Research Article

The Assessment of the Essential Trace Elements Concentration by the Instrumental Neutron Activation Analysis in Patients with Degenerative Lumbar Disc Disease

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Received: 03 February 2021; Accepted: 15 February 2021; Published: 24 February 2021

Citation: Sanjar Kochkartaev, Shatursunov Shahaydar, Abdusattarov Khurshid, Elena Danilova, Natalija Osinskaya. The Assessment of the Essential Trace Elements Concentration by the Instrumental Neutron Activation Analysis in Patients with Degenerative Lumbar Disc Disease. Journal of Spine Research and Surgery 3 (2021): 010-016.

Abstract

Objective: To evaluate the concentration of essential trace elements in herniated lumbar intervertebral disc in relationship with age of patients and stages of lumbar degenerative disc disease.

Material and methods: Prospective observational study. The specimens of the lumbar disc herniations were obtained from patients who underwent conventional transforaminal endoscopic discectomy. The instrumental neutron activation analysis was applied to determine the concentration of microelements in lumbar disc herniations. Statistical analyses performed by descriptive statistics and Student's t-
tests. The level of significance was set to \( p < 0.05 \).

**Results:** A total of 44 patients who underwent transforaminal endoscopic lumbar discectomy, of these 16 (38.09%) patients had lumbar disc herniation at the level of L3-L4, whereas 28 (62.01%) patients had lumbar disc herniation at the level of L4-L5. The instrumental neutron activation analysis was demonstrated that the mean concentrations of essential trace elements such as Au, Br, Cr, Hg, Ni, Sc, Fe, and Co were declined by aging and progression the degenerative-destructive process, at the same time the content of other trace elements such as K, Se, Zn, Mn, Zn, Cl and Ca were increased, while the concentrations of Ag and La were relatively constant (all \( p \) values<.001).

**Conclusion:** This study revealed that the significant differences in the trace element concentrations in lumbar herniated discs between different age groups of patients, as well as the different stages of lumbar degenerative disc disease.

**Keywords:** Lumbar Degenerative Disc Disease

1. **Introduction**

Lumbar degenerative disc disease is the global burden public health issue due to the high prevalence and substantial impact on health-related quality of life and rising healthcare costs [1]. A systematic review and methanalysis of the Global Burden of Diseases 2016 was reported that 266 million patients (3.63%) worldwide are diagnosed with lumbar degenerative disc diseases with low back pain annually [2]. The global lumbar disc replacement device market was estimated approximately as US$ million in 2020, and it was predicted that this figure will rise to 13.5 per cent annually over the forecast period from 2020 to 2027 years [3]. The lumbar disc herniation or an extrusion of the nucleus pulposus intruding into the lumbar spinal canal, has been suggested as the most frequent reason of the lower back pain [4]. The most-at-risk area of the formation of herniated discs either at L4-L5 or L5-S1, which is impinge on the L4, L5 or S1 nerve root [5]. The treatment modalities for degenerative lumbar disc disease cover a wide range of conservative, surgical and nonsurgical interventions. To date, a stem cell therapy cell therapy has focused great interest as it promotes a regenerative potential of intervertebral discs [6].

In spite of the significant issue of symptomatic lumbar disc herniation to the healthcare system, the issue of prevention and treatment of degenerative disc disease of the spine is remained challenging throughout the years to date [7, 8]. It should be recognized that conservative treatment of degenerative lumbar disc disease are effective in the earlier stages of disease. The surgical interventions are effective in the later stages, however the long-term risk factors such as recurrent lumbar disc herniation and subsequent lumbar disc degeneration are observed in clinical practice [9]. The prevention of the degenerative lumbar disc disease can be discovered through the fundamental investigations in fields of nanoscience and nanotechnology [10]. Thus, our research aimed to evaluate of trace element concentration in herniated lumbar intervertebral disc by the instrumental neutron activation analysis. In future, the outcome of this research can be used to a new pharmaceutical therapy or to a novel tissue engineering approaches, that might prevent the degenerative disc disease in the lumbar spine.

2. **Materials and Methods**

A prospective study, which was carried out in accordance with the International Ethical Guidelines an Declaration of Helsinki. The ethical approval for this study was obtained from appropriate bioethics committee. The database included data from patients
The study material included surgical specimens of herniated disc tissue obtained from 44 patients who underwent single-level, conventional transforaminal endoscopic lumbar discectomy. The preoperative T2- weighted magnetic resonance imaging was used to assess the degeneration status of the operated lumbar disc in accordance with Pfirrmann grading system. The database were divided into three groups according to the stage of development of disc herniation (Pfirrmann grading system) and age of patients. Sample preparation technique. The herniated disc tissue fragments were washed with distilled water and dried to constant weight in a drying cabinet at a temperature not exceeding 60°C. The dried samples were ground in a porcelain mortar to a homogeneous mass, then weighed (two weighed portions: 40 mg for analysis for short-lived radionuclides and 90-100 mg for analysis for medium- and long-lived radionuclides). All biological samples were labeled in specific plastic bags. The study biomaterials were tranfered to the laboratory of the Institute of Nuclear Physics of Academy of Science of the Republic of Uzbekistan.

