal intensity spot of lateral meniscus, scattered posterior horn of the medial meniscus and a slightly increased amount of synovial fluid around the medial femoral condyle. Relying on the results of this examination, the claimant charged three doctors who had performed a surgical operation with a commission of medical error.

The first issue that must be resolved in this case, is a proper definition of the defendant. It is necessary to indicate how the employment relationship connects doctors with the hospital where the surgery was performed. Because, the claimant sued doctors for damages. So, the doctors were employed under a contract of employment. This resulted, on the side of doctors, in the lack of passive capacity to be a party. This capacity allows to be sued in the proceeding[9]. Doctors who didn't have this capacity, they couldn't be the defendants.

If so, it had to properly identify who can be sued. In this regard, the content of the two articles is helpful, i.e. Art. 430 of the Civil Code and 120 § 1 of the Labour Code.

It is worth quoting articles mentioned above. Article 430 of the Civil Code states the following:

who, on his own account, entrusts the performance of action to a person who, while performing the action, is subjected to his management and is obliged to follow his instructions, is liable for damage caused by a fault of the person during the performing of the entrusted action.

This regulation governs the rules of liability of a superior for a subordinate. Based on the interpretation of its content it must be assumed that the doctors were subordinate to the subject entrusting them to perform operations.

Therefore, follows from the wording of mentioned regulation, a material premise of liability for damage is the relation of management and subordination between a person entrusting the execution of activities and a person entrusted with the activity. From this perspective, a supervisor is a person who, on his own account, entrusts the performance of an action to a person who, while performing the action is subjected to his management and is obliged to follow his instructions. Whereas the subordinate is a person under this management and having the duty to obey the supervisor's instructions. The subordinate performs entrusted activity "on own account" of the supervisor.

An interpretation of Art. 430 of the Civil Code, leads to the conclusion that it is particularly important, for the relation of supervisor – subordinate, that the first isn't independent in the performance of a given activity. The concept of subordination can be understood broadly and narrowly. In the first case, this is the subordination of the general organization. In the second, this is just a subordination to the supervisor's instructions, formulated in the performance of the activity.

Consequently, a determination who is doctors' supervisor may raise some doubts. In fact, that can be two subjects or, as someone prefers – two people. Physical person or legal person. The first of these would be the hierarchical superior of defendants orthopedists. The second – the hospital employing them.

In civil law doctrine and jurisdiction of common court of law, the second of these approaches is particularly prevalent. Thus, it is assumed that the doctor's supervisor is not his hierarchical supervisor, indicated in the organizational structure of the hospital (e.g. chief surgeon), but the hospital itself as an institution that is a legal person[10].

However, a liability of hospital for acts of professionals, such as doctors, may raise doubts. In fact, they are entitled to a wide range of autonomy in making decisions[11]. This liability is formed not only by an existence of headship but also by the type of employment relationship, which connects them with the hospital. As a result, although hospital will be responsible for the damage caused to the patient by a doctor, as his supervisor, the ranges of this responsibility are highly diversified.

A general and necessary condition for its responsibility is that the damage was the consequence of the performance of health services entrusted to the doctor, and doctors as subordinates of hospital bear the

[9] Art. 65 and art. 194–196 of Act of 17 November 1946 – Kodeks postępowania cywilnego, Dz. U. nr 43, poz. 296 z późn. zm.

[10] The hospital is represented by its authority defined in the statute, i.e. most frequently by the Director. Cf. art. 38 of the Civil Code.

[11] Commentary to the Civil Code, ed. G. Bieńka, Warszawa 2005, p. 365.

fault for this damage. In other words, a responsibility of the direct perpetrator i.e. doctors, must occur[12]. This includes, in some simplification, a situation when a doctor performs medical services, for example surgery, in a faulty manner. It results mostly from a medical malpractice. It can be caused by various reasons, e.g. carelessness or negligence in diagnosing, performing treatments, using of inappropriate drugs, etc.

