PŮVODNÍ PRÁCE

ORIGINAL PAPER

Changes in essential and trace elements content in degenerating human intervertebral discs do not correspond to patients' clinical status

Změny v obsahu esenciálních a stopových prvků v lidských degenerujících meziobratlových ploténkách nekorespondují s klinickým stavem pacientů

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Abstract

Aim: To date, there has been no paper considering the disc degeneration process in respect of the content of essential and trace elements in degenerating discs tissue, clinical status of patients, and imaging analysis. Concentration of essential and trace elements in disc tissue may be a consequence of both environmental and genetic factors. The study aims to analyse and assess the contents of essential and trace elements in intervertebral discs. *Patients and methods:* The material of 19 intervertebral discs was obtained from 17 patients during lumbar discectomy. Control was 9 healthy discs obtained from organ donors. Atomic absorption spectrometry was used to assess levels of Cu, Fe, Mn, Pb, Zn, Na, Mg, K, Ca, and P in the tissue, as well as dry weight (d.w.) of the tissue. *Results:* All 10 essential and trace elements were detected in all samples. A significant increase of Ca, Mg, Fe, and P, and decrease of Cu and K in operated discs was found; the remaining changes between unhealthy and healthy discs were not significant. There were no age / elements, Pfirrmann grade / elements, or Modic grade changes / elements correlations. A significant positive correlation was found between Mg and Zn, K and Fe, Ca and Zn, Ca and Mg, P and Zn, P and Mg, and P and Ca. A negative correlation was only indicated between age and Na. Ca levels were higher in the degenerating disc group than in the healthy group. *Conclusion:* A lack of correlation between the Ca content and the stage of intervertebral disc degeneration as well as the age of patients in the degenerating disc group is an unexpected result.

Souhrn

Cíl: V současné době neexistuje odborná literatura, která by se zabývala degenerativním onemocněním plotének z pohledu obsahu esenciálních a stopových prvků v tkáni degenerující ploténky, klinického stavu pacientů a zobrazovací analýzy. Koncentraci esenciálních a stopových prvků ve tkáni ploténky mohou ovlivňovat jak environmentální, tak genetické faktory. Studie analyzovala a hodnotila obsah esenciálních a stopových prvků v meziobratlových ploténkách. *Soubor a metody:* Od 17 pacientů byl v průběhu lumbární diskektomie odebrán materiál z 19 meziobratlových plotének. Jako kontrola sloužilo 9 zdravých disků získaných od dárců orgánů. Pomocí atomové absorpční spektrometrie byla určena suchá hmotnost (s.h.) tkáně a hladiny Cu, Fe, Mn, Pb, Zn, Na, Mg, K, Ca a P ve tkáni. *Výsledky:* Ve všech vzorcích bylo detekováno všech 10 esenciálních a stopových prvků. V operovaných ploténkách byl zaznamenán významný nárůst hladin Ca, Mg, Fe a P a pokles Cu a K. Ostatní rozdíly v nemocných a zdravých ploténkách nebyly významné. Nebyly nalezeny žádné korelace mezi věkem a prvky, stupněm degenerace dle Pfirrmanna a prvky nebo změnami typu Modic a prvky. Významná pozitivní korelace byla nalezena mezi Mg a Zn, K a Fe, Ca a Zn, Ca a Mg, P a Zn, P a Mg a P a Ca. Negativní korelace byla naznačena jen mezi věkem a Na. Hladiny Ca byly ve skupině degenerujících plotének vyšší než u zdravých plotének. *Závěr:* Překvapivým výsledkem je chybějící korelace mezi obsahem Ca a stupněm degenerace meziobratlové ploténky, stejně jako mezi obsahem Ca a věkem pacientů ve skupině s degenerací meziobratlové ploténky.

Introduction

The process of degeneration of human intervertebral discs is a problem which has been investigated in a number of ways, and yet there are still many elements which remain unexplained. Many putative factors concerning its aetiology have been suggested, such as genetics, which seem the most important, as well as causes of an environmental origin [1–5].

The trigger point in disc degeneration is an injury of the disc, and the changes following the injury are aberrant cell-mediated responses to the structural damage [1].

Factors like age, genetic inheritance, and a history of inadequate transport and loading of metabolites can weaken discs to such an extent that structural failure occurs during daily activities such as physical effort, or even sneezing or coughing [1,2].

At the moment, we are faced with the chicken and egg scenario – which came first, the chicken or the egg? Are the structural changes a cause or an effect of the disc degeneration process?

