JMB 2008; 27 (1) DOI: 10.2478/v10011-007-0047-z

UDK 577.1 : 61 **ISSN 1452-8258**

JMB 27: 59–63, 2008 *Original paper*
Originalni naučni rad

ALANINE AMINOPEPTIDASE, γ-GLUTAMYL TRANSFERASE AND β_2-MICROGLOBULIN AS DIAGNOSTIC MARKERS IN PATIENTS WITH RHEUMATOID ARTHRITIS

ALANIN AMINOPEPTIDAZA, γ-GLUTAMIL TRANSFERAZA I β_2-MIKROGLOBULIN KAO DIJAGNOSTIČKI MARKERI KOD PACIJENATA SA REUMATOIDNIM ARTRITISOM

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Summary: The purpose of this research is to evaluate the values of alanine aminopeptidase (AAP), γ-glutamyl transferase (γ-GT), and β_2-Microglobulin in urine (β_2-M), in untreated rheumatoid arthritis (RA) and to define the effect of untreated rheumatoid arthritis on the tubular function and brush border region. We used a kinetic assay for AAP, standard methods by the IFCC for γ-glutamyl transferase and MEIA for the determination of β_2-Microglobulin in urine in 70 participants (35 untreated RA patients, 35 healthy individuals). From the total of 35 RA patients, 24 patients had AAP (sensitivity of the test 68.57%), 16 patients had γ-GT enzymuria (sensitivity of the test 45.71%), while the presence of β_2-Microglobulin in urine was found in a very low percentage. Out of 18 RF negative patients, 14 patients are AAP positive, 10 patients were γ-GT positive, while the presence of β_2-Microglobulin in urine was not detected. Among 17 RF positive RA patients, the presence of AAP was noticed in 10, the presence of γ-GT in 6 patients, while the presence of β_2-Microglobulin in urine was not detected. AAP has higher sensitivity than γ-GT and β_2-Microglobulin in the detection of asymptomatic renal lesions in untreated RA.

Keywords: alanine aminopeptidase, γ-glutamyl transferase, β_2-Microglobulin, rheumatoid arthritis, rheumatoid factor

Kratak sadržaj: Cilj ovog istraživanja je bio da se proceni nivo alanin aminopeptidaze (AAP), γ-glutamiltransferaze (γ-GT) i β_2-mikroglobulina (β_2M) u urinu kod pacijenata sa netretiranim reumatoidnim artritisom (RA). Sekundarni ciljevi su bili određivanje efekta netretiranog reumatoidnog artritisa na tubularnu funkciju i takozvane brush border regije. Koristeći kinetičku metodu za određivanje AAP, standardizovani metod IFCC za γ-glutamiltransferazu (γ-GT), kao i MEIA za određivanje β_2-mikroglobulina u urinu, ispitani su uzorci 70 pacijenata, 35 netretiranih RA pacijenata i 35 pripadnika kontrolne grupe. Od ispitanih 35 RA pacijenata, kod 24 je pokazano prisustvo AAP enzimurije (senzitivnost testa 68,57 %) γ-GT je zastupljena kod 16 pacijenata (senzitivnost testa 45,71%), dok je prisustvo β_2-mikroglobulina u urinu bilo neznačajno. Četrnaest RF negativnih pacijenata bili su AAP pozitivni, 10 sa prisutnim γ-GT, bez prisustva β_2-mikroglobulina. Od 17 RF pozitivnih RA pacijenata, 10 je bilo pozitivno na AAP, 6 na γ-GT, dok prisustvo β_2-mikroglobulina u urinu nije detektirano. AAP ima veću senzitivnost od γ-GT i β_2-mikroglobulina za detekciju asimptomatskih bubrežnih lezija kod netretiranog RA.

Ključne reči: alanin aminopeptidaza, γ-glutamil transferaza, β_2-mikroglobulin, reumatoidni artritis, reumatoidni faktor

Introduction

There are approximately 40 different enzymes in the urine with different origin. They are excreted from the kidneys, urinary tract epithelium and urinary tract glands, plasma and blood cells (1).

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However, proximal tubules of the kidneys have a dominant function in their excretion. Examination of the brush border epithelium (BBE) of the proximal tubules confirms that alanine aminopeptidase, AAP (90%), alkaline phosphatase, AF (70%) and γ-glutamyl transferase, γ-GT (50%), constitute the biggest part of the total activity of these enzymes in the kidney (1). Because BBE is very sensitive to changes, these and other enzymes can be used as markers for secondary renal damage under the action of different diseases, medicines and toxines (2). Increased enzymatic activity can be a reflection of disease activity and of the residual functional capacity of the kidney.

The aim of this study is to define the effect of untreated rheumatoid arthritis on the brush border epithelium of the proximal tubules and the tubular function. In this regard, AAP, γ-GT and β_2-Microglobulin in urine were used as indicators for tubular damage.