For the adoption of doctors guilt, it is not needed to prove that he violated the rules on the safety of life and human health. On the contrary, according to the opinion expressed by the Supreme Court (Sąd Najwyższy), it is sufficient that he desisted the principles of carefulness and safety arising from life experience and circumstances of the accident[13]. It is worth noting that while the premise for the hospital as a supervisor doctor is always subordinate fault, it is not required any superior fault.

Another premise of liability of health care institution as a doctor superior is to establish, if damage occurred during the action, not during opportunity of execution. The requirement of causing damage in the delegated act means that "between entrusting activities and action, which resulted in damage occurred, there should be a causal link".

Considering the nature of this relationship it should be mentioned about the article 361 & 1 of the Civil Code, according to which person required to compensation is liable only for the normal consequences of an act or abandonment from which the damage occurred. The normal consequences of action in the interpretation of this provision are the ones that – based on the life experience – can be considered as the effects of type of action or abandonment, in contrast to accidents, which extend beyond this rule[14].

It should be emphasized, that in the case law the Supreme Court consistently adopts the principle of the article 430 of the Civil Code, only if matter of damage is caused during delegated act, but not if the damage is caused during opportunity of execution. The criterion for distinction between this two situations is the aim of the perpetrator or the causation between the damage caused and the delegated activity.

If it is possible to state the guilt of the doctor, and the doctor is not employed under a contract of employment, the hospital and the doctor are jointly and severally liable for damage. For a doctor the responsibility is very far-reaching, because he responses with all his property.

There are exceptions from the principle of joint and several liability, referred in article 430 of the Civil Code. Legal structure of this exceptions is based on differences in the type of employment relationship between the doctor and the hospital. The best situation for the doctor is when he signed contract of employment, because he is in fact an employee within the meaning of the Code.

In favor of the doctor liability, labor laws are corrected radically. They are included in the basic act ie. Labor Code, which regulates the issues of employment, including medical staff.

It should be recalled that the definition of employee in labor law is constraining. According to art. 2 of the Labor Code an employee is a person employed under an employment contract, appointment, nomination or a cooperative contract of employment. Only employee benefit protection from civil liability provided in this Code. So, even though the work can be perform on the other basics than contract of employment, e.g.: on specific task contract, contract of mandate and agency agreement, the person that takes it will not use the protective elements from labor laws[15]. This also includes protection against liability.

Rules which protect doctors from liability are regulated in several law of the Labor Code. They are gathered in the Department Fifth in Chapter I. The first, that should be mentioned, is article 115. According to it, the employee is liable for damage within the limits of the actual loss incurred by the employer and only for the normal consequences of acts or abandonment from which damage resulted. This rule of law is very important, because at the same time it determines the scope of civil liability of the employer, i.e. health care entity and therefore the hospital.

However, the most important issue for establishing the rule of civil liability for orthopedic doctors who operate J.N. is art. 120 § 1 of the Labor Code.

According to this article, in the event of causing harm to a third party by the employee in the performance of his duties, only the employer is obligated to compensate damage. Commenting on the importance of the rule of liability of the hospital and its employees it should be referenced to The Resolution of 7 judges

[12] It also relates to a nurse, a medical analytics and other category of medical staff.

[13] Judgement of the Supreme Court, April 9, 1975, case number: II CR 140/75 (unreleased).

[14] Judgement of the Supreme Court, December 9, 1958, case number I CR 867/58, OSPiKA 1960, poz. 292.

[15] Labor Code, Comment ed. B. Wagner, Gdańsk 2004. p. 15

of the Supreme Court, June 12, 1976, case number III CZP on 5/76[16]. It provides as follows:

art. 120 & 1 of the Labor Code is a breakthrough from the rules adopted in the Civil Code in connection with receiving a third party in bringing substantive in relation to the perpetrator, because to repair the damage in such a system is required only workplace.

