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Biolog. Journal of Armenia, 1 (69), 2017

ISOFORMS OF ADENOSINE DEAMINASE1 IN SYNOVIAL FLUIDS AT DIFFERENT ARTHRITIS

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The significant elevation of adenosine deaminase activity in synovial fluids of patients at different joint disorders was observed. It was shown that the enzyme level can be used as a statistically significant, specific and sensitive biochemical marker for distinguishing the rheumatoid and osteoarthritis. Moreover, it appeared that in the case of rheumatoid arthritisthe elevation of the enzyme activity in synovial fluidtakes place mainly via increasing of small-molecular adenosine deaminase. In the cases of ankylosing spondylitis, reactive arthritis, gout and juvenile idiopathic arthritisthis regularity was not registered.

Adenosine deaminase– arthritis– synovial fluid–biochemical marker

Հոդերի ախտաբանական խանգարումներով հիվանդություն ներիսինովիալ հեղուկում գրանցվել է ադենոզին դեամինազ 1-ի ակտիվության զգալի բարձրացում: Ցույց է տրվել, որ ֆերմենտի ակտիվությունը կարող է օգտագործվել օստեո- և ռևմատոիդային արթրիտների տարբերակման վիճակագրորեն զգալի, սպեցիֆիկ և զգայուն կենսաքիմիական ցուցանիշ: Պարզվել է, որ ռևմատոիդային արթրիտի դեպքում սինովիալ հեղուկում ֆերմենտի ակտիվությունը բարձրանում է ֆերմենտի ցածրամոլեկուլային ձևի աճի հաշվին, որը չի գրանցվել անկիլոզացնող սպոնդիլոարթրիտի, ռեակտիվ արթրիտի, հոդատապի (պոդագրա) և պատանեկանի դիոպաթիկ արթրիտների դեպքում:

Ադենոզին դեամինազ - արթրիտներ – սինովիալ հեղուկ – կենսաքիմիական մարկեր

Наблюдалось значительное повышение активности аденозин дезаминазы 1в синовиальной жидкости больных при различных суставных заболеваниях. Было показано, что уровень активности фермента может быть использован в качестве статистически значимого, специфического и чувствительного биохимического маркера для различения остео- и ревматоидного артритов. Оказалось, что в случае ревматоидного артрита повышение активности фермента в синовиальной жидкости происходит главным образом за счет увеличения низкомолекулярной аденозин дезаминазы. В случаях анкилозирующего спондилоартрита, реактивного артрита, подагры и ювенильного идиопатического артрита эта закономерность не регистрируется.

Аденозин дезаминаз–артриты–синовиальная жидкость–биохимический маркер

Arthritis is one of the common causes of people's disability in the world. More than 54.4 million adults have doctor-diagnosed arthritis, and by the year 2040 this number of US adults is expected to be 78 million. There are 100 different forms of arthritis. Inflammatory joint diseases are heterogeneous group of disorders afflicting not only joint tissues but often multiple organs in the body. The group of the joints disorders includes rheumatoid arthritis (RA), osteoarthritis (OA),ankylosingspondylitis, reactive arthritis (ReA), psoriatic arthritis, juvenile idiopathic arthritis (JIA), etc. According to the studies of European and North American populations, among these diseases RA is the most common, affecting 0.5–1% of the population. It is a chronic, progressive and disabling autoimmune disease that causes inflammation in the joints, the tissue around the joints, and other organs in the human body [14]. RA results in swollen and painful joints.It ischaracterized by extracellular matrix degradation, destruction and loss of function of joint cartilage. OAis degenerative joint disorder which results in pain and deformity, ultimately leading to chronic disability. It is becoming a significant medical and financial burden in a world. Optimal management requires early diagnosis and awareness of the risk factors affected the prognosis.

Currently, diagnosis of inflammatory diseases of the joints is carried out on the basis of the adopted classification criteria. Depending on the expected type, the clinical examination may be supported by radiology and/or blood analysis [4]. At present, a great task is the identification of biomarkers, which can help in differentiating the arthritis type. This is important for applying the appropriate treatment. Obviously, the analysis of synovial fluid (SF) might provide more targeted option to research and differentiate the arthritis. As a biomarker for differentiating the etiology of joint diseases, adenosine deaminase activity level in SF has been considered also [15].

