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Comparison of levels of toxic trace elements in two most common spinal pathologies in three different tissues

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Keywords
trace element, lumbar spinal stenosis, lumbar disc herniation, inductively coupled plasma mass spectrometry

ABSTRACT

Aim: The toxic trace element levels in serum, bone (lamina), and intervertebral disc tissues of patients with lumbar spinal stenosis and lumbar herniated nucleus pulposus (HNP) which are the two most common spinal pathologies were determined, and it was investigated whether they have a role in the pathophysiology of these pathologies.

Materials and methods: Cadmium (Cd), aluminium (Al), arsenic (As), mercury (Hg), and lead (Pb) levels in serum, intervertebral disc, and bone (lamina) tissue of patients with HNP (=20) and 30 with lumbar spinal stenosis (LSS) (n=30) were determined by Inductively Coupled Plasma Mass Spectrometry technique.

Results: LDH group Cd serum level was found to be significantly higher than LSS group Cd serum level (p=0.024). Al disc level in the HNP group was found to be significantly higher than the Al disc level in the LSS group (p=0.038). While As serum level increased in LDH group, it was determined that As bone level increased very significantly (r= 0.699, p=0.001). In the LSS group, it was determined that the Hg disc level increased significantly as the Hg serum level increased (r=0.608, p<0.01). On the other hand, as the Hg serum level increased in the LDH group, the Hg disc level also decreased significantly (r= -0.579, p<0.01).

Conclusion: The difference in toxic trace element levels seen in these pathologies has been discussed in terms of possible causes in light of current literature. The findings of our study support the hypothesis that toxic trace elements may be effective in lumbar disc degeneration.

INTRODUCTION

It is known that some environmental factors and especially toxic trace elements that are not essential for human health pass into the human body with contamination and cause physiological and pathological

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negative effects (1). However, there is not adequate and satisfactory information about the effects of presence of these toxic elements in the human body in acceptable amounts or what kind of effects they have on the organism in the amounts below the permissible levels in food, drinking water or air. Moreover, the accumulation of these toxic elements in the body and the possibility that they may play a role in the pathophysiology of some different diseases that occur in chronic degenerative processes or may appear as predisposing factors should not be forgotten.

In this study, it was investigated whether five toxic substances (aluminum, arsenic, mercury, lead, cadmium) have an effect on lumbar herniated nucleus pulposus and chronic intervertebral disc degeneration. For this reason, two patient groups with lumbar HNP and LSS thought to be caused by intervertebral disc degeneration but with different pathogenesis were compared (2). In the HNP, disc degeneration generally develops in a shorter time due to axial loading and annulus fibrosis tears. However, in LSS degenerative changes are thought to be due to a more chronic inflammatory process which affects ligaments, bones, discs and facet joints (3,4). In LSS, it is predicted that the process occurs mostly with biomechanical effects as a result of instability developing on the basis of degenerative changes due to the aging process of the spine (5). Although intervertebral disc degeneration is a common feature in both diseases, it is assumed that they have different pathophysiological basis due to their histopathological differences (6). In this way, toxic trace elements in both groups of diseases were determined in serum, bone and disc tissue, and the effects of these substances on the degenerative process were investigated.

Material and method

After receiving the approval of the Yozgat Bozok University Clinical Research Ethics Committee (12.10.2016 / 68 dated 12.10.2016), the blood, bone (lamina) and intervertebral disc samples were collected from the patients who had undergone lumbar spine pathology operation in the Department of Neurosurgery of Yozgat Bozok University Faculty of Medicine between 2016-2017. Trace element levels in the samples were measured by ICP-MS (Inductively Coupled Plasma - Mass Spectrometry). The patients were divided into two groups as lumbar spinal stenosis (LSS) and lumbar disc herniation (LDH) by preoperative magnetic resonance imaging (MRI). Patients with rheumatic diseases such as rheumatoid arthritis and ankylosing spondylitis, under the age of 18, with missing file information or missing radiological images, and who underwent surgery due to trauma were excluded from the study. Patients with the possibility of prolonged or intense exposure to the trace elements mentioned in the study were excluded from the study. The diagnosis of LSS or HNP was made according to clinical and radiological findings. 20 out of 50 patients were diagnosed with L-HNP and 30 with LSS. Patients who underwent discectomy in addition to total laminectomy in the LSS group were included. In LSS group, patients who underwent only laminectomy and did not undergo discectomy were excluded from the study. Patients who use cigarettes and alcohol, and patients with diseases that require continuous drug use such as hypertension, diabetes, and goiter were excluded from the study.

