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Renal Enzymes and Microglobulins in Patients with Rheumathoid Arthritis

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Abstract

Aim: The aim of this study was to detect and compare the enzymes, globulins and reactants of the early phase in patients with untreated rheumatoid arthritis and to reveal the effect of untreated rheumatoid arthritis on tubular function and sensitivity on brush border area.

Material and Methods: The samples of serum and urine were examined in 70 participants (35 patients with untreated rheumatoid arthritis, 35 individuals of the healthy control group). We used in the study the kinetic assay for determination of alanine aminopeptidase (AAP) (Standards methods under IFCC), γ-glutamiytransferase and MEIA (Microparticle Enzyme Immunoassay) (Abbot $A_x$sym system) for detection of β 2-microglobulin in urine.

Results: Of 35 patients with RA, 16 patients showed presence of γ-GT (sensitivity of the test - 45.71%), 24 patients showed presence of AAP enzymuria (sensitivity of the test - 68.57%), while the presence of β2-microglobulin in urine was very low (sensitivity of the test - 0%). From 18 RF negative patients, 14 patients were AAP positive, 10 patients were γ-GT positive, while the presence of β2-microglobulin in urine was not detected. Among 17 RF positive patients with RA, the presence of AAP was detected in 10 patients, the presence of γ-GT in 6 patients, while the presence of β2-microglobulin in urine was not detected.

Conclusion: In untreated rheumatoid arthritis AAP had higher sensitivity than γ-GT and β2-microglobulin in detection of asymptomatic renal damage.

Keywords: Rheumatoid arthritis; Rheumatoid factor; β2-microglobulin; Alanine amino peptidase; g-glutamyl transferase

Introduction

The urine enzymes usually originate from epithelium cells and glands of urogenital system, plasma, leucocytes, erythrocytes and kidneys [1]. Approximately 40 [2-4] different enzymes from different groups appear in urine: transferases, lyases, oxidoreductases, different isomerases and ligases which usually are not found in urine. The appearance of such great number of enzymes in urine is due to the kidney's dominant function - excretion. Brushed epithelium of proximal tubules is the location for alanine aminopeptidase (AAP) - 90%, alkaline phosphatase (AP) -70% and γ -glutamyl transferase (γ-GT) - 50% from the total activity of these kidney enzymes [5]. Brushed epithelium is very sensitive even in physiological conditions, thus definite dispensation of superficial enzymes can be used as a marker for renal damage both primary and secondary, caused by drugs or toxins [6]. Tubular lysosomal system is very dynamic system and low level of lysosomal enzymes found in normal urine is a result of normal exocytic and pinocytic activity of tubular epithelial cells [7]. The extent of enzymuria depends on location and damage intensity. Increased enzymatic activity is a reflection of disease activity and kidney's residual functional capacity [8]. Renal tubular damage initially affects lysosomal /plazma cell membrane system, causing enzymatic loss in urine in early phase. Further increase in enzymatic excretion is connected with cell structural damage causing cell necrosis. Elimination of toxic stimulus is followed by reduction of urine enzymatic activity and tubular regeneration. The aim of this study is to define the effect of untreated rheumatoid arthritis on tubular function and sensitivity of brush border epithelium of renal proximal tubules. AAP, γ -GT and β2- microglobulin (β2M) in urine are used as indicators for proximal tubular damage.

Markers for Assessment of Renal Damage

For assessment of asymptomatic renal damage some classes of protein S in urine are used:

1. Low molecular weight proteins usually filtered in glomerulus and reabsorbed in tubules (β2M) [9-14].
2. Intermediate proteins normally filtered in glomerulus in very small quantity, while the rest is reabsorbed in tubules (microalbumin, transferin).
3. High molecular weight enzymes, usually not filtered in glomerulus, originating from renal proximal tubules (microsomal AAP, NAG).

Alanine aminopeptidase (AAP) arylamidase amino acid, amino peptidase, α-aminoacyl-peptidyl hydrolase (microsomal EC 3.4.11.2, earlier 3.4.1.2) is hydrolytic product of peptides, amides and p-nitroanilide. AAP is found in many tissues such as kidneys, intestine, lung and liver. AAP in different tissues has different electrophoretic conductivity. This enzyme has at least five [5] different isoenzymes, distinguished by immunological and electrophoretic features and ion change chromatography [15]. Normal serum contains only one isoenzyme, while in liver, biliary or pancreatic disease additional fractions are found. The enzyme is found in urine [16].