Many factors and substances were examined in intervertebral discs to answer the following questions: 1. what is disc degeneration; 2. what triggers it, and 3. how does the process continue.

A degenerating intervertebral disc undergoes many changes which are different in nature, such as biomechanical (structural damage) or biochemical (levels of many complex substances as well as chemical elements in the tissue are changing).

In this paper, the author would like to focus attention on the chemical changes in degenerating intervertebral discs, and the relationship of these changes to the clinical status of the patients.

Excessive deposition of selected elements in an avascular adult intervertebral disc can lead to the acceleration of a cascade of adverse metabolic changes that normally occur during ageing; such a situation reflects negatively the stability of the intercellular matrix of disc pulpose.

To date, the correlations between changes in the contents of selected elements in the intervertebral discs and the clinical patients'

status have not been studied. Such examination might provide more light on the pathogenesis of the disc degeneration process and perhaps newer therapeutic approaches.

The first aim of this study was an evaluation of the differences of the essential and trace elements concentration in intervertebral disc tissue between healthy people and patients with degenerative changes. We decided to analyse (according to the other authors [6,7]) the following elements: Cu, Fe, Mn, Pb, Zn, Na, Mg, K, Ca, and P.

The second purpose of our study was to investigate the correlation of the content of these elements with clinical characteristics of the patients with intervertebral disc degeneration.

Patients and methods

The study was approved by the Bioethics Committee of the Medical University of Silesia (decision number KNW/0022/KB/42/15). Nineteen specimens were obtained from

Tab. 1. Gender, age, location, level of surgery and complaint level in Visual Analogue Scale in patients in the experimental group.

Sample No.	Case No.	Age	Gender	Level	Site	VAS
1	1	35	F	L5/S1	R	4
2	2	58	F	L4/L5	R	6
3	3	35	M	L5/S1	L	10
4	4	28	F	L4/L5	L	6
5	4	28	F	L5/S1	L	4
6	5	23	F	L4/L5	L	6
7	6	60	M	L4/L5	L	10
8	7	32	M	L5/S1	L	6
9	8	21	M	L5/S1	R	5
10	9	52	M	L4/L5	L	8
11	10	59	M	L4/L5	L	8
12	11	35	F	L4/L5	L	6
13	12	58	M	L4/L5	L	4
14	13	36	M	L5/S1	L	9
15	14	58	M	L3/L4	L	6
16	14	58	M	L4/L5	L	5
17	15	28	M	L5/S1	L	8
18	16	48	M	L4/L5	L	10
19	17	41	F	L4/L5	L	8

F – female; M – male; L – left; R – right; VAS – Visual Analogue Scale

Tab. 2. Degeneration changes of discs and adjacent vertebral bodies assessed in Pfirrmann and Modic scales in the presented group.

Sample No.	Case No.	Pfirrmann grade	Modic type
1	1	3	N
2	2	5	2
3	3	5	N
4	4	5	2
5	4	4	2
6	5	5	2
7	6	5	2
8	7	3	3
9	8	2	N
10	9	5	N
11	10	4	2
12	11	4	N
13	12	4	N
14	13	5	N
15	14	5	N
16	14	5	2
17	15	4	N
18	16	5	N
19	17	4	2

N – no Modic type changes

Tab. 3. The contents of elements in intervertebral discs.