Statistical Analysis: These measured levels were grouped according to radiological findings. Three replicates were obtained for each sample analyzed and their mean value was taken into account for the concluded assessment. If the required statistics are given, it is evaluated by SPSS 20.0 statistical program. A normality test was used to determine whether sample data of groups a normally distributed. The correlations between toxicological variables among groups were assessed with Pearson correlation test. The parametric and nonparametric tests were carried out to determine the association of main parameters concerning among groups. All tests were considered significant at $p < 0.05$.

Results

Median age was 53 years old (mean:53.84, std: ±12,55, min: 25, and max: 76). In L-HNP (n=20) median age was 35 and 9 of them were female and 11 were male. In LSS group (n=30), mean age was 54 and 18 of them were female and 22 were male. Mean, median, standard deviation, minimum, and maximum levels of 5 toxic trace elements were showed in three different tissue presented in Table 1.

Table 1. General descriptive values of the study

n=50 Unit - [µg/L]	Age	Cd			Al			As			Hg			Pb		
		Bone	Serum	Disc	Bone	Serum	Disc	Bone	Serum	Disc	Bone	Serum	Disc	Bone	Serum	Disc
Mean	53.84	0.31	0.49	0.54	27.13	30.57	2.47	0.82	0.53	1.16	0.26	0.21	0.03	14.76	5.93	6.34
Median	53.00	0.32	0.51	0.45	26.32	31.00	2.22	0.65	0.49	0.37	0.02	0.17	0.03	12.91	2.93	3.09
Std. Deviation	12.55	0.11	0.06	0.43	18.14	2.01	1.40	0.66	0.59	2.10	0.41	0.14	0.03	8.59	12.54	8.76
Minimum	28.00	0.02	0.26	0.07	8.64	21.15	0.39	0.03	0.00	0.02	0.00	0.00	0.00	1.75	0.06	0.34
Maximum	76.00	0.48	0.58	1.81	149.01	33.67	5.73	3.77	3.89	12.30	2.28	0.53	0.12	47.72	81.65	41.41

Table 2. The comparison of the toxic trace elements in between LSS and L-HNP groups

		LSS (n=30)		LDH (n=20)		p
		Mean	SD	Mean	Sd	
Cd	bone	0.30	0.10	0.32	0.12	p>0.05
	serum	0.47	0.07	0.51	0.04	**0.024***
	disc	0.57	0.47	0.50	0.35	p>0.05
Al	bone	28.20	23.24	25.54	4.44	p>0.05
	serum	30.39	2.25	30.85	1.58	p>0.05
	disc	2.14	1.25	2.97	1.50	**0.038***
As	bone	0.79	0.58	0.88	0.77	p>0.05
	serum	0.44	0.28	0.66	0.86	p>0.05
	disc	1.54	2.62	0.57	0.53	p>0.05
Hg	bone	0.30	0.46	0.19	0.33	p>0.05
	serum	0.22	0.16	0.18	0.11	p>0.05
	disc	0.03	0.03	0.03	0.02	p>0.05
Pb	bone	15.76	9.81	13.26	6.28	p>0.05
	serum	7.84	15.97	3.06	1.29	p>0.05
	disc	6.82	9.88	5.61	6.93	p>0.05
LSS-Lumbar Spinal Stenosis; HNP-Herniated Nucleus Pulposus; SD- Standard Deviation; *significant						

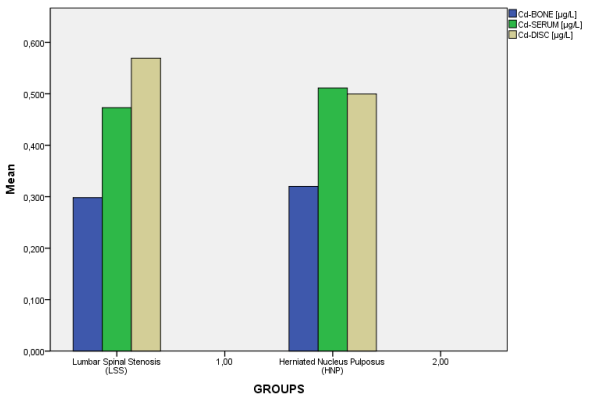
Cadmium (Cd) evaluation:

