Histamine index and clinical expression of rheumatoid arthritis activity

Histaminski indeks i stepen aktivnosti reumatoidnog artritisa

Aleksandra P. Tomić-Lučić*, Suzana B. Pantović†, Gvozden L. Rosić*, Zdravko M. Obradović†, Mirko A. Rosić*

*University of Kragujevac, School of Medicine, Department of Physiology, Kragujevac, Serbia; †Clinical Center Kragujevac, Internal Clinic, Department of Rheumatology, Kragujevac, Serbia

Abstract

Background/Aim. Many arguments prove the pathophyslogic role of histamine in the process of remodeling and joint destruction in rheumatoid arthritis. The aim of our study was to find out if there was a relation between histamine concentration in synovial fluid and blood with clinical expression of disease activity. Methods. Histamine concentration in synovial fluid and blood was determined in 19 patients with rheumatoid arthritis. Histamine concentration measurement was based on the Shore's fluorometric method. Histamine index (HI) was evaluated as a ratio between histamine concentration in synovial fluid and blood. Disease activity score, DAS 28 (3), with three variables (erythrocyte sedimentation rate, the number of swollen joints and the number of tender joints) was also evaluated. Results. Our results showed that there was no significant difference in concentration of histamine in synovial fluid and blood related to disease activity. However, there was a significant difference in the histamine index which was increased proportionally with disease activity. Conclusion. Our study indicates that histamine index could be useful in estimation of rheumatoid arthritis activity.

Key words: arthritis, rheumatoid; histamine; blood; synovial fluid; disease progression.

Introduction

Many arguments prove the pathophyslogic role of histamine in the process of remodeling and joint destruction in rheumatoid arthritis (RA). Histamine modifies behaviour of many cells in vitro including chondrocytes, fibroblasts, osteoclasts, macrophages, T lymphocytes, endothelial cells. Histamine also modifies cytokine production and receptor expression 3, 4. There are evidences of an increased production of chondrocytes matrix metalloproteinases (MMP3, MMP 13) induced by histamine, as well as chondrocytes stimulation and proliferation via H1 receptors 5, 6. Histamine stimulates synovial fibroblast proliferation and that effect is mediated by H1 receptors 5. Interaction between histamine from mast cells and macrophages and synovial fibroblasts H1 receptors has important role in the process of remodeling and joint destruction in RA 5. Osteoclasts differentiation is induced by histamine and mediated by H2 receptors 1.

Mast cells express histidine decarboxilase, an enzyme that is essential for histamine production. Human chondrocytes also produce histidine decarboxilase. It indicates that histamine originates from chondrocytes, as well as from mast
cells and takes place in inflammatory events in RA. There was an evidence of decreased expression of H2 receptors on lymphocytes, bone marrow mononuclear cells and synovial fibroblasts, as well as decreased expression of H1 receptors on RA chondrocytes. It means that beneficial effects of histamine could be decreased due to H2 receptors hypofunction. Abnormality in the function of histamine receptors could play a significant role in maintaining the inflammation process in RA. It has been suggested that histamine suppress TNFα gene expression, as well as its secretion from peripheral blood mononuclear cells. This effect was mediated by H2 receptors. As far as it is concerned, it could indicate that histamine released from mast cells could paradoxically limit the stage of inflammation and immune reaction by suppressing cytokine secretion in the H2 bearing cells. However, this effect is suppressed because of a decreased function of H2 receptors in RA patients. There is an increased number of mast cells in synovial tissue of RA patients, predominantly on the places with cartilage erosion. Mast cells contain potent mediators such as histamine, leukotrienes, proteinases, heparin and many cytokines. Therefore, their role in inflammation process and destruction of matrix in RA becomes evident. Le et al. reported that mice that lack mast cells are resistant to inflammatory and erosive arthritis induced by arthritogenic serum. It was proposed that mast cells play an important role as the cellular link among autoantibodies, the complement network, and inflammatory mediators. Therefore, the activation of mast cells makes a pivotal contribution to inflammatory arthritis.

