

Urinary Proteins Induce Lysosomal Membrane Permeabilization and Lysosomal Dysfunction in Renal Tubular Epithelial Cells in Seronegative Rheumatoid Arthritis

Dejan Spasovski*

University Clinic of Rheumatology, Clinical Center "Mother Therese", Skopje, Faculty of medicine, Republic of North Macedonia.

**Corresponding Author:* Dejan Spasovski, University Clinic for Rheumatology, faculty of medicine, University Clinical Center "Mother Therese", Skopje, Republic of North Macedonia.

Abstract

Introduction: To determine the effects of non-treated seronegative rheumatoid arthritis (RA) on proximal renal tubule, sensitivity of Alanine aminopeptidase (AAP), γ -glutamyltransferase (γ -GT), β 2 microglobulin in urine (β 2M), as well as relation with rheumatoid factor (RF) and C-Reactive protein (CRP), DAS 28 disease activity index.

Methods: RF was determined by agglutination test (Latex RF test, while kinetic methods were used for determination of Alanine aminopeptidase (AAP) and γ -glutamyltransferase (γ -GT), as well as MEIA (Microparticle Enzyme Immunoassay) to determine β 2 microglobulin in urine. Samples (serum and urine) of 70 participants were examined (35 RA not treated, 35 health control group).

Results: In 35 RF negative RA, AAP enzymuria was present in 12 (34.28%) patients, γ -GT was present in 7 patients (20%), while β 2 microglobulin was present in 3 patients (8.57%). In the healthy control group, 4 patients showed AAP positivity (11.42%), 2 patients γ -GT positivity (5.71%) and 1 patient showed presence of 2 microglobulin in urine (2.85). RF was not present in any patient (0%).

Conclusion: AAP has a higher sensitivity of γ -GT and β 2 microglobulin in the detection of asymptomatic renal lesions in non treated seronegative RA.

Keywords: Alanine aminopeptidase, (AAP); γ -glutamyl transferase (γ -GT), β 2 microglobulin (β 2M); Rheumatoid arthritis (RA), Rheumatoid factor (RF)

INTRODUCTION

Enzymes in urine can derive from plasma, glands of the urogenital tract, epithelial cells of the urinary tract, leukocytes, erythrocytes (1) and kidneys. There are about 40 different enzymes (2-6) in the urine that belong to different groups: oxidoreductase, transferase, hydrolase, lyase, while isomerases and ligases are not found in the urine. The occurrence of such large number of enzymes in the urine indicates the dominant role of kidneys in their excretion.

Examination of the cell membranes of the brush epithelium of the proximal tubules confirms the localization of alanine aminopeptidase (AAP) in 90%, alkaline phosphatase (AF) in 70% and γ -glutamyl

transpeptidase (γ -GT) in 50% of the total activity of these enzymes in the kidney. (7-9)

AIM

The aim of this study is to determine the effects of non-treated Rheumatoid arthritis on the tubular function AAP, γ -GT and β 2M being used as indicators for proximal tubular damage.

MATERIALS AND METHODS

In patients included in the study, disease diagnosis is based on the revised diagnostic criteria for classification of Rheumatoid arthritis proposed in 1987 by the American Rheumatism Association (ARA) (10-13). For the classification, i.e. the patients to be

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included in the RA group it is necessary to satisfy at least 4 of the predicted 7 criteria.

Criteria from 1 to 4 were present at least 6 weeks. The study included 35 patients (age 28, age 7) who were diagnosed with seronegative RA, as well as 35 patients (age 18, age 17) as a healthy control group. The average mean age was 48.5 years (± 4.13) (37-65 years) for the RA group, 36.2 years (± 10.78) (29-65) for the healthy group. The average time of onset of disease in months from the beginning was 14.97 (± 15.23), in the interval of (1-14) months. None of the patients in the study had a history of previous or current renal impairment. The others negate use of other drugs before sample were taken. The samples were collected in a period of 1 year.

INCLUDING CRITERIA

In the study were included patients with RA at the age of 18-65 years, who were not previously treated with NSAIDs or DMARDs.

EXCLUDING CRITERIA

In the study were excluded patients with symptoms or conditions that can directly or indirectly affect the results, such as:

1. Patients with a history of gonorrhoea, mild to moderate hepatic, renal, hematologic, cardiovascular, neurological diseases, nausea, vomiting, autoimmune disease.
2. Patients with diabetes mellitus, acute infections, malignant neoplasms, febrile conditions.
3. Patients with urinary tract arthritis, urinary tract infections, SLE, mixed connective tissue disease, vasculitis.
4. Patients with a history of blood transfusion, and excessive body weight.
5. Patients who receive baseline therapy are excluded from the study.
6. Ppatient with a history of glycemia or increased levels of product degradation in the 0-th range: serum creatine and urine, serum urea, hypertension, arterial hypertension. and hematological and enzyme status.
7. Patients previously treated with salicylates, antibiotics, gold salts, or diuretics.