Sample No	Dry matter %	Cu	Fe	Mn	Pb	Zn	Na	Mg	K	Ca	P
						mg × kg⁻¹ dry matter					
1	28.9	3.05	128.7	< 0.15	< 0.6	12.9	16,500	3,300	1,100	3,700	1,400
2	32.8	< 0.6	80.1	< 0.15	< 0.6	51.1	10,500	14,000	1,000	76,900	39,800
3	24.3	0.58	154.4	< 0.15	< 0.6	19.5	16,700	5,500	1,400	26,600	13,500
4	30.9	0.99	182.3	0.27	< 0.6	13.2	21,000	4,500	1,300	9,600	4,100
5	35.4	0.25	190.7	0.24	< 0.6	28.0	16,300	3,800	1,200	7,100	3,400
6	29.4	< 0.6	68.6	0.26	< 0.6	15.5	16,900	2,900	1,000	2,900	700
7	28.2	1.05	185.9	< 0.15	1.75	18.4	15,700	2,600	1,300	4,300	1,700
8	30,0	1.33	141.9	< 0.15	1.65	16.4	15,500	3,600	1,200	8,700	4,100
9	29.0	< 0.6	168.2	< 0.15	< 0.6	14.6	16,900	5,000	1,400	17,500	8,700
10	29.9	< 0.6	161.0	< 0.15	< 0.6	30.1	14,100	7,500	1,300	41,700	20,100
11	31.4	< 0.6	90.6	< 0.15	< 0.6	12.3	14,400	5,700	900	8,000	3,800
12	28.1	< 0.6	171.5	< 0.15	< 0.6	15.6	16,700	3,700	1,600	6,100	2,800
13	35.8	1.54	79.2	< 0.15	< 0.6	13.7	16,400	3,100	1,000	2,700	400
14	30.7	1.29	139.5	< 0.15	< 0.6	16.4	15,700	3,500	1,400	6,500	3,300
15	37.1	< 0.6	125.9	< 0.15	0.88	22.7	13,800	6,100	1,200	27,100	13,100
16	31.9	3.19	224.7	1.22	2.45	62.2	14,900	3,800	1,300	16,200	7,700
17	35.1	2.56	254.3	< 0.15	< 0.6	21.0	21,200	10,500	2,000	3,000	1,200
18	34.2	< 0.6	101.3	< 0.15	< 0.6	7.6	9,500	2,600	1,000	4,400	1,800
19	34.2	1.30	152.1	0.20	< 0.6	24.9	21,900	5,500	1,800	11,600	4,800

17 patients (7 women, mean age 41.7 years) during lumbar discectomies. All specimens were prolapsed lumbar intervertebral disc. All patients matched two basic criteria: 1. discectomy was their first spinal surgery; 2. standard pre-operational 2-month pharmacotherapy was not effective. Sixteen samples were collected from the left site, and three from the right side. Seven specimens were obtained from L5/S1, eleven from L4/L5, and one from L3/L4 level. All patients were examined and interviewed; data regarding age, gender, level and site of operation, as well as intensity of the pain measured by means of Visual Analogue Scale (VAS) [5,8] were collected and are presented in Tab. 1.

Before surgery, all patients underwent a MRI examination. In this examination, each intervertebral disc and vertebral body were analysed in terms of degeneration stage (Pfirrmann grade) [9], level of surgery, and Modic type endplate changes in adjacent vertebral bodies [10] (Tab. 2).

The control group was constituted of nine intervertebral discs collected from three patients diagnosed with brain death, who were referred to be organ donors. All these discs

were then evaluated for the presence of degenerative changes by two qualified board-certified pathologists. Only completely healthy discs, with no degenerative changes, were included as controls in the research.

Laboratory analyses

Immediately after surgery, specimens were deep frozen at –80 °C and stored. All samples of intervertebral discs were determined in terms of: dry matter (d. m.) and contents of Cu, Fe, Mn, Pb, Zn, Na, Mg, K, Ca and P.

The d. m. content of analysed discs was determined by weighing a sample before and after complete drying at 105 °C. On the basis of the difference in sample mass before and after drying, the percentage of dry mass was calculated.

In order to determine the content of elements, material was digested using the wet method in a closed system in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). About 0.5 g of d. m. of samples was treated with a 7 cm³ mixture (1 : 6 v/v) of concentrated acids, HCl and HNO₃ (Suprapure, Merck, Darmstadt, Germany). Digestion was conducted in teflon vessels with maximum power of the

oven (1,400 W) for 25 min. The concentration of elements in the obtained filtrate was determined with the use of a PerkinElmer Optima 7300 DV (PerkinElmer, Inc., Waltham, MA, USA), inductively coupled plasma optical emission spectrometer. Each analysis was repeated to ensure the accuracy of the result. If the replication analyses results differed from one another by more than 5%, another two analyses were conducted for that same sample. The results were given in mg × kg⁻¹ d. m.

Statistical analysis

Data were analysed using the Statistica 8.0. computer software (StatSoft, Inc., Tulsa, OK, USA). All variables were tested for normality of distribution using the Shapiro-Wilk test. Statistical analysis was conducted using the nonparametric Kruskal-Wallis / Mann-Whitney U test with post-hoc Dunna test as well as the analysis of variance for parametric data (Analysis of Variance [ANOVA] test with post-hoc RIR Tukey test). The correlation rate was calculated using the Spearman's test. The Spearman rank correlation coefficient was determined. Statistical significance was set at a P value of less than 0.05.

Tab. 4. The comparison of elements content in healthy and degenerating discs (mg × kg⁻¹ dry matter).