The lowest Cd level was observed in bone tissue in both LSS and HNP groups (0.30±0.10 µg/L and 0.32±0.12 µg/L, respectively). While the Cd serum level in the L-HNP group was found to be significantly higher than the Cd serum level in the LSS group (0.51±0.04 µg/L and 0.47±0.07 µg/L, p=0.024, respectively), the Cd bone level was also found to be higher, although not significantly, in the HNP group (p>0.05). On the other hand, Cd disc level was higher in the LSS group (p>0.05). However, for both groups, it was observed that Cd accumulated more in disc tissue rather than bone. In addition, while Cd serum and bone levels were inversely proportional in the LSS group, this was directly proportional in the HNP group.

Table 3. Correlations of Cd levels in bone, serum and disc tissues in both groups

Groups		Cd_Bone [µg/L]	Cd_Serum [µg/L]	Cd_Disc [µg/L]
LSS (n=30)	Cd_Bone [µg/L]	1	-,119	-,036
	Cd_Serum [µg/L]	-,119	1	,106
	Cd_Disc [µg/L]	-,036	,106	1
HNP (n=20)	Cd_Bone [µg/L]	1	,182	,284
	Cd_Serum [µg/L]	,182	1	,319
	Cd_Disc [µg/L]	,284	,319	1

Graphic 1. Graphical view of mean Cd levels in bone, serum and disc tissues



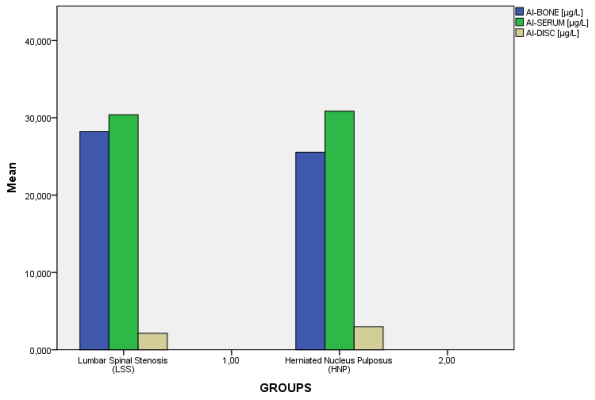
Aluminum (Al) evaluation:

The lowest Al level was observed in the disc tissue in both LSS and HNP groups (respectively, 2.14±1.25 µg/L and 2.97±1.50 µg/L). Al disc level in the HNP group was found to be significantly higher than the Al disc level in the LSS group (respectively, 2.97±1.50 µg/L and 2.14±1.25 µg/L, p=0.038). In both groups, the means were close to each other for each matrix. However, Al was found to accumulate in the bone tissue for both groups.

Table 4. Correlations of Al levels in bone, serum, and disc tissues in both groups

Groups		Al_Bone [µg/L]	Al_Serum [µg/L]	Al_Disc [µg/L]
LSS (n=30)	Al_Bone [µg/L]	1	,062	,269
	Al_Serum [µg/L]	,062	1	-,001
	Al_Disc [µg/L]	,269	-,001	1
HNP (n=20)	Al_Bone [µg/L]	1	-,053	-,205
	Al_Serum [µg/L]	-,053	1	,202
	Al_Disc [µg/L]	-,205	,202	1

Graphic 2. Graphical view of mean Al levels in bone, serum, and disc tissues



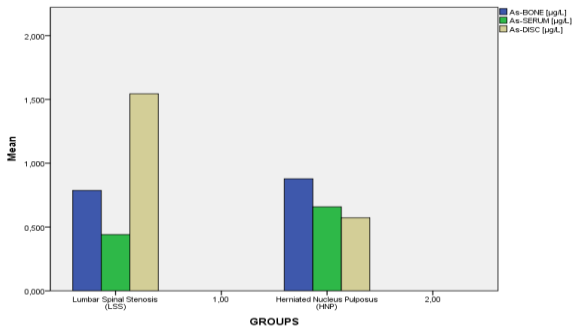
Arsenic (As) evaluation:

In LSS group, when as serum level increased, As bone level decrease was detected (r= - 0154, p=0.416). Whereas in L-HNP group, As serum level increased in HNP group, As bone level increased significantly (r= 0.699, p=0.001). LSS group As mean levels were found to be approximately 3 times higher than HNP group As mean levels.