It is likely that direct migration of mast cells within tissues is a very important mechanism of increasing the number of mast cells in synovial tissue in RA. There was an evidence that several factors in the synovial fluid can act as mast cell chemoattractants, such as the stem cell factor (SCF), transforming growth factor (TGFβ), C5a, and platelet activation factor (PAF). Stem cell factor regulates growth, differentiation, adhesion and activation of the mast cells. Besides that, histamine influences chemotaxis of mast cells by the H4 receptors, their activation leads to mobilisation of intracellular Ca++, and includes Gαi/o protein mechanism and phospholipase C. The expression of H4 receptors was reported on the two populations of cells from synovial tissue (fibroblast-like cells and macrophage-like cells) in RA patients. It is likely that there is a difference in H4 receptors expression in the different stages of RA activity.

Recent investigations indicate that the histamine releasing factor (HRF) significantly influences the releasing of histamine. Histamine releasing factor is an intracellular protein (as cytokine) that modulates the secretion of cytokines from human basophills, eosinophills, T and B lymphocytes. The expression of HRF and its mRNA is evidenced on the pannus (fibroblasts, macrophages) which destroyed cartilage in RA patients, but not in the healthy population. That confirms the role of histamine in pathogenesis of RA and other autoimmune diseases.

As far as histamine concentration in synovial fluid of RA patients is concerned there are many contradictory results.

The aim of our investigation was to detect histamine concentration in synovial fluid and blood in RA patients and to explore its relation with clinical expression of disease activity.

**Methods**

The investigated patients suffered from RA that was diagnosed in accordance with American College of Rheumatology (ACR) criteria. The mean age of patients was 57.45 ± 3.56 years, (min 30 max 79, years). There were 15 female patients, and 4 male patients. All patients underwent routine clinical and laboratory investigations. Laboratory data including erythrocyte sedimentation rate, C-reactive protein, rheumatoid factors (latex test and hemaglutination test), blood count, blood urea, blood creatinine, aspartataminotransferasis, alanin aminotransferasis, were obtained. In clinical assessment radiography of hands and clinical activity of the disease were registered. Clinical activity was determined according to the Disease Activity Score – DAS-28 (3) with three variables (erythrocyte sedimentation rate, the number of swelled joints and number of tender joins). All patients were classified in three groups according to the disease activity: very active (VA) with DAS-28 (3) score more than 5.1, moderate activity (MA) with DAS-28 (3) score between 3.2 and 5.1, and inactive with DAS-28 (3) score below 3.2. The control group comprised age- and sex-matched healthy persons.

Synovial fluid was obtained from the knee under sterile procedure during therapeutic arthrocentesis, collected in heparinized tubes and centrifuged. Histamine concentration in the synovial fluid and blood of the RA patients was detected. Concentration of histamine in the blood of the 19 healthy individuals were taken as control, too. Histamine index was evaluated as a relation between synovial fluid histamine concentration and blood histamine concentration multiplied by 100.

\[
\text{Histamin index} = \frac{\text{Synovial fluid histamine conc. (ng/mL)}}{\text{Blood histamine conc. (ng/mL)}} \times 100
\]

Detection of histamine was based on the Shore’s fluorometric method. The mean values of biological parameters were compared using the Student’s t-test, where p value of < 0.05 was considered as a statistically significant difference. The data in tables are presented as mean ± standard error of the mean.

**Results**

Our results showed histamine concentration in synovial fluid in all RA patients about 1.548 ± 0.10 ng/mL. Histamine level in blood of the same group of the patients was 48.84 ± 3.05 ng/mL, while histamine index was 3.155 ± 0.035. Histamine concentration in blood of healthy subjects in the control group was 51.35 ± 3.75 ng/mL. There was no significant difference in histamine concentration in blood between healthy population and RA patients.
Among the investigated patients, 73.6% (14 patients) had positive rheumatoid factors, and 26.3% (5 patients) had negative. There was no significant difference between the seropositive and seronegative patients in histamine concentration in synovial fluid and blood, as well as in histamine index (Table 1).