Adenosine deaminase (ADA, EC 3.5.4.4) is an important enzyme in purine metabolism. It is involved in the breakdown of adenosine (Ado), catalyzing its irreversible hydrolytic deamination to inosine [6]. The enzyme is presented in all mammalian tissues. Its primary function in humans is development, differentiation, and maturation of the lymphoid system [7]. Two molecular isoforms of the enzyme are known. The small form of human ADA (SADA) is a catalytically active protein with a relative molecular mass of 36-38 kDa. The large form (LADA, Mm of 280 kDa) represents a complex of SADA with so called ADA binding protein, which is identical with dipeptidyl peptidase IV (DPPIV), a multifunctional enzyme, appearing in many tissues in cell membrane immobilized and circulating forms [8, 3]**.** The ADA substrate Ado suppresses and mediates the inflammatory processes and is provided with the protective properties against injuries. Its anti-inflammatory function consists in decreasing of pro-inflammatory and increasing of anti-inflammatory cytokines, in cytokine modulation of macrophages and monocytes and regulation of inflammatory function of endothelial cells [5]. Hence, DPPIV-bound extra cell LADA participates in regulation of Ado concentration in physiological fluids [9]. In inflammation, a tissue injury brings to the enhancing of ADA activity in the extra cellular medium: SF and the effusions of different pathology. This promotes the decreasing of

concentration of the anti-inflammatory Ado and aggravation of inflammation. The increase of ADA activity has been observed in such pathologies as tuberculosis [11], cancer [13], rheumatoid arthritis [12], etc.

The evaluation of ADA activity level is considered as a reasonable approach in the diagnosis of some diseases. It was assumed also as a reasonable test for distinguishing at least of two type arthritis, RA and OA. ADA activity in SF from patients at inflammatory joint diseases is significantly higher than at non inflammatory diseases [15]. We studied the activity of ADA in SFs of 140 patients of Armenian clinics (57 RA and 83 OA) [1].

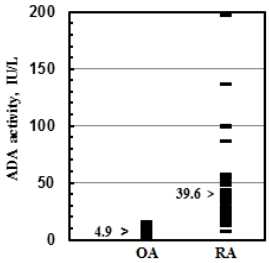
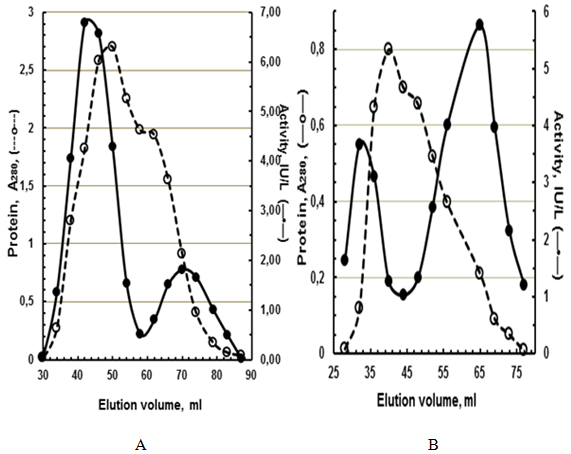


Fig.1.The ADA activity in SFs of patients, diagnosed as osteoarthritis (OA) and rheumatoid arthritis (RA). The mean values of the activities are indicated by arrows.

The analysis evidenced a statistically significant ($p<0.0001$) difference between the activity levels in SFs from knee joint of two groups. Fig. 1 shows the experimental points, representing the enzyme activity in SFs of patients, diagnosed as OA and as RA. The mean values of the enzyme activity for these groupswere 4.9 ± 0.5 IU/Land 39.6±4.5 (mean ± S.E.M.) IU/L, respectively.Based on the ADA cutoff value of 12 IU/L, the parameters for RA and OA differentiation were evaluated as: 94.4% – sensitivity, 93.9% – specificity and 94.1% – efficiency. Hence, our study contributed into consideration of the ADA activity level in SFs as an additional, suitable for fast and low-cost differential diagnostic test of RA and OA, particularly, in Armenian population.The difference between ADA activities in SFs of RA and OA patients had been recommended also in the arthritis diagnosis in Japan, Iran, etc. [12, 10]. It is worth of note that the cut off values in different populations are different.