All participants voluntarily took part in this study, so that the criteria to do it are met.

CLINICAL ASSESSMENT OF DISEASE ACTIVITY

Clinical assessment and interpretation was made from the subspecialist in the given area. The disease activity was assessed using DAS 28 index. (Disease Activity Score (DAS 28)) (14). Indexes use mathematical formula to use the unique composite quantitative score consisting of palatable painfully sensitive joints (maximum number 28) and swollen joints (maximum number 28), global assessment for disease activity (0 - 100 mm Visual Analog Scale VAS), as well as morning stiffness (minutes). DAS 28 index ranges from 0 to 10 and score below 3.2 qualifies the disease as low active.

LABORATORY ASSESSMENT

For clinical assessment of disease, it is necessary to consider the following laboratory variables: Complete blood count (CBC) and differential, acute phase reactants, such as C-reactive protein (CRP), Rheumatoid factor (RF), Erythrocyte sedimentation rate (ESR), alkaline phosphatase (AF), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), urea / serum, creatinine / serum.

Urine samples were taken not only for routine urinary examination, but also for determination of AAP, γ -GT, β 2M.

Serum urea is determined by the method of "Kassirer"

Reference values: Serum urea (3-7.8 mmol / L).

Creatine in serum and urine and determined by the method of: "Jaffe"

Reference values: Serum creatine 45 - 109 μ mol / L; Creatine in urine 7 - 17 mol / dU.

C-reactive protein (CRP) determined by agglutination test (Latex CRP test)

Reference values: < 6 mg / L CRP in serum

Rheumatoid factor (RF) determined by agglutination test (Latex RF test)

Reference values: < 8 IU / ml in serum

Determination of Alanine Aminopeptidase Activity (AAP): Kinetic Method

Reference values: AAP in urine 0.25-0.75 U / mmol creatinine

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Determination of γ -glutamyltranspeptidase (γ -GT) activity: Ifcc method

valuable referents

γ -GT (urine) 0.84-1.80 u / mmol creatinine

Determination of β 2 microglobulin (β 2M) concentration in urine by the method „meia” (“microparticle enzyme immunoassay”)

Reference values:

β 2 microglobulin (urine) = 0.02-0.19 mg / L

STATISTICAL ANALYSIS

For testing the significance of the differences between two arithmetic means, i.e. the corresponding proportions, the Student t-test is used, when comparing the mean values of the given number of parameters between two groups, such as Wilcoxon- matched test for independent samples. Sensitivity and predictivity for positive and negative tests of the examined markers is determined with tests for sensitivity and specificity. The P value of between 0.05 and 0.1 is considered statistically significant. The data processing is made with the statistical package Statistica 7.0.

RESULTS

In the group of 35 patients with RA, RF seronegative RA, AAP enzyme was present in 12 (34.28%) patients, γ -GT was present in 7 patients (20%), while β 2 microglobulin in urine was not present at all (0%).

In the healthy control group, 4 patients showed AAP positivity (11.42%), 2 patients γ -GT positivity (5.71%)

and 1 patient presented with β 2 microglobulin in urine (2.85). RF was not present in any patient (0%)

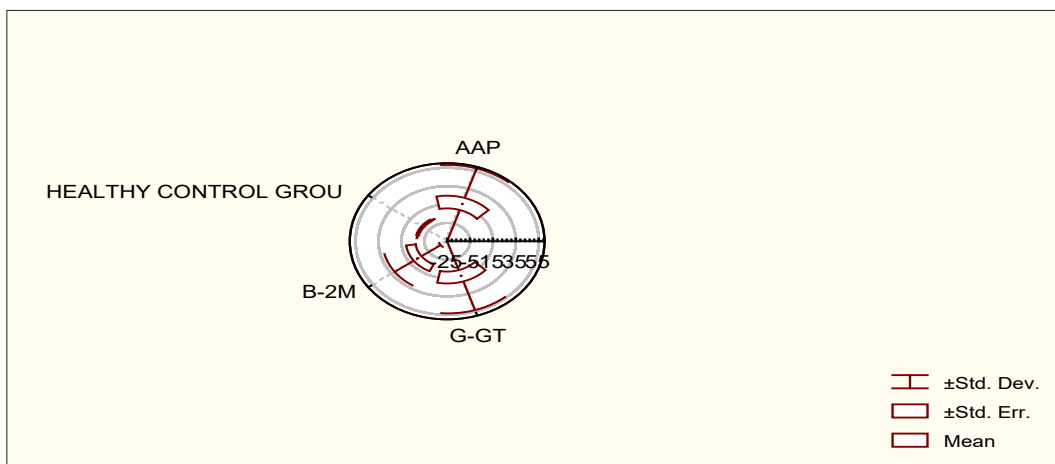
AAP, γ -GT, B2M AND DAS 28 INDEX OF DISEASE ACTIVITY

In the group of 35 patients with RA, DAS 28 > 3.2 was present in 28 patients (80%). In these 28 patients DAS 2 > 3.2, AAP positive 10 (35.71%) and their M \pm SD (1.25 \pm 0.43) range (0.85-2.46), γ -GT positive were 5 (17.85%) their M \pm SD (2.65 \pm 0.46) range (0.95-3.45), while β 2M was not present in any patient.