It should be emphasized that this provision applies to the hospital, regardless of whether it is public or not, the cooperative medical clinics and even private clinic. This means that in every case when a doctor is employed under a contract of employment for any committed by him mistakes and offenses, in particular for errors in medical malpractice, is responsible – and exclusively responsible– an institutional therapeutic entity that employs him.

The provision of article. 120 & 1 of the Labor Code is repealed, therefore, liability of doctor or other medical staff as employees to the injured patient. In other words, it deprives them locus standi, referred to in the cited resolution. If so, operating plaintiff doctors were not employed in orthopedics center under a contract of work, they would have such legitimacy. This means that they could be sued and be liable for the damage caused to him jointly and severally with the hospital, where they were operating.

The defendant doctors, however, were originally employed in the hospital under a contract of employment. So they used from the benefits of protection from civil liability provided in the article 120 § 1 of the Labor Code. It is always entitled to employees, regardless of whether a hospital is public or private.

Summing up all the comments that have been done so far, one request can be formulated that the doctor employed under a contract of employment is in a comfortable situation. His liability for defective treatment, negligence, etc. is in fact very seriously reduced. The hospital has indeed so recourse to the doctor, but the claims may be taken only when the hospital repaired the damage caused to the patient. Usually by paying him appropriate compensation, established either by agreement with him, or during court proceedings.

Regardless of the amount of compensation, construction of recourse to the doctor in the provisions of the Labor Code allows the hospital to call a doctor to return only a portion of its parts. It cannot exceed 3 – monthly salary (cf. article 119 of the Labor Code). So if hospital decides to investigate claims of recourse, it may with the consent of the employee deduct the amount of recourse from salary up to the amount of free classes in accordance with the provisions of the execution to the extent specified in the provisions of the Labor Code[17]. It is worth recalling that the amount of compensation is determined with salary at the time of injury[18] and calculated in accordance with § 3 of the Regulation of the Minister of Labor and Social Policy, May 29, 1996 on the method of determining the remuneration for the period of inactivity, and the remuneration constituting the basis for calculating the compensation, severance pay, compensatory allowances to salaries and other charges provided for in the Labor Code[19].

Payment of the amount of recourse in the manner indicated above allows to avoid high court costs. It also avoids the costs of enforcement proceedings initiated by the bailiff on the judgment of the court. In the absence of consent by the doctor, however, the hospital would have to take court proceedings.

It should be noted that the regression is the law of the hospital. So it may use it or not. If the hospital did not occur to the doctor with recourse claim, but does not have to do this, the doctor is not liable at all. An exception to this rule comes only when a doctor would do harm intentionally. Experience shows, however, that such situations are extremely rare. Another situation is when the hospital is insolvent or has been incorrectly insured, and finally, when the damage was caused not by performing medical services, but on the occasion of their execution (i.e. perform various duties of the employee).

To sum up, the patient, in our case the plaintiff, who had defective knee surgery can enforce his claims only from the center of orthopedics. The doctors who operated him, working on a contract of employment, are subject to the compensation regime specified in the labor code, but not much further-reaching rules of the Civil Code.

The last mentioned rules would apply in the case of employment doctors on the basis of a contract or a contract of mandate. So let's talk a bit more about this issue.

The legal situation in the employment of doctors and other medical personnel on the basis of a contracts is completely different. This contract is in fact

[16] OSNCP 1977, nr 4, poz. 61.

[17] Cf. art. 832 of Labor Code.

[18] Cf. The Resolution of 7 judges of the Supreme Court, case number: V PZP 4/75, OSNCP 1976, z. 1 . poz. 2.

[19] Dz. U. Nr 62, poz. 289.

a civil law agreement. Its essence is defined in art. 734 § 1 and 735 § 1 of the Civil Code. It is worth to cite their content.

Art. 734 § 1 – Person who accepts contract of mandate for work commits to provide a specific legal act for the principal

and

art. 735 § 1. If neither the contract nor the circumstances do not suggest that person who accepted the order committed to do it without pay, for the execution of the order should be paid.

