Journal of Trace Elements in Medicine and Biology 48 (2018) 20–24

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Applied methodology

Essential elements in synovial fluid samples obtained from patients living in Northern Poland

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ARTICLE INFO

Keywords:
Essential elements
Synovial fluids
Osteoarthritis
FAAS
Statistical methods

ABSTRACT

Many people after 50 year of life suffer pains caused by osteoarthritis (OA), which make their life difficult and painful. There is a probable association of OA with the concentration of certain elements in body fluids. Therefore the aim of the study was to relate the level of essential elements in synovial fluids of patients with OA with the treatment using antiarthritic drugs. Thus, flame-atomic absorption spectrometry was used to determine the concentrations of Fe, Zn, Cu, Mn, Na, K, Ca and Mg in 74 samples of synovial fluids taken from 36 patients. Prior to analysis, the samples of synovial fluids were centrifuged and obtained supernatants were digested by microwave technique. It was found that the level of investigated essential elements was differentiated in patients suffering with OA. High correlation between Zn and K, Na and K, as well as Cu and Mn were revealed. Based on cluster analysis, synovial fluids of patients treated with Diprophos, Euflexxa and Synocrom, can be found almost in all clusters. ANOVA has shown that there is a significant difference between Na level in synovial fluids of patients treated with antirheumatic drugs, and those who were not treated. For the patients treated with Euflexxa, similar relations for Ca and K exist. Principal component analysis has shown that essential elements responsible for variability within studied samples, are Ca, Mg, Cu, Mn and Fe. However, there were no statistically significant relations between the studied macro- and microelements, and treatment using specific preparations.

1. Introduction

Nowadays osteoarthritis (OA) and rheumatoid arthritis (RA) are common problems for societies in most of countries of the world [1]. Especially people after 50 year of life suffer pains caused by these inflammatory diseases, and the symptoms connected to this types of illnesses make their life difficult and painful. Arthritis manifests itself in multiple joints in human organism and it usually affects the synovial membranes of patients. Etiology of RA and OA was primarily related to the process of human ageing, but recent studies have demonstrated that synovitis and the resultant pro-inflammatory mediators should also be taken into consideration as important factors in their pathogenesis [1]. One of the main differences between these two diseases is the fact that RA may begin any time in life, and OA usually begins later in life time. Another difference is that RA is an autoimmune disease, while OA is caused by mechanical reasons, such as wear and tear on joints. For the treatment of RA and OA, drugs of different groups are used, involving non-steroidal anti-inflammatory drugs, but also corticosteroids, immunosuppressive drugs, as well as biological agents are applied [2]. In many severe cases of RA or OA surgery is necessary. However, several investigations point on possibility of relation of rheumatoid diseases with the concentration of certain elements in their body fluids [3–5]. This approach is justified, because trace elements participate as cofactors in many enzymatic reactions, and deficiency of micro- and macroelements can be associated to undesired effects on health of humans.

Selected essential elements, such as Se, Zn, Cu and Fe have been determined in synovial fluids and plasma of patients with RA and OA [6]. The results have shown that Se level in healthy controls were significantly lower in comparison with results obtained for patients with OA. On the other hand, Cu concentrations were higher in patients with RA than in those suffering with OA, as well as when compared with healthy people [6]. Other studies have shown that Zn and Fe levels were significantly lower in the serum of patients with RA in comparison with healthy ones, but serum Cu concentrations were found higher in the RA patients [7]. It was also recently found that patients with RA represented a higher serum level of Cu, and lower of Zn in the same biological fluid than the healthy people [8]. Another study has shown that the average levels of toxic metals, such as As, Cd, Hg and Pb were

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https://doi.org/10.1016/j.jtemb.2018.02.027
Received 5 October 2017; Received in revised form 16 February 2018; Accepted 26 February 2018
0946-672X/

significantly higher in blood samples of RA smoking patients, whereas other elements – Zn, Mn, Cu and Se were found lower in RA patients when compared with healthy control group [9].

Therefore the aim of the studies was to recognize the level of essential elements in synovial fluids of patients with the occurrence of OA, and relate their concentrations with the use of antirheumatic drugs. The choice of elements was done based on their indispensable role in human organism [10]. Among microelements, Fe is built in hemoglobin and is responsible for oxygen transport, Zn plays its role in numerous enzymes and is indispensable for organism growth and reproduction. Mn is needed for proper function of brain and also protects organism against free radicals, and Cu plays important role in antioxidant reactions. On the other hand, macroelements are involved in synthesis of proteins, such as Mg, whereas Ca is indispensable element in skeleton and physiological fluids, Na assures acid-base balance in human organism, while K takes part in a sodium-potassium pump and regulates water balance in human organism [10]. Realizing this aim of studies, flame atomic absorption spectrometry was used to determine the concentrations of Fe, Zn, Cu, Mn, Ca and Mg in 74 samples of synovial fluids obtained from 36 patients treated with Diprophos, Euflexxa and Synocrom.

2. Experimental

2.1. Reagents

Redistilled water was obtained in water distillation system (Heraeus, Switzerland), other reagents were as follows: HNO_3 concentrated solution, 30% H_2O_2 solution (both from POCh, Poland), standard solutions of Fe, Zn, Cu, Mn, Mg, Ca, Na, K (1 mg/mL) (Merck, Germany), 1% solution of La obtained from La_2O_3 (Sigma, USA), 1% solution of Cs obtained from Cs_2CO_3 (Sigma-Aldrich, Germany).