Table 5. Correlations of as levels in bone, serum and disc tissues in both groups

Groups		As_Bone [µg/L]	As_Serum [µg/L]	As_Disc [µg/L]
LSS (n=30)	As_Bone [µg/L]	1	-,154	-,200
	As_Serum [µg/L]	-,154	1	,320
	As_Disc [µg/L]	-,200	,320	1
HNP (n=20)	As_Bone [µg/L]	1	**,699****	,288
	As_Serum [µg/L]	**,699****	1	,156
	As_Disc [µg/L]	,288	,156	1

Graphic 3. Graphical view of mean As levels in bone, serum and disc tissues



Mercury (Hg) evaluation:

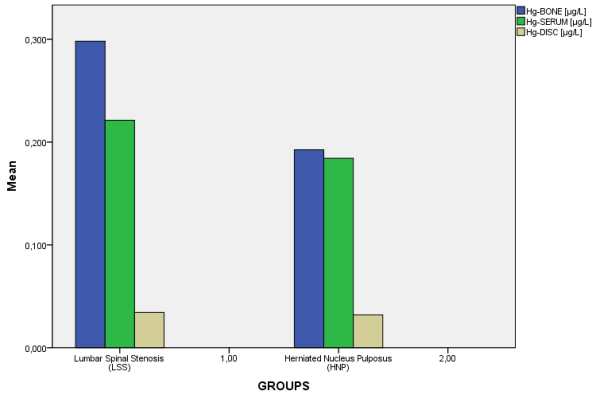
The lowest Hg levels were observed in the disc tissue in both the LSS and HNP groups (0.03±0.03 µg/L and 0.03±0.02 µg/L, respectively). The highest Hg levels in all tissues were detected in bone tissue in both groups (0.30±0.45 µg/L and 0.19±0.33 µg/L, respectively). Although not statistically significant, the Hg level in the LSS group was found to be higher in all three tissues than in the HNP group (p>0.05). In the LSS group, as the Hg serum level increased, the Hg disc level increased significantly (r=0.608, p<0.01). On the other hand, as the Hg serum level increased in the HNP group, the Hg disc level decreased significantly (r= -0.579, p<0.01). Again, an increase in Hg bone level was detected with the increase in Hg disc level in both groups (p>0.05).

Table 6. Correlations of Hg levels in bone, serum and disc tissues in both groups

Groups		Hg_Bone [µg/L]	Hg_Serum [µg/L]	Hg_Disc [µg/L]
LSS (n=30)	Hg_Bone [µg/L]	1	,190	,053

	Hg_Serum [µg/L]	,190	1	**,608****
	Hg_Disc [µg/L]	,053	**,608****	1
HNP (n=20)	Hg_Bone [µg/L]	1	**-,579****	,023
	Hg_Serum [µg/L]	**-,579****	1	,348
	Hg_Disc [µg/L]	,023	,348	1

Graphic 4. Graphical view of mean Hg levels in bone, serum and disc tissues



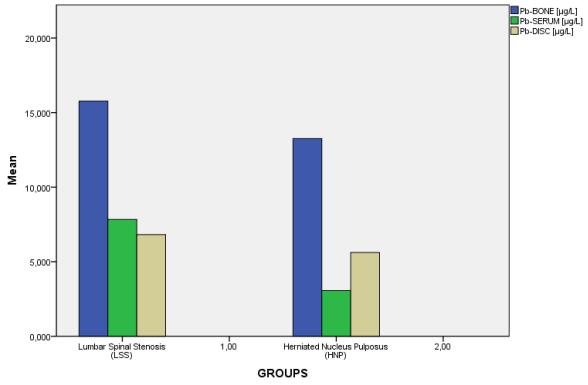
Lead (Pb) evaluation:

It was detected that the Pb bone level was approximately 2 times the Pb disc level for both the LSS and HNP groups (15.76±9.81 µg/L and 6.82±9.88 µg/L for LSS, respectively; 13.26±6.28 µg/L and 5.61±6.93 µg/L for HNP). The highest Pb level in all tissues was detected in bone tissue in both groups (15.76±9.81 µg/L and 13.26±6.28 µg/L, respectively). Although not statistically significant, it was observed that as Pb serum level increased in both groups, Pb bone and Pb disc levels decreased (p>0.05). Again, an increase in Pb bone level was detected with the increase in Pb disc level in both groups (p>0.05). While the lowest Pb level was observed in the disc tissue in the LSS group, it was found in the serum in the HNP group.