The Instrumental neutron activation analysis (INAA) was used to determine the concentrations of the trace elements such as iron, manganese, nickel, strontium, zinc, bromine, calcium, chlorine, chromium, potassium, sodium, nickel, rubidium, selenium, lanthanum, antimony, mercury, scandium, hafnium, europium, cobalt, gold, and silver, which were calculated using the dry weight (dw) of the disc. To calculate the half-life of a radioactive element the different time modes were used for irradiation, cooling and measurements. A gamma-spectrometry system for activation analysis was used; the following time modes of analysis were proposed: I) irradiation time-15 seconds, cooling time-240 minutes (4 hours measurement time-100 seconds, III) irradiation time-900 minutes, cooling time-240 minutes (4 hours), measurement time-100 seconds, IV) irradiation time-900 minutes (15hours), cooling time-720 hours (30 days), measurement time-400 seconds.

- Determination of short-lived radionuclides. The samples together with the standards were packed in a polyethylene container and irradiated in the vertical channel of the reactor with a neutron flux of 5.1013 neutron / cm 2.sec for 15 sec. Measurement of the induced activity was carried out twice - 15–10 min after irradiation to determine chlorine and 4 hours later - to determine sodium, copper, potassium and manganese;

- Determination of medium-lived radionuclides. To determine the calcium, bromine, lanthanum, gold; the samples were wrapped in aluminum foil and irradiated in the wet channel of the reactor for 15 hours. Measurement of the induced activity was performed on the tenth day after irradiation using the corresponding nuclides.

- Determination of long-lived radionuclides. To determine the content of long-lived radionuclides, samples irradiated for 15 hours were measured a month after irradiation by the corresponding γ-lines.

To register the induced activity, a detector made of high-purity germanium (V = 120 cm³) with a resolution of 1.8 keV along the Co-60 gamma line and a gamma spectrometer with computer software were used. Data processing was carried out using the Genie™ 2000 Basic Spectroscopy Software. The maximum error of the activation method for determining the elements did not exceed 12%. The accuracy of the determination of this or that element was checked by comparing the

Journal of Spine Research and Surgery
obtained data with the certified values of the IAEA standard reference samples (IAEA-336, IAEA-375) and NIST Standard Reference Material 1572 - CITRUSLEAVES. Statistical analyses were performed using Microsoft Excel 2019 and SPSS for Windows, version 18.0 (IBM SPSS Inc., New York, USA). Statistical analyses performed by descriptive statistics and Student’s t-tests. Data are presented as Mean ± SD (standard deviation) for continuous variables. The level of significance was set to p < 0.05.

3. Results

The mean age of patients in the (A) group was 29.5 years (range 24-36 years); it was included 12 tissue fragments, which was the corresponding Pfirrmann grade III. The mean age of patients in the (B) group was 41.2 years (range 37-44 years), it was included 16 tissue fragments, which was the corresponding Pfirrmann grade IV. The mean age of patients in the (C) group was 53.4 years (range 45-60 years), it was included 16 tissue fragments, which was the corresponding Pfirrmann grade V. The results of this study demonstrated that the mean concentrations of essential trace elements such as Au, Br, Cr, Hg, Ni, Sc, Fe, and Co were decreased by aging and progression the degenerative-destructive process, however, at the same time the content of K, Se, Zn, Mn, Zn, Cl and Ca were increased, while Ag and La were relatively constant (all p values < .001) (Table 1).

