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ADENOSINE DEAMINASE ACTIVITY IN SYNOVIAL FLUID AT ARTHRITIS

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It was found that the levels of Adenosine deaminase activity in joint effusions of patients with rheumatoid arthritis and osteoarthritis differ significantly ($p < 0.0001$). To differentiate the arthritis of these two etiologies, the cutoff value of the enzyme activity in synovial fluid was evaluated as 12 IU/*L* with sensitivity 0.96, specificity 0.89 and diagnosis efficiency 0.93. Based on this observation, we can offer using in clinics of Armenia the Adenosine deaminase activity in synovial fluid for quick diagnosis of rheumatoid arthritis as a sensitive, specific, low-cost and technically convenient test.

***Keywords*:** adenosine deaminase, ADA-test, differentiation of arthritis, rheumatoid arthritis, osteoarthritis, synovial fluid.

Introduction. Arthritis is the initial manifestation of many joint disorders. Rheumatoid arthritis (RA) is a chronic disease, characterized by extracellular matrix degradation, destruction of joint cartilage and loss of function. It is a chronic multisystem autoimmune disease of unknown cause. The characteristic feature of RA is persistent inflammatory synovitis, usually involving peripheral joints. It is characterized by infiltration of T-cells and monocytes into the synovium, proliferation of synoviocytes and presentation of secreted products of activated lymphocytes, macrophages, fibroblasts and leucocytes [1, 2].

Osteoarthritis (OA) also known as degenerative arthritis or degenerative joint disease, is a group of mechanical abnormalities involving degradation of joints, including articular cartilage and subchondral bone [3]. OA possesses substantionally lower inflammatory component in terms of aggressiveness, progressivity, errose potential and systemic infiltration.

During recent years a great attention is paid to the identification of bio-markers, which can help in differentiating the type of arthritis. Synovial fluid (SF) analysis is expected to provide an easy option to differentiate arthritis.

Endogenous adenosine, a purine nucleoside with protective property against injuries, suppresses and mediates the inflammatory system. Its anti-inflammatory

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function consists in decreasing pro-inflammatory cytokines, increasing anti-inflammatory cytokines, cytokine modulation of macrophages and monocytes, and regulation of endothelial cell inflammatory functions [4]. Adenosine deaminase (ADA, E.C. 3.5.4.4) is an enzyme converting adenosine to inosine [5]. It plays a central role in the development of immune system [6]. During inflammations ADA is released in extra cellular and SF and in effusion of different pathology, resulting in the considerable increase of its activity. ADA as a marker of cellular immunity activation may help to better understanding of some pathophysiologic aspects of a disease and may help to relieve the triggering factors of inflammation and promote new therapeutic approaches [8]. The determination of ADA activity is considered as an appropriate technique for diagnosis of some diseases [7]. Particularly, several works appeared devoted to the valuation of application of ADA activity for differentiating the etiology of joint diseases [9, 10].

The goal of the present work is to compare the levels of ADA activity in SF of patients with RA and OA, to determine the proper cutoff level of the enzyme activity suitable for differentiating these diseases, to calculate the sensitivity and specificity for using the ADA-test in Armenia.

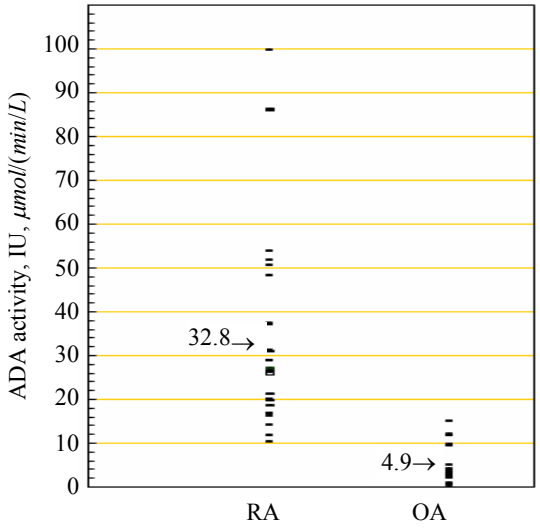

Fig. 1. The experimental points, representing the activity of adenosine deaminase in SF of patients, diagnosed as rheumatoid arthritis and osteoarthritis. Near the data the mean values of ADA activity for both groups are indicated.

Experimental Part. Synovial fluid samples were obtained from patients in Erebuni Medical Center (45 patients, 26 and 19 with RA and OA respectively were studied). They were matched in sex and age and were diagnosed according to the accepted classification criteria of the diseases. The samples were stored at -20^0C, then refrozen, centrifuged and diluted three times to conduct the assay. The ADA activity was determined by evaluation of the amount of ammonia, liberated in the reaction of adenosine deamination catalyzed by the enzyme. The earlier described [11] phenol-hypochlorite colorimetric method was used with some modification. Briefly, the assay mixture in 0.5 *mL* of 40 *mM* K-Na-phosphate buffer, pH 7.4,

contained 0.4 *mM* adenosine. The aliquots of the synovial fluid per 50 and 100 *μL* in RA and OA respectively were added, and the mixture was incubated for 30 *min* at 37^0C. Then phenol-nitroprusside and hypochlorite reagents, 1 *mL* each, were added, and the absorbance at 630 *nm* was registered. Sulfate ammonia solution was used as a standard. An International Unit of the enzyme activity (IU) was expressed as *μmol* of substrate deaminated in 1 *min* per 1 *L* of SF.