Elements	Healthy discs					Degenerating discs					
	N	mean	SD	min.	max.	N	mean	SD	min.	max.	P
Cu	9	5.90	1.51	3.50	9.22	19	1.19	0.88	0.25	3.19	0
Fe	9	104.60	16.84	75.00	125.60	19	147.42	49.90	68.60	254.30	0.016075
Mn	9	0.20	0.16	0.15	0.62	19	0.23	0.24	0.15	1.22	0.595047
Pb	9	0.81	0.32	0.60	1.47	19	0.83	0.52	0.60	2.45	0.498235
Zn	9	21.76	6.91	13.79	32.29	19	21.90	13.59	7.60	62.20	0.410187
Na	9	14,300	200	10,800	17,800	19	16,000	3,100	9,500	21,900	0.076289
Mg	9	300	100	200	400	19	5,100	2,900	2,600	14,000	0
K	9	300	1,100	1,700	4,500	19	0.13	300	900	2,000	0.000001
Ca	9	400	200	200	700	19	1.50	18,200	2,700	76,900	0
P	9	1,300	300	800	1,600	19	0.72	9,500	400	39,800	0.004277

N – number of patients; SD – standard deviation

Results

The content of Fe, Zn, Na, Mg, K, Ca, and P in the discs were detected in high levels in all samples. Trace elements (Cu, Mn, Pb) were considered as present when their concentration exceeded 0.6 mg × kg⁻¹ d.m.

Mn was detected in 5 (26%) samples, Cu in 8 (42%) samples, and Pb in 4 (21%) samples.

The ranges of concentrations for particular elements were as follows (mean value ± standard deviation, range resp.) (Tab. 3).

Cu mean was 1.19 ± 0.88 mg × kg⁻¹ d.m., range of 0.25–3.19 mg × kg⁻¹ d.m. in degenerating discs, while in the healthy ones the mean was 5.9 ± 1.51 mg × kg⁻¹ d.m., range of 3.5–9.22 mg × kg⁻¹ d.m.

Ca mean was 1.50 ± 1.82% of d.m., range of 0.27–7.69% of d.m. in degenerating disc, while in healthy ones the mean was 0.04 ± 0.02% of d.m., range of 0.02–0.07% of d.m.

Fe mean was 147.42 ± 49.94 mg × kg⁻¹ d.m., range of 68.60–254.30 mg × kg⁻¹ d.m. in degenerating discs, while in healthy ones the mean was 104.57 ± 16.84 mg × kg⁻¹ d.m. range of 75.00–125.60 mg × kg⁻¹ d.m.

K mean was 0.13 ± 0.03% of d.m., range of 0.09 –0.20% of d.m. in degenerating disc while in healthy ones the mean was 0.30 ± 0.11% of d. m., range of 0.17–0.45% of d.m.

Mg mean was 0.51 ± 0.29% of d.m., range of 0.26–1.40% of d.m., in degenerating disc, while in healthy ones the mean was 0.03 ± 0.01% of d.m., range of 0.26 –1.4% of d.m.

Mn mean was 0.23 ± 0.24 mg × kg⁻¹ d.m. range of 0.15–1.22 mg × kg⁻¹ d.m. in degenerating disc, while in healthy ones the mean was 0.20 ± 0.16 mg × kg⁻¹ d.m., range of 0.15–0.62 mg × kg⁻¹ d.m.

Na mean was 1.60 ± 0.31% of d.m. range of 0.95–2.16% of d.m. in degenerating disc, while in healthy ones the mean was 1.43 ± 0.20% of d.m., range of 1.08–1.78% of d.m.

P mean was 0.72 ± 0.95% of d.m. range of 0.04–3.98% of d.m., in degenerating disc, while in healthy ones the mean was 0.13 ± 0.03% of d.m., range of 0.08–0.15% of d.m.

Pb mean was 0.83 ± 0.32% of d.m., range of 0.60–1.47% of d.m. in degenerating disc, while in healthy ones the mean was 0.83 ± 0.52% of d.m., range of 0.60 –2.45% of d.m.

Zn mean was 21.90 ± 13.59 mg × kg⁻¹ d.m., range of 7.60–62.20 mg × kg⁻¹ d.m. in degenerating disc, while in healthy ones it was 21.76 ± 13.59 mg × kg⁻¹ d.m., range of 7.60–62.20 mg × kg⁻¹ d.m.

The differences between healthy and degenerating discs appeared significant for Cu, Fe, Mg, Ca, K and P (Tab. 4).

The levels of K and Cu were higher in healthy discs, while levels of P, Ca, Mg and Fe were higher in degenerating discs.