Table 7. Correlations of Pb levels in bone, serum and disc tissues in both groups

Groups		Pb_Bone [µg/L]	Pb_Serum [µg/L]	Pb_Disc [µg/L]
LSS (n=30)	Pb_Bone [µg/L]	1	-,302	,341
	Pb_Serum [µg/L]	-,302	1	-,148
	Pb_Disc [µg/L]	,341	-,148	1
HNP (n=20)	Pb_Bone [µg/L]	1	-,189	,109
	Pb_Serum [µg/L]	-,189	1	-,060
	Pb_Disc [µg/L]	,109	-,060	1

Graphic 5. Graphical view of mean Pb levels in bone, serum and disc tissues



Discussion

Mechanical effects, aging, genetic, systemic and toxic factors are held responsible for the pathophysiology of intervertebral disc degeneration (7). However, the accumulation of toxic trace elements in intervertebral discs and the effects they may create have been little studied in the literature. Kubaszewsk et al. compared a number of trace element levels in intervertebral disc and bone tissue and concluded that disc tissue provides a more stable environment for elemental analysis (8). In another study, Nowakowski et al. determined the trace element in the degenerative intervertebral disc and pointed out that the levels are very different from the levels in other tissues and the existence of positive and negative correlations between the elements (9). Although several trace element level determinations and comparisons have been made in the literature in the form of human and animal studies, especially for bone, in this study, which we have done for the first time, the amount of toxic trace elements in intervertebral disc degeneration, which is thought to develop with different pathophysiological mechanisms in two different diseases, has been compared (10-12).

The effects of essential and non-essential trace elements on human bone and their toxic threshold limits are stated in plasma, blood, and urine, and it is said that no toxic side effects are observed below

these amounts (13). However, the effects of these trace elements on body metabolism at under the toxic levels and their relationship with some chronic diseases have not been clearly demonstrated yet. In our study, the serum, bone and intervertebral disc levels of five toxic trace elements (Cd, Al, Pb, Hg, and As) were compared and the results were tested to see if they were statistically significant. Cd element was found to be lowest in bone in both LSS and HNP groups (0.30±0.10 µg/L and 0.32±0.12 µg/L, respectively).

On the other hand, it was found that it accumulated more in the intervertebral disc tissue than in the bone in both groups and was slightly higher in the LSS group. The serum level of Cd in the HNP group was significantly higher than the serum level of the LSS group. In addition, while Cd serum and bone levels were inversely proportional in the LSS group, this situation was found to be directly proportional in the HNP group. It is known that the Cd can have toxic effects in many organs in humans (14). Osteotoxic and osteoporotic effects were observed especially in the male and female groups made on bone (15,16). Experimental cell culture studies have shown that the Cd element triggers the apoptosis effect on rat osteoblasts with its mechanisms (17).

Again, in experimental studies, it was determined that the Cd element decreases the bone collagen content and increases the collagen solubility (18). In another experimental study performed on male rats, it was concluded that the compact bone microstructure was changed, the bone weight was affected due to decreased the vascular structure which resulted in secondary osteoporosis and decreased potential bone mechanical properties (19). The higher Cd element in the intervertebral disc in our study suggests its relationship with degenerative processes.

Studies have shown that Cd element increases osteoclastic differentiation and turns the process in favor of degeneration (20). Disc degeneration is a multifactorial process, and effects such as the initiation of disc degeneration by one or more of these factors and the insufficiency of the regeneration process, especially in HNP cases developing as a result of axial loading with acute process, by preventing regeneration with the negative effect of the Cd element, rapidly expanding the annulus tear and as a result suggesting the hypothesis that the disc may be mechanically protruding. The high level of serum Cd in the HNP group in our study supports this hypothesis. In addition, it has been determined that the Cd element has a vasoconstrictor effect on the vascular system (21). For this reason, it was thought that the nutrition of the intervertebral disc may be impaired secondarily and accelerate the degenerative process. Since the osmotic supply of the intervertebral disc would be disrupted, it has been hypothesized that the Cd element may continue to accumulate in the disc space and increase the degenerative process. As a result, it is predicted that the Cd element may directly initiate the degenerative process, either as a cofactor or as a predisposing factor in this process.