As it can be seen, the contract of mandate is in many ways similar to the contract of employment. Nevertheless, there are several differences between them which are of a fundamental nature. The two most important differences based on the fact that the contractor does not perform the work under the direction of the principal, so the health care facility, but entirely on their own account, moreover, he does not need to work in a place designated by the client.

With these two differences, for existence and for the principles of civil liability for a doctor more important is first. If he does not provide medical services under the direction of a superior designated by the client, the latter is responsible for the damage together with the doctor by the principle of solidarity. This means that the patient can sue for damages both institutional therapeutic entity and a particular person providing health services, which directly caused the damage.

It is worth noting that for the doctor this situation is incomparably less favorable. In the case of employment on a contract of employment he is by law excluded from participation in the process (Labor Code). Quite different is his process situation in the case of employment on contract of mandate. The provisions of the Civil Code which regulate this situation, do not provide any limitations of it. As a result, the doctor must participate in the process, in solidarity with the health care facility that hired him. Solidarity in this case means, however, that the injured patient has the right to sue the doctor only. Patient may do it particularly in the case when therapeutic entity becomes, e.g. insolvent. In this case also, even if the judgment order to pay for damages jointly and severally from the hospital and doctor, the doctor is required to pay total compensation.

The same situation is in the case of employment on the basis of the so-called. contract. In either case, the doctor liability is essentially unlimited.

Ending this short presentation of the problems associated with the liability of doctors for any damage caused to patients during and in relation to medical services, it is worth asking a few conclusions.

1. Present labor market and health services are very flexible. They are a subject to the sharp rules of the economic game. This situation has its advantages and disadvantages. They are shown in today's legal regulations. It is worth to know them.
2. Legal regulations are very flexible, what allows people to customize various legal structures, in particular the various contracts to perform medical services adopted to the needs of employers doctors, as well as themselves. We must also remember that the various contracts not only provide varied benefits, but involve various risks. One of the most important risks is to reduce or increase the scope of liability of doctors for all sorts of errors and shortcomings, especially for malpractice. In this article I tried to show that risk, as well as consider how it can be reduced to a minimum. The greatest opportunities in this area gives the contract of employment. The problem is that not always the employer and the doctor want it to contain.
3. The most important, practical conclusion that follows from the article, is recommendation to insure against civil liability in connection with the medical profession. Especially against responsibility for medical malpractice.
4. The rules and scope of insurance must be determined in order to optimally protect doctor from medical liability in situations where malfunctioning would cause a patient harm[20].

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REVIEW PAPER

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The value of electron microscopy in the diagnosis of renal disease

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ABSTRACT

In the literature of recent years there are few publications on the importance of research in electron-microscopic pathomorphological diagnosis of kidney disease. The most important, which is diagnosed only on the basis of these studies are: minimal change disease, assessment of mesangial cell proliferation as well as the differentiation of the types of membranoproliferative glomerulonephritis, fibrillary glomerulonephritis, lupus nephritis, thin basement membrane disease, Alport syndrome, hemolytic-uremic syndrome. This report presents the most characteristic features of the ultrastructure allowing for diagnosis of these diseases.

Keywords: renal biopsy; kidney; electron microscopy.

Percutaneous kidney biopsy was introduced in clinical practice in the early 1950s. The first biopsies were carried out by Alwall on 13 patients already in 1944, however, the death of one of his patients discouraged Alwall from using biopsy as he considered it too risky [1]. He published his experience with renal biopsy only in 1952, one year after the publication by Iversen and Brun, who are considered the pioneers in this area [2]. From then on, biopsy of the kidneys was applied more and more often. At the same time, the technique of electron microscopy was introduced.

In Poland, electron microscopic examination was first introduced in Poznań by professor Janusz Groniowski in 1961. Kidney biopsy was applied earlier in Gdańsk, but without ultrastructural analyses.