In the surgery group, the correlation analysis revealed a significant negative relationship between age and sodium content. A positive correlation was indicated between VAS and Pfirrmann grade, Fe and K, Zn and Mg, Cu and P, Na and K, Mg and Ca, Mg and P (Tab. 5).
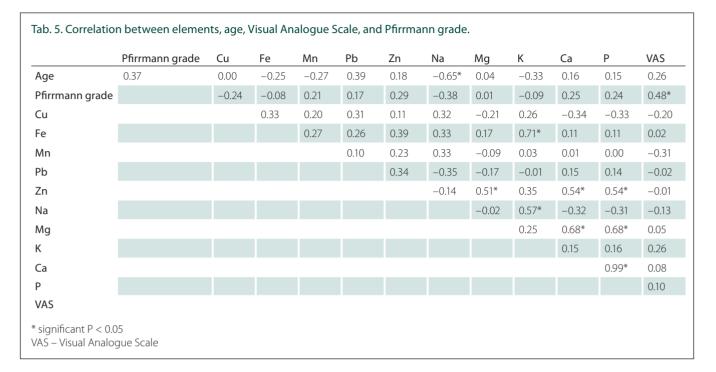
Discussion

Analysis of selected trace elements in degenerating intervertebral discs showed a significant increase of Fe, Mg, Ca and P, and a decrease in Cu and K as compared with healthy ones.

Calcium

Calcium is one of 21 essential elements for humans [11]; it regulates many intracellular and extracellular processes. Dysregulation in production, level, or transport of Ca is always associated with a disease. Ca deposits are well known elements of disc degeneration; however, the role of the deposits in the degeneration process is still unclear. Several papers showed that there was a correlation between the presence of Ca crystals and disc degeneration phenomenon [12–14]. Some data advocate that deposits present in degenerated intervertebral discs are made of calcium pyrophosphate dihydrate, and this phenomenon is more associated with the previous history of trauma or surgery [12]. On the other hand, there are data which state that the calcium pyrophosphate dihydrate is both the cause and effect of disc degeneration [13]. In the literature, there is still a lack of quantitative analysis; therefore, data contained in this paper may be helpful in understanding the process of disc degeneration. In the degenerating disc group, there was no correlation between age and Ca levels, as well as between Ca levels and Pfirrmann grade of degeneration. Considering the differences in Ca concentration between healthy and degenerating discs, there was a higher Ca content in degenerating discs than in healthy ones.

Copper

Copper is an active metal characteristic for organisms living in an oxygen-rich environment. It is associated with animal pro-

Tab. 5. Correlation between elements, age, Visual Analogue Scale, and Pfirrmann grade.

	Pfirrmann grade	Cu	Fe	Mn	Pb	Zn	Na	Mg	K	Ca	P	VAS
Age	0.37	0.00	−0.25	−0.27	0.39	0.18	−0.65*	0.04	−0.33	0.16	0.15	0.26
Pfirrmann grade		−0.24	−0.08	0.21	0.17	0.29	−0.38	0.01	−0.09	0.25	0.24	0.48*
Cu			0.33	0.20	0.31	0.11	0.32	−0.21	0.26	−0.34	−0.33	−0.20
Fe				0.27	0.26	0.39	0.33	0.17	0.71*	0.11	0.11	0.02
Mn					0.10	0.23	0.33	−0.09	0.03	0.01	0.00	−0.31
Pb						0.34	−0.35	−0.17	−0.01	0.15	0.14	−0.02
Zn							−0.14	0.51*	0.35	0.54*	0.54*	−0.01
Na								−0.02	0.57*	−0.32	−0.31	−0.13
Mg									0.25	0.68*	0.68*	0.05
K										0.15	0.16	0.26
Ca											0.99*	0.08
P												0.10
VAS												

* significant P < 0.05
VAS – Visual Analogue Scale

teins involved in reduction-oxygenation processes [3]. Many enzymes harness the changes in the Cu oxidation stage to catalyse redox reactions in a numerous range of biochemical transformations [15]. Cu plays an important role in cell haemostasis, and cell signalling processes. Moreover, Cu handling and Cu utilising proteins control metabolic changes in cancer cells known as the Warburg effect – the down-regulation of cell respiratory capacity observed in cancer cells [15]. In examined groups, there was a higher concentration of Cu in the healthy group, which may be a consequence of improved oxygenation and improved blood supply to healthy disc tissue [4].