γ-glutamyl transferase (γ-GT), (γ-glutamyl-peptid aminoacid; γ-glutamyl-transferase, EC 2.3.2.2, is catalyzing transmission of γ-glutamyl groups with peptides (as glutathione) and other peptides or amino acides. γ-GT has an important place in metabolism of

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glutathione. High concentrations of enzymes are found in kidneys (renal proximal tubules), pancreas (acinar cells), prostate and liver. γ-GT is located mainly in external parts of plasma membrane. Isoenzymes of γ-GT in serum are consequence of different post-translational modifications such as modification of carbohydrate part of molecule of γ-GT, formation of complexes with lipoproteins [17,18]. Due to the differences in carbohydrate parts of the γ-GT molecule the isoenzymes are found in different tissues (liver, pancreas, kidney and duodenum). Although the peptide part of enzyme is the same in the tissue that originate, these isoenzymes differ in kinetic, electrophoretic and immunologic features [19]. Renal tubular function could be evaluated by measuring the excretion of low molecular proteins in urine.

β 2-microglobulin (*β*2M) in urine is used for detection of tubular malfunction in glomerulonephritis [20] and is often used as sensitive marker for evaluation of renal function [21-25]. *β*2M is a polypeptide with small molecular weight (11.815 daltons). It contains light chain of main histocompatibility antigen (HLA) and influences the production of rheumatoid factor (IgM class) [26]. *β*2M is found in serum and urine in healthy individuals [27]. 95% of free *β*2M is ultra-filtrated in renal glomerulus and almost complete (99.99%) is reabsorbed via proximal tubular endocytosis and finally is catabolized in amino acid in healthy individuals. Usually it is detected in traces in urine. Disturbance in glomerular filtration leads to increase in serum β2M, while tubular damage leads to increase in urine *β*2M. Serum *β*2M concentration is dependent on GFR and shows significant negative correlation with inulin clearance. Serum *β*2M level could serve as an index of damage intensity of renal glomeruli. In pathological conditions increased amount of *β*2M are excreted in urine. This happens when serum β2M concentration exceeds renal threshold. Serum level of *β*2M is dependent on synthesis or excretion in serum and is in relation with clearance. This happens in patients with inflammatory diseases such as rheumathoid arthritis [28], SLE [29], Sjogren's syndrome, Crohn's disease [30], cancer and liver damage. Urine *β*2M concentration can be increased in conditions when reabsorption is decreased due to renal proximal tubule damage. It results in urine *β*2M increased concentration and enables distinction between renal proximal tubular and glomerular damage.

*β*2M is used for GFR and for renal tubular function evaluation, especially for toxic tubular damage caused by heavy metals (cadmium and lead) and as screening test for early detection of Balkan's nephritis in endemic regions. *β*2M is unstable in urine pH<6 and it is recommended the urine to be alkalized with bicarbonates before it is processed. *β*2M is considered the earliest protein in tubular proteinuria. It suggests an asymptomatic renal dysfunction in RA patients.

Material and Methods

Diagnosis of rheumatoid arthritis (RA) was based of the revised diagnostic criteria for classification of RA, suggested in 1987 by the American Association for Rheumatism (ARA) [31]. Patient has to fulfill 4 out of 7 ACR criteria in order to be included in the group with RA. Criteria from one 1 to four 4 have to be present at least 6 weeks. 70 participants were included in the study: 35 RA patients (28 female, 7 male) and 35 participants (18 female, 17 male) as healthy control group. Their average age in the group with RA was 56-68 years (±6,79) (range 40-65 years), but in healthy control group 46,2 years (±12,49) (range 29-65 years). The average period from the beginning of disease was 43, 97 months (±45,23) (in interval 1-168). All the participants denied past or present renal disease. Three patients were previously treated with oral steroids, while nobody used NSAIL. The rest of the patients denied drug use before inclusion in the study.

Inclusion criteria

newly diagnosed patients with RA, not treated, age 18-65 years.