Aluminum (Al), another toxic trace element, was found to be higher in the intervertebral disc of the HNP group than in the LSS group in our study (2.97±1.50 µg/L and 2.14±1.25 µg/L, respectively, p=0.038). Al element is known to cause dementia, osteomalacia and microcytic anemia especially in dialysis patients at toxic doses (22). However, the effects on the human body in amounts below toxic limits and in chronic processes have not been clearly determined yet, and the perception of a safe metal with little effect on human health continues (23). Al element is found in almost all human body organs and tissues. The highest concentration was found in the bones after the lungs, and it was reported that nearly half of the body Al element was in the skeletal system (24). Although these rates change in patients exposed to excessive Al, such as dialysis patients, the amount of Al may also change with osteoporosis that develops with aging, and the increase in osteoclast cell activity and resorption.

There are also studies showing that it is not associated with the development of osteoporosis in elderly patients exposed to normal amounts of Al (25). In our study, bone Al element levels in both groups were found to be higher than other tissues, which is consistent with the literature. However, the fact that Al element, which was found significantly in our results, was at higher levels in the intervertebral discs belonging to the HNP group, again suggests that it may cause a degenerative event in the formation of disc herniation in the acute period, as well as the fact that the intervertebral disc, which has degenerated for some reasons and lost its normal osmotic nutrition feature, may have accumulated more Al element.

The second possibility seems more logical since there was no significant difference in bone and serum levels of Al element.

Another toxic trace element, arsenic (As), can have a toxic effect on almost all body organs and tissues (26). Although the effect of the As element, which can be stored in the bone, on the bone has not been clearly demonstrated clinically, it has been shown to cause osteoporosis by inhibiting osteoblastic activity in a few experimental studies (27,28). In our study, while there was no significant difference between the element levels between the two patient groups, it was found that while the serum level of As increased in the HNP group, the bone level also increased in direct proportion. As mentioned before, the degenerative event in the LSS group is not limited to the intervertebral disc, but develops as a result of an osteoporotic degenerative process that also affects the facet joints and the spine. On the other hand, the HNP group is a group that causes intervertebral disc degeneration and protrusion of the nucleus in a shorter time, but no osteoporotic degeneration of the bones is observed. Therefore,

As toxic element forms apatite arsenate and possibly other calcium arsenate crystals by competing with the phosphate groups in hydroxyapatite crystals via alkaline phosphatase in the HNP group where bone turnover continues (29,30). In the LSS group formed on an osteoporotic background, it will not be possible for the As element to form crystals in the bone as a result of loss of mineralization and the shift of metabolism to catabolism. For this reason, it was thought that the increase of As element in bone tissue of HNP group along with the increase in serum level may be due to the increase in arsenate crystals in the bone. In support of this assumption, in the literature, in an elderly female patient who was chronically exposed to As element, with the dissolution of calcium arsenate crystals as a result of increased bone resorption during and after menopause, As blood levels increased to toxic levels and gave clinical symptoms and signs as a result of many organ damage (31).

The other toxic trace element mercury (Hg), which we investigated in our study, was found to be the lowest at the disc level for both groups. In the literature, there is no clear data or many studies on the effects of Hg element on human bone, and bone toxicity is not mentioned. In a study conducted on men, no correlation was found between the increase in Hg level in the blood and lumbar bone mineral density, and even as in some studies, it was observed that the incidence of bone fracture development decreased with the increase of the Hg element level in the blood (32,33,34). Another data we obtained is that as Hg serum level increases in the LSS group, the Hg disc distance level also increases, and it decreases inversely with the serum level in the HNP group. Although these data suggest that the bone density is tried to be preserved with Hg increase in the LSS group, which is an osteoporotic group, this would be a weak assumption that needs proof.

Our last toxic trace element lead (Pb) was found in the bone and in the intervertebral disc distance in both groups, approximately twice its levels. It is known that Pb element has many systemic effects and is stored more in the bone in adults (35). In a study on growing rats, it was found to impair bone development (36,37). The result we found is that the Pb element is mostly found and stored in the bone tissue.

As a result, today toxic elements pose a great threat to the environment and human life (38,39). Determining the standard amounts of toxic trace elements in different organs and tissues in the human body presents difficulties. In the literature, toxic trace element amounts have been detected in some tissues obtained from cadaver studies (40,41). However, within the framework of ethical rules, it is obvious that toxic trace element research can be done and a connection with diseases can be made in materials such as biopsies taken in some diseases that require surgery. Supporting meaningful results from clinical studies with experimental studies will accelerate research on toxic elements.

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