To be emphasized is the fact that it was in kidney biopsies, that electron microscopy was applied for the first time to solve problems of human pathology.

Thanks to that research, a series of morphological and functional connections were discovered, in particular concerning the mesangium, the glomerular basement membrane and the juxtaglomerular apparatus.

Electron microscopic analyses made it possible to explain the character and location of series of changes observed in optical microscopy and revealed changes not discerned in optical microscopy.

At present, for 10–13% of biopsies, electron-microscopic analyses improve the initial histopathological diagnosis, and in 30–40% of the cases they serve to broaden the information obtained by optical microscopy.

Without examinations of the ultrastructure, the classification of glomerular diseases would have been impossible.

The best examples of diseases the diagnosis of which is based exclusively on electron microscopy are: minimal change disease, assessment of mesangial cell proliferation as well as the differentiation of the types of membranoproliferative glomerulonephritis, fibrillary glomerulonephritis, lupus nephritis, thin basement membrane disease, Alport syndrome, hemolytic-uremic syndrome.

Minimal change disease

The diagnosis of this glomerulopathy is based on changes concerning the podocytes consisting in the enlargement and subsequent effacement of the foot processes as well as the growth of microvilli on the sur-

face of the podocytes. The latter is of essential significance, as it lasts longer than the foot process effacement and helps to establish the diagnosis when the biopsy is performed after the application of treatment. The process effacement then in general no longer concerns 70% of the capillary loops, which is required for the diagnosis of this glomerulopathy [3, 4].

Assessment of mesangial cell proliferation

Sometimes very difficult to assess by histologic examination is mesangial cell proliferation. Endothelial cells and/or podocytes can, due to their location in the immediate proximity of the mesangial areas, erroneously be included in the mesangial areas and lead to the diagnosis of mesangial cell proliferation. This occurs in particular when the changes are not very pronounced and can even result in an erroneous diagnosis of mesangial proliferative glomerulonephritis. Obviously, immunofluorescence is equally of basic significance for the diagnosis of this glomerulopathy, but the material for this examination cannot always be obtained or the result of the reaction is uncertain. Then, a reliable diagnosis depends on the correct assessment of the mesangial hypercellularity (from 4 cells up) and the discovery of deposits in the mesangium [5–9].

Membranoproliferative glomerulonephritis

Electron microscopy is also essential for the diagnosis of the relevant type of membranoproliferative glomerulonephritis. All 3 types present the same optical microscopy image, i.e. a splitting of the glomerular structure, an increase in the number of mesangial cells, double contouring of the capillary loops found in specimens stained with silver salts. The most important ultrastructural feature here is the transposition of the mesangial cell processes on the loop perimeter between the endothelium and the basement membrane least marked in type II as well as the presence of subendothelial deposits (in type I) and subepithelial deposits (in type III). The definition of the location of deposits as either subendothelial or subepithelial cannot be achieved by histologic examination nor by immunofluorescence, but only by electron microscopy. Electron microscopy is particularly important for the diagnosis of type II, which is based on the appearance of a blackening of the lamina densa. This is possible only with electron microscopy. Regardless of possible further developments regarding the classification of this glomerulopathy (exclusion of type II and association of type I with type III), these finds are still of indisputable significance.

The basic feature of membranoproliferative glomerulonephritis, i.e. the transposition of mesangial cell processes, occurs also in other glomerular changes, for example in case of a rejection of a transplanted kidney and in the hemolytic-uremic syndrome [10–14].

Fibrillary glomerulonephritis

This glomerular disease can only be diagnosed by electron microscopy. It is characterized by the presence of fibrillar deposits measuring 18 to 20 nm. These deposits are found in the mesangial matrix and in the glomerular basement membranes [15–17].

Lupus nephritis

Ultrastructure examinations can be helpful also in the assessment of lupus-related changes.