All patients were classified into three groups according to the disease activity (Table 2): very active (VA) with DAS-28 (3) score more than 5.1, moderately active (MA) disease with DAS-28 (3) score between 3.2 and 5.1, and inactive (IA) with DAS-28 (3) score below 3.2. Histamine levels in synovial fluid and blood, and histamine index, as well as a number of tender and swelled joints and erythrocyte sedimentation rate are presented in Table 2.

The mean number of tender joints in all patients suffering from RA was 6.57 ± 1.07 (median = 6, minimum = 2, maximum = 16). The mean number of swelled joints was 4.42 ± 0.54 (median = 4, minimum = 1, maximum = 9). The mean erythrocyte sedimentation rate was 25.89 ± 3.51 (median = 25, minimum = 6, maximum = 60). The mean value of DAS 28 (3) score was 4.48 ± 0.26 (median = 4.61, minimum = 2.79, maximum = 6.52).

Histamine concentration in synovial fluid tended to increase in relation to RA activity. Besides, there was no statistically significant difference in histamine levels in synovial fluid and blood related to disease activity. However, there was a significant difference in histamine index and it was increased proportionally to the disease activity (Figure 1).

**Discussion**

Histamine level in synovial fluid of all the patients with RA was in accordance with the previous literature data 24, 25 that are related to determination of histamine concentration using the fluorometric assay method of Shore 33. These authors reported increased histamine levels in synovial fluid of patients with osteoarthritis in comparison with RA. On the other hand, there is data that shows very low (almost undetectable) amounts of histamine in synovial fluid in RA, using the radioimmunoassay method for histamine detection 26. The authors, who used ELISA method for histamine detection reported decreased plasma histamine levels in RA patients compared to healthy population, while synovial fluid levels were even lower 27. Other authors detected higher plasma histamine levels in RA patients compared to population without inflammatory arthritis 28. They notified higher histamine levels in synovial fluid than in correspondent plasma samples. Radioenzyme assay for histamine detection was used in their investigations 28. Results of various investigations indicate that histamine levels in synovial fluid and plasma depend on the method for histamine detection that is used.

![Histamine index and rheumatoid arthritis activity according to DAS 28 (3) score: very active (VA) with DAS-28 (3) score more than 5.1; moderate activity (MA) with DAS-28 (3) score between 3.2 and 5.1, and inactive (IA) with DAS-28 (3) score below 3.2. (Student's t-test, *p < 0.05)](image-url)

**Table 1**

<table>
<thead>
<tr>
<th>Rheumatoid factor</th>
<th>Synovial fluid (ng/ml)</th>
<th>Blood (ng/ml)</th>
<th>Histamine index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1.54 ± 0.13</td>
<td>48.33 ± 4.05</td>
<td>3.170 ± 0.043</td>
</tr>
<tr>
<td>Negative</td>
<td>1.56 ± 0.16</td>
<td>49.71 ± 4.93</td>
<td>3.131 ± 0.064</td>
</tr>
</tbody>
</table>

**Table 2**

Dependence of DAS 28(3) score, histamine concentration in synovial fluid and blood, histamine index, number of tender and swelled joints, and erythrocyte sedimentation on rheumatoid arthritis activity

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>very active</th>
<th>moderately active</th>
<th>inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS 28 (3)score</td>
<td>6.056 ± 0.23</td>
<td>4.52± 0.12</td>
<td>3.07± 0.05</td>
</tr>
<tr>
<td>Histamine concentrations (ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>synovial fluid</td>
<td>1.68 ± 0.13</td>
<td>1.57 ± 0.14</td>
<td>1.4 ± 0.24</td>
</tr>
<tr>
<td>blood</td>
<td>50.36 ± 3.88</td>
<td>49.61 ± 4.35</td>
<td>46.56 ± 7.71</td>
</tr>
<tr>
<td>Histamine index</td>
<td>3.331 ± 0.041†</td>
<td>3.171 ± 0.023†</td>
<td>2.988 ± 0.038</td>
</tr>
<tr>
<td>Number of tender joints</td>
<td>13.4 ± 1.2</td>
<td>5.12 ± 0.58</td>
<td>2.16 ± 0.16</td>
</tr>
<tr>
<td>Number of swelled joints</td>
<td>7.4 ± 0.67</td>
<td>4.0 ± 0.37</td>
<td>1.66 ± 0.33</td>
</tr>
<tr>
<td>Sedimentation rate</td>
<td>46.5 ± 5.33</td>
<td>24.5 ± 1.42</td>
<td>9.0 ± 0.87</td>
</tr>
</tbody>
</table>