Exclusion criteria

1. Patients with autoimmune diseases, SLE, uric arthritis, Sjogren's syndrome, mixed conjunction texture disease and vasculitis.
2. Patients with previous history of renal, hematologic, cardiovascular, neurologic, liver and lung damage, diseases of the spleen and thyroid gland.
3. Patients who take drugs from basic line.
4. Patients treated with antibiotics and salicylates in period <6 months from the beginning of the study.
5. Patients with arterial hypertension, diabetes mellitus, acute infections, cancer, febrile conditions, AIDS.
6. Patients treated with antihypertensive, diabetic and cardiac therapy.
7. Hypersensitive to some drugs or their component.
8. Patients with previous history for blood transfusion and overweight.
9. Patients with glycemia in 0 spot or increased level of degraded products: creatinine in serum and urine, hematuria/proteinuria, urea in serum and disorder of hematologic and enzymatic status.
10. Patient's age < 18 years.

All patients took part voluntarily in the study, so the ethical criteria were fulfilled.

Clinical evaluation of disease activity

The disease activity was evaluated by subspecialist using DAS 28 index (Disease Activity Score - DAS 28) [32-35]. The index is mathematical formula, so we can get uniquely composed quantitative score, which consists of palpation of painful sensitive joints (max number 28), swollen joints (max number 28), ESR and patient's global assessment of disease activity (0-100mm Visual Analogous Scale - VA) and the morning stiffness. DAS 28 index is ranked from 0 to 10 and score <3.2 ranks the disease as low active. The assessment of glomerular filtration rate (GFR) is calculated with Cockroft & Gault's formula. [36]

Laboratory Assessment

Clinical assessment of RA comprised: erythroid sedimentation rate (ESR), complete blood count (CBC) and differential, anti CCP 2, C-reactive protein (CRP), Rheumatoid factor (RF), alkaline phosphatase (AP), aspartate amino-transferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), serum urea and serum creatinine. Samples of urine are taken not only for routine urine analysis, but also for detection of creatinine in urine, AAP, γ-GT and *β*2M.

Serum urea was detected with the "Kassirer" method.

Reference values: 3-7,8 mmol/L.

Creatinine in serum and urine was detected with "Jaffe" method:

Reference values: serum creatinine - 45 - 109 μmol/L; urine creatinine - 7 – 17 μmol/dU.

CRP was detected with agglutination test (Latex CRP test) (BioSystems S.A. Reagens&Instruments Costa Brava 30, Barselona (Spain) [37-39].

Reference value: <6mg/L CRP in serum.

RF was detected with the agglutination test (Latex RF test) (BioSystems S.A. Reagens&Instruments Costa Brava 30, Barselona - Spain) [40,41].

Reference value: <8mg/L RF in serum.

ESR was determined using Westergren's quantitative method for ESR.

Reference values: 7-8mm for male, 11-16mm for female.

Anti CCP 2 (second generation) is detected using the ELISA technology of DIA-STAT™ Anti-CCP (Axis–Shield Diagnostics).

The test's recommended values: < 0.95 negative; ≥0.95 to ≤1.0 borderline (repeated test recommend); >1.0 positive.

Alanine aminopeptidase (AAP) is detected with kinetic methods similar to those for detection of leucin aminopeptidase. L-alanin-4-nitroanilid was used in this method as substratum. Catalytic concentration of AAP is directly proportional of p - nitroanilin absorption measured on 405 nm.

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

γ-glutamil transpeptidase (**γ-GT**) is detected with IFCC method [42]. The methods of measuring the activity of this enzyme in serum use aromatic amides as substratum (γ-glutamylanilide and γ-glutamylnaftilamide). The most often used artificial substratum peptide analog is γ-glutamyl-p-nitroanilide, offering possibility for kinetic and colorimetric determination of the enzymatic activity [43,44]. γ-glutamyl-p-nitroanilide later is changed with L-γ-3-karboksi- 4-nitroanalid (Glukan), because of higher dissolution [45]. As acceptor of the substratum and puffer is used glycin-glycin, getting higher catalytic activity. This method is standardized by International Federation for Clinical Chemistry (IFCC) and is considered as reference method. IFCC method for measurement of the concentration of catalytic activity of γ-GT in serum and urine is based on the principles developed by Orlowski and Meiser [43] and Szasz [42]. This method has modifications developed by Persijin and Van der Slik [44]. L-γ-glutamyl-3-carboxi -4-nitroanilide is used as a donor of the substratum. In IFCC method Tris (hydroxymethyl) aminoethane is changed with glycin-glycin, used as a buffer and acceptor of the substratum. Magnesium (Mg) earlier used for keeping L-γ-glutamyl-4-nitroanilide in solution in IFCC method is omitted.