Among other ultrastructural changes of sometimes essential diagnostic significance, the presence of so-called virus-like inclusions must be mentioned. This change occurs in glomerular endothelial cells and is caused by a deformation of the channels of the endoplasmic reticulum which is typical for lupus nephritis. This nephritis is accompanied by fingerprint-like deposits which are probably the result of changes in the DNA [18–23].

Thin basement membrane disease

Thin basement membrane disease, like minimal change disease, cannot be diagnosed by histologic examination. Sometimes the thinning of the basement membranes can be observed in specimens stained with the Jones' method, but this occurs extremely rarely. Also, this result does not provide sufficient basis for a definite diagnosis of this syndrome. For this, an assessment of the ultrastructure is absolutely necessary.

A thickness of maximum 250 nm of the lamina densa of the basement membrane has been assumed for the diagnosis of this syndrome. In addition, the change must involve the majority of the capillary loops [24–30].

Alport syndrome

In case of the Alport syndrome the light microscopy image can be diverse. The presence of immature glomeruli together with mature glomeruli and various glomerular changes, as well as the presence of interstitial cells with a foamy cytoplasm are considered as fairly characteristic. However, these changes appear also in other diseases and do not allow for a final diagnosis. Significant are, on the other hand, changes observed in electron microscopy consisting in an uneven thickness

of the basement membrane of the renal glomeruli and the characteristic splitting of the lamina densa. Other symptoms of this syndrome, apart from kidney disorders, such as hearing loss or ocular manifestations, do not always occur and usually appear late, and genetic analyses are performed very rarely. Electron microscopy is therefore decisive in these cases.

Structural changes of the lamina densa of the basement membrane are sometimes observed also in other glomerulopathies, for example in thin basement membrane disease. They are then, however, discrete, concern only small sections and consist in a thinning rather than a splitting of the structure [31–34].

Hemolytic-uremic syndrome

The light microscopy image of the hemolytic-uremic syndrome has no specific features, the glomerular changes are quite diverse: presence of thrombi, detachment of endothelial cells, double contouring of loop walls, loop wall thickening. Sometimes, these changes are indiscernible. The electron microscopy image is more characteristic. Apart from the transposition of the mesangial cell processes in the initial phase in the space created by the detachment of endothelial cells, it reveals the presence of a plasma-like matter. This matter encloses small fibrin fibers, fine myofibrils, platelets or platelet fragments, endothelial cells or endothelial cell fragments, detached mesangial cell processes. Detached endothelial cells retain some of their functions, such as the production of basement membrane. The mesangium shows changes called „netting", mesangiolysis and the presence of fibrin deposition. All these changes are identified above all by electron microscopy. They can persist for a certain time in recovering patients. In case of the occurrence of exponents of kidney damage in such patients a certain time after the acute symptoms have subsided, only the result of an electron microscopy can determine, if they are connected with the hemolytic-uremic syndrome or if they are symptoms of another glomerulopathy. This may involve difficulties with regard to the differentiation from the extremely rarely diagnosed fibrillary glomerulonephritis [35–38].

Focal segmental glomerulosclerosis

An important role is played by electron microscopy in detecting early changes, yet elusive in the light microscope. Here one should mention first of all, the early phase of glomerular sclerosis [39–42].

In recent years, the literature reveals little of the position concerning the importance of electron- microscopic study. Outweigh issues of immunology.

Some even say that the role of electron microscopy in pathomorphological practice is declining. Kidney pathology and in particular the pathomorphology of glomerulopathies represent a very strong argument against this view. Here, the value of electron microscopy cannot be overestimated. This is why pathomorphologists insist so strongly on securing biopsy material for such analyses.

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All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

List all authors if there are six or fewer; if there are seven or more, list first six follower by "et al.". Journal names should be abbreviated according to Index Medicus.

Some examples
Standard journal articles

1. Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet. 2012 Sep;49(9):578–590.

2. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013 Mar;45(3):279–284.

Books
Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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