*values of all parameters are given as X ± SD; †p < 0.05 vs inactive
Our results indicate that there are no significant differences in histamine blood concentration between the RA patients and the healthy population. Histamine levels in blood are within the previous reported values in the literature.

Rheumatoid arthritis activity was determined in accordance with DAS 28 (3) score observing the erythrocyte sedimentation rate, the number of swollen joints and the number of tender joints. We registered the lowest synovial fluid histamine levels in the patients with inactive disease (DAS-28 < 3.2). On the other hand, the patients with very active disease (DAS-28 > 5.1) had the highest levels of histamine in synovial fluid. However, these differences in histamine concentration were not statistically significant because of the large individual variabilities. Although it could be expected that there is a relationship between histamine level in the synovial fluid and disease activity, this "hypothesis" is shown to be incorrect. The definition of the previously mentioned "hypothesis" has been confirmed by many literature data: increased histidine decarboxylase (HDC) activity influenced by cytokines IL-1 and TNF-α which are produced in larger amounts in evolutive forms of RA. Increased number of synovial tissue mast cells in patients with active and evolutive form of RA, compared to patients with inactive form and the end stage of the disease. Possible different H4 receptor expression in dependence of RA activity, increased histamine levels in bronchoalveolar lavat (BAL) as a useful marker for pulmonal disease activity in RA.

Considering the increased histamine concentration in synovial fluid of RA patients with very active disease, it can be suggested that there is a different expression of histamine releasing factor and/or different chemotactic (SCF, TGF-β, C5α, PAF) concentration that influences mast cells migration according to the disease activity. It was also suggested that histamine could play an important role in autocrine regulation of cytokine secretion from mast cells. That indicates a possible pathway of inflammatory response modulation in certain diseases.

Although histamine level in synovial fluid tends to elevate according to increased RA activity, there was no significant difference between the patients with a distinct disease activity. There was no difference in blood histamine concentration, as well. That was the reason to involve "histamine index" in our investigation as a more sensitive tool in determination of histamine role in RA.

The patients were treated in accordance with therapeutic strategies for RA, with disease modifying antirheumatic drugs (DMARD) methotrexate and/or chloroquine, nonsteroid antiinflammatory drugs (NSAID), and some of them with low doses of prednisone (≤ 10 mg per day).

There has been literature data that suggests decrease of histamine release influenced by methotrexate and prednisolone. On the other hand, a decrease of histamine metabolism by chloroquine inhibition of histamine N-methyltransferase may lead to the increase of histamine concentration. Our results showed no difference in histamine index in relation to the mentioned therapeutic protocols. The reason for this may be the fact that patients were in the various phases of illness activity at the moment of our investigation. There were no difference in DAS 28 score in relation to various therapeutic protocols applied before our investigation. To investigate the effects of various therapeutic protocols on histamine index we have to measure histamine blood and synovial fluid concentrations before and after the applied therapeutic protocol. That will be the subject of our further investigations.

Our results obviously suggest that histamine index is significantly increased in patients with high disease activity. This new parameter is increased proportionally with disease activity expressed by DAS-28 score. Values of histamine index (HI) below 3.02 represent inactive disease, values of HI between 3.02 and 3.29 moderate activity of the RA and HI more than 3.29 very active disease. According to this, histamine index could be a new, additional parameter in the evaluation of disease activity.

Conclusion

It can be concluded that, although there is no difference in histamine concentration in blood and synovial fluid related to RA activity, histamine index is increased proportionally with the disease activity. According to this, histamine index can be considered as a useful parameter in the evaluation of RA activity.

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