Reference values: γ-GT in urine: 0.84-1.80 U/mmol creatinin.

Detection of *β*2-microglobulin (*β*2M) in urine by MEIA method (Microparticle Enzyme Immunoaassay) (Abbot $A_x$sym system)

$A_x$sim *β*2M detection is based on MEIA technology (Microparticle Enzyme immunoassay), used for quantitative detection of *β*2M in serum, plasma and urine in patients with RA and renal dysfunction. The reaction consists of interaction of *β*2M with anti- *β*2M antibody, forming an interrelated complex. The complex reacts with Matrix cell and is tightly connected to them. A conjugate of alkaline phosphatase is added, which connects with the complex forming sandwich-complex. 4-Methylumbelliferyl Phosphate (4-MUP) is added on this complex; it reacts with alkaline phosphatase from the complex and fluorescent product Methylumbelliferon with light blue color is obtained. The degree of optical fluorescence directly proportionally determines *β*2M concentration. It is determined automatically (Abbot $A_x$sym system). *β*2M is very sensitive to pH changes in urine, because it decomposes rapidly in low pH values (<pH 6.0). So, if the urine is acid, it has to be alkalized.

Reference values: *β*2M in urine - 0.02-0.19 mg/L

Statistical Analysis

The difference between two arithmetic means was tested with Student's "t-test", comparing the middle values of the certain numerical parameters between two groups. Wilcoxon-matched test was used for independent samples. Sensitivity and prediction for positive and negative test of examined values were defined with the test of sensitivity and specificity. P value under 0.05 was taken as statistically significant. Data processing was done with statistical package -Statistica 7.0.

Results

Of the 35 patients with RA, 16 patients (45.71%) showed presence of γ-GT, 24 patients (68.57%) presence of AAP enzymuria and no presence of *β*2M (0%) in urine. RF appeared in 17 patients (48.57%). 6 patients (17.14%) were γ-GT and RF positive, 10 patients were AAP and RF positive (28.57%), while *β*2M in urine in RF positive patients showed very low percentage (0.05%). From 18 RF negative patients, 10 patients (28.57%) were γ-GT positive, 14 patients (40%) were AAP positive, while presence of *β*2M in urine was not detected (0%). Among 7 RF positive patients (20%) AAP enzymuria was not detected. Among 11 RF positive patients (31.42%) γ-GT was not detected. In 17 RF positive patients (48.57%) *β*2M was not detected in urine. Of 18 RF negative patients AAP enzymuria was detected in 14 (77.77%), while in 10 (55.55%) presence of γ-GT was detected. The presence of β2M in urine showed very low percentage (0%). From 11 patients without AAP enzymuria, 7 patients (63.63%) were RF positive. From 19 patients with no presence of γ-GT enzymuria, 11 patients (57.89%) were RF positive. From 35 patients without changes in *β*2M concentration in urine, 17 patients (48.57%) were RF positive. Among 18 RF negative patients, AAP enzymuria was present in 14 patients (77.77%), γ-GT was present in 10 patients (55.55%), while *β*2M in urine was not present at all (0%). Among 35 RA patients sensitivity of AAP was 68.57%, sensitivity of γ-GT was 45.71%, sensitivity of *β*2M was 0%, while sensitivity of RF was 48.57%. Among 17 RF positive patients, the presence of γ-GT was detected in 6 patients (35.29%), the presence of AAP was in 10 patients (58.82%), while the presence of *β*2M was not detected in urine (0%). Among healthy control group 7 patients (20%) were AAP positive, 6 patients (17.14%) γ-GT positive and 1 patient (2.85%) showed presence of *β*2M in urine. RF appeared in 2 patients (5.71%) (Table 1).

Diagnostic value of alanine aminopeptidase (microsomal AAP), γ-GT and β2M in urine in RA patients

For AAP, γ-GT and *β* 2M and for other laboratory variables in RA, sensitivity, specificity, predictable value for positive and negative test are show in (Table 2). AAP had better diagnostic performances than -GT and *β*2M in terms of sensitivity (sensitivity 68.57% vs 45.71% vs 0%) and specificity (specificity 80% vs 82.85% vs 97,14%) in detection of renal tubular damage in untreated RA.