Iron
The role of Fe in many processes is difficult to overestimate. As a very important component of haemoglobin, it plays a significant role in oxygen transport. There are four classes of Fe-related proteins: Fe containing haeme proteins (haemoglobin, myoglobin, cytochromes), iron sulfur enzymes (flavoproteins, haemaflavoproteins), proteins for Fe storage and transport (transferrin, lactoferrin), and other Fe-containing and Fe-activated enzymes. The role of Fe in the disc degeneration process is still unknown, and there are no papers describing this topic [16]. The content of Fe was higher in degenerating discs, and this result was statistically significant.

Sodium and potassium
Sodium and potassium as well as their attendant anions are important components of all body fluids [17]. Na and K play a principal role in maintaining body fluid homeostasis. Levels of these ions are important for water balance, and disc dehydration is one of the components of disc degeneration. These two ions are important in the creation of nerve impulses, using concentration gradients across plasma membrane produced by Na(+), K(+) adenosine triphosphate (ATP)-ase [18].

Our results show higher levels of K in healthy discs, and a negative correlation between age and the content of Na; both results are statistically significant.

Magnesium
Magnesium is the second most abundant intracellular cation, and fourth cation in terms of abundance for the whole body. This cation is essential for the synthesis of nucleic acids and proteins, and plays a role in Ca metabolism by competing with Ca for membrane binding. Mg has many important biological functions, such as intracellular energy metabolism cell replication, and protein synthesis [19]. Levels of Mg were higher in degenerating discs and the difference was statisticallys ignificant.

Manganese
Manganese is essential for bone formation. It plays an important role in the metabolism of amino acids, lipids, and carbohydrates. Glycosyltransferases and xylotransferases are important in proteoglycan synthesis and they are very sensitive in the presence of Mn. Thus, the latter can play a role in the disc degeneration process [6]. The levels of Mn in healthy discs and degenerating discs were similar, and the difference was not statisticallys ignificant.

Phosphorus
Numerous normal physiologic functions are dependent on P, including skeletal development, cell membrane phospholipid content and function, cell signalling, platelet aggregation, and energy transfer through mitochondrial metabolism [6]. P is essential for the bone mineralisation process [20]. The level of P was higher in degenerating discs, and the difference was statistically significant.

Lead
Lead accumulates in bones and its concentration tends to increase with age, because lead is difficult to remove from the tissue [21]. More than 90% of the body's Pb burden is found in the skeleton [8]. The biological half-life of lead is about one month for soft tissue, it is longer – years – for trabecular bones, and decades for cortical bones [22]. Pb can cause several adverse health effects, such as neuropathy, encephalopathy, and kidney damage. Pb levels in intervertebral discs

should not be high, because most Pb cumulates in bones. This is the case in the presented group, where only four specimens showed Pb levels higher than 0.60 mg × kg⁻¹; the difference between healthy and degenerating discs was not significant.

Zinc

Zinc is a component of various enzymes; it forms and helps to maintain the structural integrity of proteins and regulates gene expression [16]. The biological role of Zn can be divided into three categories: structural, catalytic, and regulatory. Zn plays a crucial role in the immune system, and Zn-deficient individuals present increased susceptibility to infection [23]. The inflammatory process in disc degeneration is still to be examined and at the moment we know that it is a part of the whole degeneration process [1,24,25]. Moreover, matrix metalloproteinases are Zn-dependent enzymes, and these enzymes are responsible for extracellular matrix synthesis and degradation. Balance between these two processes is a basic condition to stop the degeneration process [1,24]. Zn levels may indirectly indicate a metalloproteinase concentration and activity in the disc tissue. The difference in Zn levels between healthy and degenerating tissue was not statistically significant.

Conclusions

The study is one of only a few to present elements concentration in vertebral disc tissue; moreover, there are no papers analysing either a healthy control group or the clinical status of the patients analysed (MRI images and elements contents).

The results showing differences between healthy and degenerating discs in terms of Ca levels are particularly important. There is a statistically significant difference be-

tween healthy and degenerating discs (the level of Ca is higher in degenerating discs), while there is no correlation between Ca levels and the age of the patient, and Ca levels and disc degeneration stage. The examined group may be too small to demonstrate such correlation, but if this fact is confirmed upon further examination, questions regarding Ca chemistry and its role in disc degeneration process should be formulated.

Levels of other elements may be influenced by diet, and other exogenous factors such as contamination which is associated with technological development of the dwellingl ocation.

Identification of significant factors as well as their influence on the disc degeneration process are both issues still demanding further investigation.

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