Patients and Methods

The study involved 70 patients (18–65 years of age), 35 of whom fulfilled ACR criteria for RA (3), and who were not treated with disease modifying agents. The average age was 56.68 years (± 6.79) in the RA group, 40–65 years, and 46.2 years (± 12.49) in the healthy control group. The average duration of the disease was 43.97 (± 45.23) months. RA patients were not treated with disease modifying agents or NSAIDs. Only 3 patients had been previously treated with oral glucocorticoids.

Patients with a medical record for past or present renal disease were excluded from the study.

The activity of the disease was evaluated using the DAS28 index, which is a unique composite quantitative score, constituted from the tender joint count, swollen joint count, ESR, and the patient's global assessment of the disease activity (VAS 0-100). DAS > or = 3.2 denotes high disease activity (4).

Glomerular Filtration Rate (GFR) is calculated with the Cockroft@Gault equation.

A kinetic assay for AAP, standard methods by the IFCC for γ-glutamyl transferase (γ-GT) and MEIA (Microparticle Enzyme Immunoassay) for the determination of β_2-Microglobulin in urine were used. The reference values were as follows: AAP urine 0.25–0.75 U/mmol creatinine, γGT urine 0.84–1.80 U/mmol creatinine, β_2-Microglobulin (urine) = 0.02–0.19 mg/L.

We used Statistica 7.0 for data processing. The participants signed their informed consent.

Results

From the total of 35 patients with RA, 24 (68.57%) showed the presence of AAP, 16 (45.71%) the presence of γ-GT, while in none of them appeared the presence of β_2-Microglobulin in the urine. Among the total of 35 examined RA patients, sensitivity of AAP was 68.57%, sensitivity of γ-GT was 45.71%, sensitivity of β_2-Microglobulin was 0, while sensitivity of RF was 48.57%.

Among the 17 RF positive RA patients, 10 patients showed the presence of AAP (58.82 %), 6 patients the presence of γ-GT (35.29 %), while the presence of β_2-Microglobulin was not detected in the urine.

Among 18 RF negative RA patients, AAP enzymuria was present in 14 patients (77.77%), γ-GT was present in 10 (55.55%), while β_2-Microglobulin in urine was not present at all (*Table I*).

RF was positive in 17 patients (48.57%), and 10 patients (28.57%) were positive for both RF and AAP. Six patients (17.14%) were γ-GT and RF positive. RF was negative in 18 patients, 14 of whom were AAP positive, 10 of whom (28.57%) were γ-GT positive. The presence of β_2-Microglobulin in the urine was unsignificant in seropositive and seronegative patients (0.05%).

AAP enzymuria was not detected in 7 RF positive patients (20%) and γ-GT enzymuria was not detected in 11 RF positive patients (31.42%). β_2-Microglobulin was not detected in the urine of all 17 patients (48.6%) who were RF positive.

AAP and γ-GT enzymuria were detected in 14 (77.77%), and in 10 patients (55.55 %) out of 18 RF negative patients, respectively. There was no presence of β_2-Microglobulin in the urine.

From the of total 11 patients without AAP enzymuria, 7 patients (63.63%) were RF positive. From the total of 19 patients presence of γ-GT enzymuria was not detected, 11 patients (57.89%) were RF seropositive.

In the HC group, 7 patients (20%) appeared as AAP positive, 6 patients as (17.14%) γ-GT positive, and 1 patient (2.85%) β_2-M positive. Two patients were RF positive (5.71%).

Sensitivity, specificity, positive and negative predictive values and accuracy were calculated for AAP, γ-GT, β_2-Microglobulin. They are shown in *Table II*.

AAP has better diagnostic performances than γ-GT and β_2-Microglobulin in relation to sensitivity (sensitivity 68.57% vs. 45.71% vs. 0%) and almost equal specificity (80% vs. 82.85% vs. 97.14%) in the detection of renal tubular damage in untreated RA.

Among the total of 35 patients with RA, high activity of the disease DAS 28 > 3.2 was present in 28 patients (80%). DAS 28 > 3.2 was calculated in 15 out of 17 (88.23%) seropositive RF patients. In this group of 15 patients, AAP was positive in 8 patients (53.33%), mean 1.20 ± 0.49 SD in the range of 0.80–2.30, γ-GT was positive in 4 patients (26.66%), mean values 2.61 ± 1.07 SD in the range of 1.90–4.20. β_2-M was not present in the patients (*Figure 1)*.

Among 18 RF negative patients, the presence of DAS 28 > 3.2, was found in 13 patients (72.22%).

Table I AAP, γ-GT, β_2-Microglobulin and other laboratory markers in RA and healthy control (HC) group.