AAP, γ-GT and β2M and DAS 28 INDEX of disease intensity

Among 35 patients with RA, DAS 28>3.2 was present in 28 patients (80%). In 17 RF positive patients the presence of DAS 28>3.2 was in 15

	RA NOT TREATED GROUP N^{O} 35 VALUE (M ± SD)	RA $^{sero-}$ N^{O} 18 VALUE (M ± SD)	RA $^{sero+}$ N^{O} 17 VALUE (M ± SD)	CONTROL HEALTHY GROUP N^{O} 35 VALUE (M ± SD)
	Positive / Negative	Positive / Negative	Positive / Negative	Positive / Negative
AAP + > 0,75 (U/mmol/creatinin)	24/11 1,06 (± 0,54) (0,35-2,46)	14/4 1,14 (± 0,48) (0,45-2,46)	10/7 0,98 (± 0,59) (0,35-2,30)	7/28 0,74 (± 0,43) (0,02-1,75)
γ-GT + >1,80 (U/mmol/creatinin)	16/19 1,80 (± 0,97) (0,45-4,40)	10/8 1,81 (± 0,80) (0,70-3,50)	6/11 1,79 (± 1,15) (0,45-4,40)	6/29 1,51 (± 0,70) (0,35-2,84)
β2 MICROGLOBULIN +> 0,19 (mg/L)	0/35 0,05 (± 0,03) (0,01-0,15)	0/18 0,06 (± 0,04) (0,01-0,15)	0/17 0,04 (± 0,03) (0,01-0,13)	1/34 0,08 (± 0,06) (0,02-0,25)
CREATININE SERUM ≤ 49-109 ≥ μmol/L	3/32 67,55 (± 14,76) (41-108)	1/17 68,24 (± 14,16) (44-108)	2/15 66,82 (± 15,77) (41-99)	2/33 74,95 (± 19,72) (44-135)
CREATININE URINA ≤ 7-17≥ mol/dU	9/26 10,41 (± 4,71) (3,1-25,4)	6/12 9,26 (± 4,54) (3,1-18)	3/14 11,62 (± 4,72) (5,8-25,4)	5/30 9,15 (± 4,22) (1,8-20,4)
UREA SERUM + ≥ 7,8 mmol/L	4/31 5,66 (± 1,46) (3,00-8,60)	0/18 5,52 (± 1,33) (3,00-7,5)	4/13 5,82 (± 1,62) (3,80-8,6)	1/34 4,94 (± 1,28) (2,50-7,2)
GFR + >90 ml/min	14/21 99,19 (± 24,46) (56,08-157,30)	7/11 99,19 (± 24,46) (64,67-142,59)	7/10 99,19 (± 25,22) (56,08-157,30)	4/31 113,80 (± 30,86) (69,98-177,74)
DAS 28 + ≥ 3,2	28/7 4,79 (± 1,56) (1,85-7,03)	13/5 4,56 (± 1,76) (1,85-7,03)	15/2 5,04 (± 1,33) (2,47-6,83)	0/35 0,00 (± 0,00) (0,00-0,00)
MORNING STIFFNESS + > 0 min	26/9 43,20 (± 65,13) (0-300)	14/4 57,50 (± 81,40) (0-300)	12/5 28,05 (± 38,72) (0-120)	0/35 0,00 (± 0,00) (0,00-0,00)
RF +30 ≥ IU/ml	17/18 346,15 (± 625,22) (0,00-1920)	0/18 0,00 (± 0,00) (0,00-0,00)	17/0 712,67 (± 743,72) (30-1920)	2/33 13,71 (± 38,73) (0,00-120)
CRP +12 ≥ mg/L	14/21 46,86 (± 79,19) (0,00-384)	3/15 8,66 (± 24,62) (0,00-96)	13/4 87,31 (± 96,44) (0,00-384)	4/31 5,48 (± 12,80) (0,00-48)
ESR +≥ 16	27/8 48,62 (± 39,81) (2,0-120)	13/5 43,94 (± 39,82) (2,0-120)	14/3 53,58 (± 40,39) (5,0-120)	4/31 9,42 (± 8,21) (2,0-44)
ACPA ≥ 1,26	23/12 1,71 (± 0,69) (0,92-3,0)	11/7 1,56 (± 0,59) (0,93-2,6)	12/5 1,87 (± 0,77) (0,92-3,0)	1/34 0,95 (± 0,10) (0,90-1,38)

Table 1: AAP,γ-GT, β2 M and other laboratory variables in RA and control healthy group.