Statistical analyses were performed with GraphPad software, version 3 for Windows (USA). Unpaired two-tailed *t*-test with Welch correction was applied. The data are expressed as mean ± standard error.

Results and Discussion. In Fig. 1 the ADA activities obtained in the SF samples of 45 patients, involved in the investigation are shown. The data correspond to the enzyme activity at 26 patients with RA and 19 patients with OA. The analysis evidenced a statistically significant ($p < 0.0001$) difference between the enzyme activity levels in knee joint SF of two groups of patients. The mean ADA activity for RA was 32.8±4.1 IU/*L* (10.5–99.8), and for OA – 4.9±1.07 IU/*L* (0.3–12.7). It is worth noting that the mean value at RA is nearly 7 times as big as that at OA (while in the report of Zakieri et al. [9] it is only 2 times).

In Fig. 1 we can see that most of the ADA-activity values for RA are above 10–14 IU/*L*, while most of the values for OA are below it. Taking the level of 12 IU/*L* as a cutoff value for ADA activity, we estimated the parameters, which prove its significance as a test for differentiating RA and OA:

- *The sensitivity*, determined as the ratio of the number of positive diagnoses (26) to the sum of this number and the number of false negative diagnoses (1 value of ADA activity is below 12 IU/*L*), was 25/26=0.96.
- *The specificity*, calculated as the ratio of the number of negative diagnoses (17) to the sum of this number and the number of the false positive diagnoses (2 value of ADA activity is above 12 IU/*L*), was 17/19=0.89.
- *The efficiency*, i.e. the ratio of the sum of the positive diagnoses (25) and the negative diagnoses (17) to the total number of the cases involved in the examination (45), was 42/45=0.93.

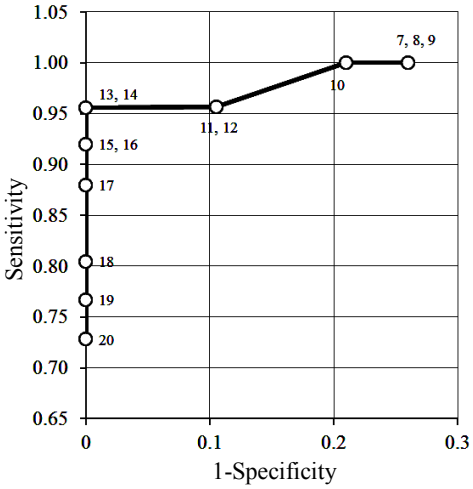

Fig. 2. Dependence of sensitivity on the 1-specificity, calculated for ADA-test cutoff values are in the interval 7–20 IU/*L*. At points the appropriate cutoff values are indicated.

The performance of cutoff value of ADA activity for differentiating RA and OA was judged using receiver operating characteristics curve, which is presented as the dependence of "sensitivity" of ADA-test from "1-specificity" in Fig. 2. The points are calculated for cutoff values of ADA activity in the interval 7–20 IU/*L*. It is seen that the cutoff values between 10 and 14 IU/*L* correspond to the suitable characteristics at diagnosing arthritis: sensitivity is in the interval 0.96–1 and specificity in the interval 1–0.79. Hence, 12 IU/*L* seems to be a reasonable cutoff value.

Discussion. Differing cutoff values for ADA-test in our research (12 IU/*L*) and in the Iranian work (20 IU/*L*) [9], manifest that, prior to using this test to diagnose RA and OA, special investigations are to be performed for every population to define the cutoff value, applicable for that particular population. A similar situation was observed at reviewing the application of ADA-test to the diagnosis of pleural tuberculosis. The cutoff values for the test differed significantly in the reports of scientists from different countries: Mexico – 70 IU/*L* [8]; South Africa – 50 IU/*L* [12]; Spain – 47 IU/*L* [13]; Russian Federation – 35 IU/*L* [14]; Republic of Armenia – 20 IU/*L* [15].

Even at limited number of the studied samples (45), the obtained statistically significant data on ADA activity evidence that ADA-test can be applied for differentiating the rheumatoid arthritis and osteoarthritis as a sensitive (0.96), specific (0.89) and high efficiency (0.93) test. Using cutoff value 12 IU/*L*, ADA-test appeared suitable for fast and low-cost differential diagnosis of RA and OA among the population of Armenia.

A further study involving a bigger number of patients with inflammatory and degenerative diseases of joints is definitely required to recommend applying this test in clinics with greater confidence.

Dedicated to the memory of L.T. Sharambeyan

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