	RA Total 35 Value (M ± SD)	RA RF negative 18 Value (M ± SD)	RA RF positive 17 Value (M ± SD)	HC group 35 Value (M ± SD)
	Positive / Negative	Positive / Negative	Positive / Negative	Positive / Negative
AAP (U/mmol/creatinin)	24/11 1.06 (± 0.54)	14/4 1.14 (± 0.48)	10/7 0.98 (± 0.59)	7/28 0.74 (± 0.43)
γ-GT + >1.80 (U/mmol/creatinine)	16/19 1.80 (± 0.97)	10/8 1.81 (± 0.80)	6/11 1.79 (± 1.15)	6/29 1.51 (± 0.70)
β_2-Microglobulin +> 0.19 (mg/L)	0/35 0.05 (± 0.03)	0/18 0.06 (± 0.04)	0/17 0.04 (± 0.03)	1/34 0.08 (± 0.06)
Creatinine serum < 49–109 > μmol/L	3/32 67.55 (± 14.76)	1/17 68.24 (± 14.16)	2/15 66.82 (± 15.77)	2/33 74.95 (± 19.72)
Creatinine urine < 7–17> mol/dU	9/26 10.41 (± 4.71)	6/12 9.26 (± 4.54)	3/14 11.62 (± 4.72)	5/30 9.15 (± 4.22)
Urea serum + > 7.8 mmol/L	4/31 5.66 (± 1.46)	0/18 5.52 (± 1.33)	4/13 5.82 (± 1.62)	1/34 4.94 (± 1.28)
GFR + >90 mL/min	14/21 99.19 (± 24.46)	7/11 99.19 (± 24.46)	7/10 99.19 (± 25.22)	4/31 113.80 (± 30.86)
DAS 28 + > 3.2	28/7 4.79 (± 1.56)	13/5 4.56 (± 1.76)	15/2 5.04 (± 1.33)	0/35 0.00 (± 0.00)
Morning stiffness + > 0 min	26/9 43.20 (± 65.13)	14/4 57.50 (± 81.40)	12/5 28.05 (± 38.72)	0/35 0.00 (± 0.00)
RF +30 > IU/mL	17/18 346.15 (± 625.22)	0/18 0.00 (± 0.00)	17/0 712.67 (± 743.72)	2/33 13.71 (± 38.73)
CRP +12 > mg/L	14/21 46.86 (± 79.19)	3/15 8.66 (± 24.62)	13/4 87.31 (± 96.44)	4/31 5.48 (± 12.80)
ESR +> 16	27/8 48.62 (± 39.81)	13/5 43.94 (± 39.82)	14/3 53.58 (± 40.39)	4/31 9.42 (± 8.21)
Anti CCP 2 > 1.26	23/12 1.71 (± 0.69)	11/7 1.56 (± 0.59)	12/5 1.87 (± 0.77)	1/34 0.95 (± 0.10)

Table II Diagnostic performance of AAP, γ-GT, β_2-Microglobulin and other laboratory variables in RA.

	AAP RA (all)	AAP RA RF^-	AAP RA RF^+	γ-GT RA (all)	γ-GT RA RF^-	γ-GT RA RF^+	β_2 Microglobulin RA (all)	β_2 Microglobulin RA RF^-	β_2 Microglobulin RA RF^+
Sensitivity %	68.57	77.77	58.82	45.71	55.55	35.29	0	0	0
Sensitivity %	80	80	80	82.85	82.85	82.85	97.14	97.14	97.14
Positive predictive values %	77.41	66.66	58.82	72.72	62.50	50	0	0	0
Negative predictive values %	28.20	2.5	0	39.58	21.62	27.5	89.74	34.61	33.33
Accuracy %	74.28	79.24	73.07	64.28	73.58	67.30	48.57	64.15	65.38

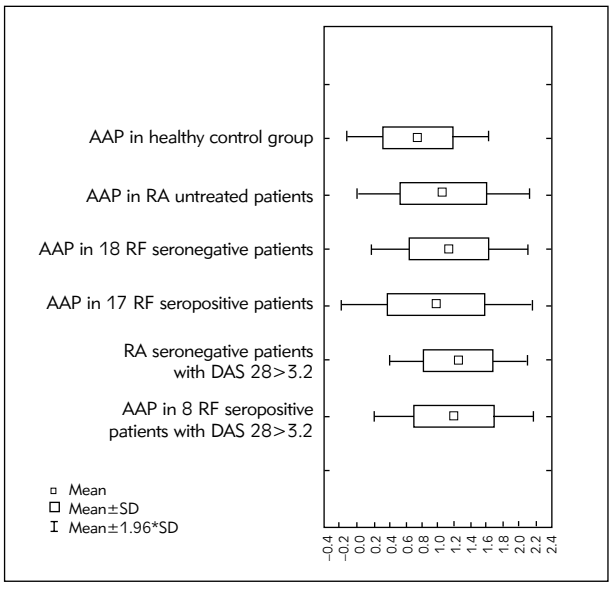

Figure 1 Distribution of alanine aminopeptidase (AAP) (U/mmol creatinine) between the groups.