	AAP RA No35	AAP RA No18	AAP RA^{+}No17	γ GT RA No35	γ GT RA^{-} No18	γ GT RA^{+} No 17	β2 Microglobulin RA No 35	β2 Microglobulin RA^{-} No 18	β2 Microglobulin RA^{+} No 17
SENSITIVITY %	68,57	77,77	58,82	45,71	55,55	35,29	0	0	0
SPECIFICITY %	80	80	80	82,85	82,85	82,85	97,14	97,14	97,14
PREDICTIVE VALUES FOR THE POSITIVE TEST %	77,41	66,66	58,82	72,72	62,50	50	0	0	0
PREDICTIVE VALUES FOR THE NEGATIVE TEST %	28,20	2,5	0	39,58	21,62	27,5	89,74	34,61	33,33
PRECISION %	74,28	79,24	73,07	64,28	73,58	67,30	48,57	64,15	65,38

	KREATININE SERUM RA No 35	KREATININE SERUM RA^{-} No 18	KREATININE SERUM RA^{+} No 17	KREATININ URINA RA No 35	KREATININ URINA RA^{-} No 18	KREATININ URINA RA^{+} No 17	**UREA SERUM RA No 35**	UREA SERUM RA^{-} No 18	UREA SERUM RA^{+} No17
SENSITIVITY %	8,57	5,55	11,76	25,71	33,33	17,64	11,42	0	23,52
SPECIFICITY %	94,28	94,28	94,28	85,71	85,71	85,71	97,14	97,14	97,14
PREDICTIVE VALUES FOR THE POSITIVE TEST %	60	33,33	50	64,28	54,54	37,5	80	0	80
PREDICTIVE VALUES FOR THE NEGATIVE TEST %	49,23	34	31,25	46,42	28,57	31,88	47,69	34,61	27,65
PRECISION %	51,42	64,15	67,30	55,71	67,92	63,46	54,28	64,15	73,07

	GFR RA No 35	GFR RA^- No 18	GFR RA^+ No 17	RF RA No 35	RF RA^- No 18	RF RA^+ No 17	CRP RA No35	CRP RA^- No 18	CRP RA^+ No 17
SENSITIVITY %	40	38,88	41,17	48,57	0	100	66,66	16,66	76,47
SPECIFICITY %	88,57	88,57	88,57	94,28	4,28	94,28	88,57	88,57	88,57
PREDICTIVE VALUES FOR THE POSITIVE TEST %	77,77	63,63	63,63	89,47	0	89,47	77,77	42,85	76,47
PREDICTIVE VALUES FOR THE NEGATIVE TEST %	40,38	26,19	24,39	35,29	35,29	0	40,38	36,60	11,42
PRECISION %	64,28	71,69	73,03	71,42	62,26	96,15	64,28	64,15	84,61

	SER RA No35	SER RA^- No 18	SER RA^+ No 17	MORNING RIGID RA No 35	MORNING RIGID RA^- No 18	MORNING RIGID RA^+ No 17	DAS 28 RA No 35	DAS 28 RA^- No18	DAS 28 RA^+ No 17
SENSITIVITY %	77,14	72,22	82,35	74,28	77,77	70,58	80	72,22	88,23
SPECIFICITY %	88,57	88,57	8,57	100	100	100	100	100	100
PREDICTIVE VALUES FOR THE POSITIVE TEST %	87,09	76,47	77,77	100	100	100	100	100	100
PREDICTIVE VALUES FOR THE NEGATIVE TEST %	20,51	13,88	8,82	20,45	10,25	12,5	16,16	12,5	5,40
PRECISION %	82,85	83,01	86,53	87,14	92,48	90,38	90	90,56	96,15

	ACPA RA No 35	ACPA RA^- No 18	ACPA RA^+ No 17
SENSITIVITY %	65,71	61,11	70,58
SPECIFICITY %	97,14	97,14	97,14
PREDICTIVE VALUES FOR THE POSITIVE TEST %	95,83	91,66	92,30
PREDICTIVE VALUES FOR THE NEGATIVE TEST %	26,08	17,03	12,82
PRECISION %	81,42	84,90	88,46

Table 2: Diagnostic performance of AAP, γGT, β2 M and other laboratory variables in RA.