| Chemical elements | I Group (A) | II Group (B) | III Group (C) |
|-------------------|-------------|--------------|---------------|
| Iron, 26Fe        | 52.000 ± 9.2000 | 48.000 ± 4.9000 | 36.00 ± 7.0000 |
| Manganese, 25Mn   | 1.3000 ± 0.3600 | 0.9800 ± 0.2200 | 1.600 ± 0.4300 |
| Nickel, 28Ni      | 26.00 ± 16.000  | 11.00 ± 0.5600 | 14.00 ± 4.9000 |
| Strontium, 38Sr   | 11.00 ± 2.8000  | 1.4000 ± 0.3600 | 7.500 ± 2.50000 |
| Zinc, 30Zn        | 15.000 ± 0.9000 | 14.00 ± 1.10000 | 19.00 ± 1.50000 |
| Bromine, 35Br     | 18.000 ± 3.2000 | 7.1000 ± 0.79000 | 9.200 ± 0.54000 |
| Calcium, 20Ca     | 3400.0 ± 1100.0 | 1700.0 ± 290.000 | 5500.0 ± 330.00 |
| Chlorine, 17Cl    | 1400.0 ± 610.00 | 1000.0 ± 310.000 | 1200.0 ± 520.00 |
| Chromium, 24Cr    | 0.7800 ± 0.1300 | 0.5300 ± 0.06000 | 0.5200 ± 0.04400 |
| Potassium, 19K    | 760.00 ± 240.00 | 870.00 ± 120.000 | 950.00 ± 230.000 |
| Sodium, 23Na      | 6100.0 ± 1500.0 | 5600.0 ± 670.000 | 6900.0 ± 1100.0 |
| Nickel, 28Ni      | 26.00 ± 1.6000  | 11.000 ± 0.56000 | 14.000 ± 4.90000 |
| Rubidium, 37Rb    | 0.720 ± 0.02000 | 0.830 ± 0.13000 | 0.740 ± 0.14000 |
| Selenium, 34Se    | 0.300 ± 0.02500 | 0.3600 ± 0.07300 | 0.3700 ± 0.04100 |
| Lanthanum, 57La   | 0.018 ± 0.00750 | 0.0120 ± 0.00210 | 0.0140 ± 0.00390 |
| Antimony, 51Sb    | 0.0360 ± 0.0059 | 0.0330 ± 0.00850 | 0.0260 ± 0.00740 |
| Mercury, 80Hg     | 0.0210 ± 0.0065 | 0.0100 ± 0.00250 | 0.0130 ± 0.00390 |
| Scandium, 23Sc    | 0.0065 ± 0.00150 | 0.0059 ± 0.00096 | 0.0030 ± 0.00049 |
| Hafnium, 72Hf     | 0.0077 ± 0.00310 | 0.0070 ± 0.00027 | 0.0036 ± 0.00150 |
| Europium, 63Eu    | 0.0012 ± 0.00023 | 0.0017 ± 0.00054 | 0.0023 ± 0.00035 |
| Cobalt, 27Co      | 0.093 ± 0.015000 | 0.063 ± 0.011000 | 0.069 ± 0.020000 |
4. Discussion

The precise etiopathology of the lumbar degenerative disc disease is still under a great discussion [11]. The evaluation of chemical composition of the human lumbar intervertebral discs in different development stages of degenerative process can represent the clue for the some etiopathological reasons, which can be used for the prevention and treatment of patients with lumbar degenerative disc disease [12]. This study demonstrated that the concentration of $^{56}$Fe in lumbar herniated intervertebral discs is statistically significant decreased following aging and increasing the stages of degenerative lumbar disc disease. The mean concentration of $^{56}$Fe was 52 ± 9.2 μg/g in the first group patients, the mean concentration of $^{56}$Fe was 48 ± 4.9 μg/g in the second group, whereas the mean concentration of $^{56}$Fe in the third group patients was significantly decreased to 36 ± 7.0 μg/g in comparison with the first and second group of patients (p<0.05). The deficiency of $^{56}$Fe can accelerate intervertebral disc degeneration through affecting the stability of DNA polymerase epsilon (Polε) complex. The latest scientific investigations demonstrated that increased IncPolE level was associated with progression of chronic lumbar degenerative disc disease [13].

The lumbar intervertebral disc calcification is the most common reason of intervertebral disc degeneration and aging [14]. Our research revealed that the mean concentration of $^{40}$Ca was 3400 ± 1100 μg/g in the first group of patients, then the mean concentration of $^{40}$Ca was decreased until 1700 ± 290 μg/g in the second group of patients, whereas the mean concentration of $^{40}$Ca was 5500 ± 3300 μg/g in the third group patients, which was two folds higher than the second group of patients (p < 0.05). It can be explained by the fact that in the early stages of the lumbar degenerative disc disease, $^{40}$Ca is washed out from the bone component of the intervertebral disc, but as the disease progresses and the transition to a advanced stage of disease, the calcification of the soft tissues of the disc occurs. The calcification of lumbar intervertebral disc is a consequence of a variety of biochemical processes, the issue of prevention is still problematic. One of the recent experimental investigations demonstrated that MSC/HP-anti-miR-199a/NS/NF-SMS constructs may contribute the nucleus pulposus phenotype and confront calcification in vitro and in a subcutaneous environment. The injection of MSC/HP-anti-miR-199a/NS/NF-SMS can produce functional extracellular matrix, maintain disc height and prevent intervertebral disc calcification [15].

The $^{87}$Sr has a significant impact on cartilage metabolism though a potential chondroprotective effect [16]. This study outcome demonstrated that the mean concentration of $^{87}$Sr was 11.0 ± 2.8 μg/g in the first group of patients, whilst during the development of the advance stage of degenerative process in the third group patients, its content sharply decreases to 7.5 ± 2.5 μg/g (p < 0.05). The experimental and clinical models were demonstrated that $^{87}$Sr can reduce the inflammatory process, which beneficially reduced the progression of discs degeneration [16]. The recent investigations revealed that the strontium ranelate can effectively decrease cartilage degeneration and subchondral bone remodeling in patients with osteoarthritis [17, 18].
5. Conclusion

This study demonstrated the significant differences of the trace element concentrations in herniated disc of the lumbar spine in different age group of patients, who had different stages of the lumbar degenerative disc disease.

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Journal of Spine Research and Surgery

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