2.2. Biological material and sample preparation

The analyzed material consisted of 74 samples of synovial fluids obtained from 36 patients living in Northern Poland. For comparison purposes, 10 samples of plasma originating from healthy volunteers, were used. Ethics committee at the Medical University of Gdansk, Poland has approved the investigations with patients and volunteers (Decision No NKBBN/61/2016). The studied group of 36 patients from whom synovial fluids were taken for analysis of elements, consisted of 74 samples of synovial fluids taken from 10 men and 26 women at the age interval from 29 to 87 years of life (Table 1). Most of patients were seniors, since the arithmetic mean of investigated population was 70, and the median was equal to 74 years. Some of them were at the beginning of treatment using one of two antirheumatic drugs, derivatives of hyaluronic acid – Euflexxa and Synocrom, while others were treated with corticosteroid drug – Diprophos (Betamethasone). Moreover, in Table 1 the WOMAC points are presented obtained after interview with a patient. WOMAC scale is a measure of monitoring the progress of rehabilitation treatment, and when this value is closer to 100%, this means that a patient feels more discomfort and pains [11].

Analyzed samples of synovial fluids were taken in sterile conditions by a nurse, and were kept in a refrigerator (Polar, Poland) at 4 °C. Prior to analysis of metals by flame-atomic absorption spectrometry (FAAS) technique, the samples were centrifuged (3200 rpm per 15 min) using a laboratory centrifuge EBA 20S (Hettich Zentrifugen, Germany). Then obtained supernatants were digested using microwave 3-step digestion system Jupiter-A (Sineo Microwave Chemistry Technology, China), and the digestive mixture: 5 ml of HNO_3 + 4 mL of 30% H_2O_2 solution. The obtained digests were used directly for FAAS measurements employing an Atomic Absorption Spectrometer 250 Plus (Varian, Australia) in case of microelements (Fe, Zn, Cu, Mn) and diluted 100 times in case of macro-elements (Mg, Ca, Na, K). For each sample of synovial fluid, the completed assay procedure was repeated in triplicate, and the standard deviation was calculated.

2.3. Metallic elements determination

Essential metallic elements under study were assayed by FAAS using standard analytical conditions (air/acetylene), absorption mode and the following analytical wavelengths (nm): Fe (248.3), Zn (213.9), Mn (279.5), Cu (324.8), Mg (285.2), Ca (422.7), and emission mode for K (766.5) and for Na (589.0). In case of Mg and Ca determinations, to each digested and diluted sample 1 mL of 1% La solution was added, and to the digest prior to determination of Na and K 1 mL of 1% solution of Cs was added.

2.4. Validation of the analytical methods

The methods were validated in terms of their precision and accuracy using certified reference materials (SERO203105 Seronorm Trace Elements Serum L-2), as shown in Table 2.

2.5. Data analysis

Statistical analysis – one-way analysis of variance (ANOVA), correlation, cluster (CA) and principal component (PCA) analyses were calculated after standardization of experimental data and auto-scaling of independent variables using *Statistica 7.1* (Statsoft, Poland) software [12].

3. Results and discussion

3.1. Levels of essential elements in synovial fluids

The concentrations of essential elements in synovial fluids are shown in Table 3. Taking into consideration the arithmetic mean and the median of the obtained data, the microelements represented the following order: Zn > Fe > Cu > Mn. For macroelements, this order was as follows: Na > K > Ca > Mg. To compare the concentrations of elements in synovial fluids with those in other natural fluid in human organism, the same elements were analyzed in plasma obtained from healthy volunteers, as presented in Table 4. The range of concentrations represents the same level, not counting higher content of Fe in plasma samples in comparison with synovial fluids. In the case of microelements (Fe, Zn, Cu and Mn) the order, in which the concentrations are decreasing is different in synovial fluids and plasma samples. For the macro-elements, it was noticed that the order is slightly different, because K represented the highest level in blood plasma, whereas Na in synovial fluids.

Yazar et al. [6] have noticed higher level of Cu in synovial fluids of patients with RA, and higher concentrations of Zn and Fe in plasma of healthy individuals in comparison with the level of these elements in RA patients. This is concurrent with the results found in this study. Similar conclusions can be drawn based on results described by Krachler et al. [3], where high concentrations of Cu in plasma and synovial fluids of RA patients were determined.

ANOVA test was used to check whether the differences in essential elements levels were significant. It was revealed that in the case of Na level determined in patients, for whom the treatment was not begun, and those treated with three anti-rheumatoid drugs (Diprophos, Euflexxa and Synocrom), the difference was significant, as shown in Fig. 1. The statistically significant differences were also obtained for levels of K and Ca in synovial fluids between the patients treated with Euflexxa, and those who were not treated. Perhaps these differences occurred due to the fact that macro-elements (Na, K and Ca) are found in high concentrations in synovial fluids of patients, who are just at the beginning of their treatment. Probably this is the effect of the use of anti-rheumatoid drugs, and therefore levels of these elements decrease.

To identify relations among studied elements, correlation analysis