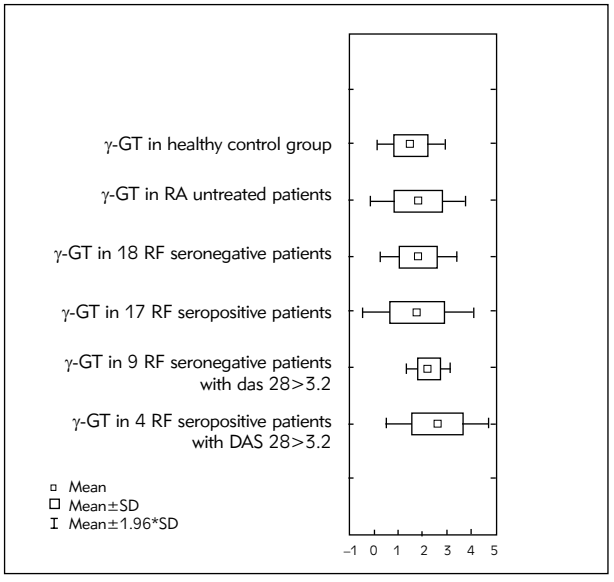

Figure 2 Distribution of γ-glutamyl transferase (γ-GT) (U/mmol creatinin) between the groups.

In these patients, DAS 28 > 3.2, 11 were AAP positive (84.61%) and their M ± SD (1.25 ± 0.43) extent (0.85–2.46), 9 were γ-GT positive (69.23%) and their M ± SD (2.55 ± 0.46) extent (0.95–3.45), while β_2-M was not present in the patients *(Figure 2)*.

Seronegative RA patients had higher values of AAP than seropositive patients, and the lower DAS 28 index, but there was no statistical difference between the results (p=0.18). Seronegative and seropositive RA patients had similar values of γ-GT in the urine (p= 0.67).

Seropositive RA patients with DAS 28 > 3.2 have much higher γ-GT induction than seronegative RF patients, with DAS 28 > 3.2, which was also not statistically significant (p=0.71).

Seronegative RA patients had a bit higher values of β_2-M than seropositive ones (p=0.22).

Using a Wilcoxon-matched test, we found statistically significant difference between AAP values in RA patients and the healthy control group p=0.02.

There was a significant correlation between AAP and γ-GT p<0.01; AAP and β_2-M (p<0.01); γ-GT and β_2-M (p<0.01) in the RA group.

We did not find a significant correlation for γ-GT and β_2-M in RA patients and the healthy control group (p=0.3 and p=0.05).

There was found a statistically significant positive correlation when AAP, β_2-M and γ-GT were correlated with age (p<0.01), duration of the disease in months (p< 0.01), DAS28 (p<0.01), RF (p=0.018), CRP (p=0.04) anti-CCP (only for γ-GT and β_2-M < 0.01), serum creatinine (p<0.01); creatinine in the urine (p<0.01); serum urea (p<0.01).

Discussion

Previous studies have shown that elevation of the urinary enzymes can indicate renal tubular damage. Urinary enzymes such as microzomal AAP and γ-GT can be used for early diagnosis of acute renal tubular damage which may be provoked by immunosuppressive drugs, contrast, antibiotics, and chronic inflammatory disorders such as RA. Renal tubular damage can be one of the visceral manifestations of the disease (5).

AAP showed higher sensitivity in untreated RA, compared to γ-GT and β_2-M (68.57% vs. 45.71% vs. 0%), but all three of them had a very similar specificity (80% vs. 82.85% vs. 94%).

Other standard routine analyses which were used for the evaluation of renal function, such as serum creatinine and serum urea, showed only very low sensitivity (Cr 8.57%, urea 25.71%).

Seronegativity for RF has showed an influence on the presence of AAP enzymuria. That is not the case with γ-GT induction, where γ-GT was higher in seropositive patients. That was a very interesting finding which should be evaluated further (6, 7).

In conclusion, untreated RA damages the tubular brush border region and is made visible by the high urinary enzymes with tubular genesis.

AAP has shown higher sensitivity than γ-GT and β_2-M in the detection of asymptomatic renal damage in untreated RA.

AAP and γ-GT can be used in everyday clinical practice in diagnosing early, asymptomatic renal damage in patients with untreated RA disease. Their presence may be used for immunosuppressive treatment tailoring and monitoring (8, 9).

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Received: January 18, 2008

Accepted: March 2, 2008