In 7 seronegative RF patients with DAS 28 < 3.2 (20%). In these 7 patients DAS 28 < 3.2, AAP was positive in 2 patients (28.57%) and their M \pm SD (1.20 \pm 0.49) range (0.80-2.30), γ -GT positive in 2 patients (28,57%) and their M \pm SD (2.50 \pm 1.07), range (0.90-2.20). β 2M was not present in any patient.

1. Sero negative RF patients with DAS 28 > 3.2 have higher AAP values than RF seronegatives with DAS 28 < 3.2. (1.25 (\pm 0.43) vs 1.20 (\pm 0.49), that had lower DAS 28 index. Between these 2 groups of AAP there was not statistical correlation (p = 0.185017);

2. Sero negative RF patients with DAS 28 > 3.2 have slightly higher value of γ -GT than RF seronegative with DAS 28 < 3.2. (2.65 \pm 0.46) vs (2.50 \pm 1.07). Between these 2 groups of γ -GT there was not statistical correlation (p = 0.670077); This group had larger γ -GT induction than the seronegative RF patients with DAS 28 < 3.2. **Graph 1**



Graph 1. Distribution of aline aminopeptidase (AAP), γ -glutamyl transferase (γ -GT), β 2 microglobulin (urine) in Rheumatoid arthritis

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There was no statistical correlation between DAS 28 index in RF negative patients with DAS 28 < 3.2 and DAS 28 > 3.2 ($p = 0.323$).

1. There was statistical correlation using Wilcoxon-matched test between AAP in RA and healthy control group for $p < 0.05$ ($p = 0.026113$). Within the RA group, there was statistical correlation between AAP and γ -GT for $p < 0.05$ ($p = 0.000003$).

2. There was no statistical correlation using Wilcoxon-matched test between: β 2M in RA and the healthy control group for $p < 0.05$ ($p = 0.054759$).

3. There was statistical correlation using Wilcoxon-matched test between AAP and γ -GT in RA and age, disease duration in months, CRP, SER, morning stiffness, serum creatine, urine creatine and serum urea in the same group for $p < 0.05$: (AAP vs. age $p = 0.000000$; AAP vs. disease duration in months $p = 0.000000$, AAP vs. CRP $p = 0.040620$; AAP, γ -GT vs SER $p = 0.000000$; AAP, γ -GT vs morning stiffness $p = 0.000010$; AAP, γ -GT vs serum creatine $p = 0.000000$; AAP, γ -GT vs creatine in urine $p = 0.000000$; AAP, γ -GT, vs serum urea $p = 0.000000$).

DISCUSSION

In standard medical rheumatology, the greatest emphasis is put on Rheumatoid arthritis as the most exposed disease. Seronegative RA is an rare form, difficult to recognize and most often confused with degenerative rheumatism, probably due to their frequency.

Urinary enzyme activity is normally low in the urine and increases when renal tubular cells are excreted (15). Urinary enzymes, especially NAG, AAP, AF are very sensitive indicators of parenchymal renal damage in comparison with functional measurements such as glomerular filtration rate (GFR), creatinine and inulin clearance. The relatively low sensitivity of the GFR can be explained by the large renal functional reserve and its large capacity for compensation (16). There are indications that elevations in urinary enzyme activity may indicate the location of the primary renal tubular damage due to their localization in the brush border area (microsomal AAP) and tubular lysozyme (NAG). They can be used in early diagnosis of acute renal failure because nephrotoxicity is induced by immunosuppressive drugs, contraceptives, antibiotics and cadmium exposure (17-19).

The sensitivity of AAP is higher in comparison with γ -GT and β 2M. Other standard routine tests used to assess renal function show low sensitivity: creatine in serum and urine, urea in serum. Seronegativity has an impact on the occurrence of AAP enzymuria. This is also present for seronegative patients with DAS 28 > 3.2 who have a much larger AAP induction than DAS 28 < 3.2. Statistical correlation of disease duration in months indicates that the non-treated RA affects kidney tissue as one of the visceral manifestations of disease.

Non-treated RA primarily affects tubular brush border area and enzymes that derives from this area have increased sensitivity.

CONCLUSION

AAP has a higher sensitivity than γ -GT and β 2M in the detection of asymptomatic renal lesions in the non-treated seronegative RA. AAP and γ -GT can be used in the everyday clinical practice to diagnose early, asymptomatic renal lesions.

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