A B

Fig. 2. The elution diagrams at gel-filtration through the column with Sephadex G-200of SFs with the initial ADA activity of 30.5 IU/L (A) and 98.9 IU/L (B)

The ratios of small- to large-molecular forms of ADA in the SFs of RA patients with different levels of the total activity were compared [2]. Two forms of ADA were separated by gel-filtration through the column with Sephadex G-200. The SF samples with the initial ADA activity below and above the statistical mean value for RA patients (39 IU/L) were analyzed in triplicate. Fig. 2 shows the presentable couple of the diagrams obtained at gel filtration of SFs with the initial ADA activity of 30.5 IU/L (A) and of 98.9 IU/L (B). These diagrams manifested the increasing of small- to large- molecular isoforms ratio from 0.27 in (A) to 1.57 in (B). Apparently, for the increasing of ADA-activity in RA-SF mainly SADA is responsible.

Unfortunately, we did not have the opportunity to investigate the SFs from patients with these diseases in amounts sufficient for performing statistical analysis.

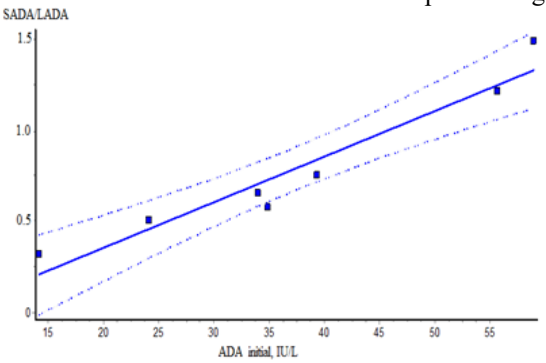


Fig. 3.The linear correlation curve between the total ADA activity and the ratio of SADA to LADA isoforms of the enzyme in the SFs of RA patients, ($r = 0.96$; $p = 0.0005$).

Table 1. The ratios of ADA isoforms in the SFs at different arthritis.

Arthritis type	Activity	SADA/LADA
Reumathoid arthritis	55.7	1.12
Reactive arthritis	63.5	0.20
Ankylosing spondylitis	56.9	0.15
Gout	53.7	0.31
Juvenile idiopathic arthritis	60	0.26

Table 1 demonstrates the ratios of the isoforms in the SFs with the close levels of ADA from the patients of different arthritis, evaluated after gel filtration.

Conclusions: The ADA activity in SFscan be used as a statistically significant, specific and sensitive biochemical marker for differentiating the RA and OA. In RA, the ADA activity elevation takes place mainly via increasing of SADA. In the cases of the other arthritis (e.g. ankylosings pondylitis, reactive arthritis, gout, juvenile idiopathic arthritis) this regularity is not registered.

Acknowledgment. This work was supported by Ministry of Education and Science of the Republic of Armenia (Agreement Ъ-16).

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Biolog. Journal of Armenia, 1 (69), 2017

THE RADICAL SCAVENGING ACTIVITY OF SOME SPECIES OF ARTEMISIA GENUS, REPRESENTED IN ARMENIAN FLORA

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Plants are valuable sources of antioxidants which could have beneficial effect on human health. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. In this respect flavonoids and other polyphenolic compounds have gained the greatest attention. The present study was undertaken to evaluate the *in vitro* antiradical activity of different extracts (ethanol, hexane, acetone, chlorophorm and methanol) of *Artemisia vulgaris* (L.), *A. fragrans* (Willd.), *A. absinthium* (L.) and *A. splendens* (Willd.) represented in Armenian flora. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay was used to measure the radical scavenging activity of extracts obtained from Artemisia subspecies aerial parts. Catechin was used as a positive reference. Different concentrations of extracts were used and results were expressed with IC_{50} values.