patients (88,23%). Of these 15 patients (DAS 28>3.2), AAP was positive in 8 patients (53.33%) and its M ± SD (1.20 ± 0.49) in extent (0.80-2.30), γ-GT was positive in 4 patients (26,66%) and its M ± SD (2.61 ± 1.07) extent (1.90-4.20). β 2M was not present in these patients. Of 18 RF negative patients, DAS 28>3.2 was present in 13 patients (72,22%). Among these patients DAS 28>3.2 AAP were positive in 11(84,61%) and their M ± SD (1.25 ± 0.43) extent (0.85-2.46), γ-GT was positive in 9 (69,23%) and their M ± SD (2.55 ± 0.4) range (0.95-3.45), while β2M was not present among them.

1. RF negative patients had higher value of AAP than RF positive patients (Table 1).

(1,14 ± 0,48) (0,45-2,46) vs 0,98 (± 0,59) (0,35-2,30), but lower DAS 28 index (4.56 ± 1.76 (1.85-7.03) vs (5.04 ± 1.33) (2.47-6.83). Between these two groups of AAP, there was not statistical correlation (p=0,185017). However, RF negative patients with DAS 28>3.2 have much higher AAP than positive RF patients with DAS 28>3.2 (1,25 ± 0.43) (0.85-2.46) vs (1.20 ± 0.49) (0.80-2.30). Between these two group of AAP there was not statistical correlation (p=0,16). (Figure 1)

2. RF negative patients did not have higher values of γ-GT than RF positive (Table 1). (1,81 ± 0,80) (0,70-3,50) vs 1,79 (± 1,15) (0,45-4,40). Between these two groups in regard of γ-GT there was not statistical correlation (p=0,67). However, RF positive patients with DAS 28>3.2 had much higher γ-GT than RF negative patients with DAS 28>3.2 (2.61 ± 1.07) (1.90-4.20) vs (2.55 ± 0.46) (0.95-3.45). Between these two groups in regard of γ-GT there was not statistical correlation (p=0,72) (Figure 2).

3. RF negative patients had a little higher value of β2M than RF positive patients. (Table 1) (0.06 (± 0,04) (0,01-0,15) vs 0,04 (± 0,03) (0,01-0,13). Between these two groups in regard of β2M there was not statistical correlation (p=0,22). However, 13 RF negative patients with DAS 28>.2 had much higher β2M than 15 RF positive patients with DAS 28>3.2 (0.07 ± 0.04) (0.01-0.15) vs (0.047 ± 0.039) (0.01-0.13). Between these two groups in terms of β2M there was not statistical correlation (p=0,22) (Figure 3). In RF positive and negative patients there was not statistical correlation between DAS 28 index (p=0,379).

4. There was statistical correlation between AAP in RA patients and control healthy group using Wilcoxon-matched test (p= 0.026113). In RA patients there was statistical correlation between AAP and γ-GT (p= 0.00); AAP and β2M (p= 0.00); γ-GT and β2M (p= 0.00).

5. There was not statistical correlation between γ-GT in RA patients and control healthy group (p= 0.308160), β2M in RA and healthy control group (p= 0.05).

6. There was not statistical correlation using Wilcoxon-matched test between AAP, age, duration of disease in months, DAS 28 index, RF, CRP, ESR, anti CCP 2, morning stiffness, creatinine in serum and urine and serum urea in the same group: AAP vs age (p=0.00); AAP vs duration of disease in months (p=0.000000); AAP vs DAS 28 p=(0,00); AAP vs RF (p=0,01); AAP vs CRP (p=0,04); AAP vs ESR (p=0,00); AAP vs anti CCP 2 (p=0,00); AAP vs morning stiffness (p=0,00); AAP vs serum creatinine (p=0,00); AAP vs urine creatinine (p=0,00); AAP vs serum urea (p=0,00).

7. There was not statistical correlation using Wilcoxon-matched test between γ-GT, age, duration of disease in months, DAS 28 index, RF, CRP, ESR, morning stiffness, creatinine in serum and urine and serum urea; γ-GT vs age (p=0.00); γ-GT vs RF (p=0,019); γ-GT vs duration of disease in months (p=0.00); γ-GT vs DAS 28 (p=0,00); γ-GT vs CRP (p=0,04); γ-GT vs ESR (p=0,00); γ-GT vs morning stiffness (p=0,00);

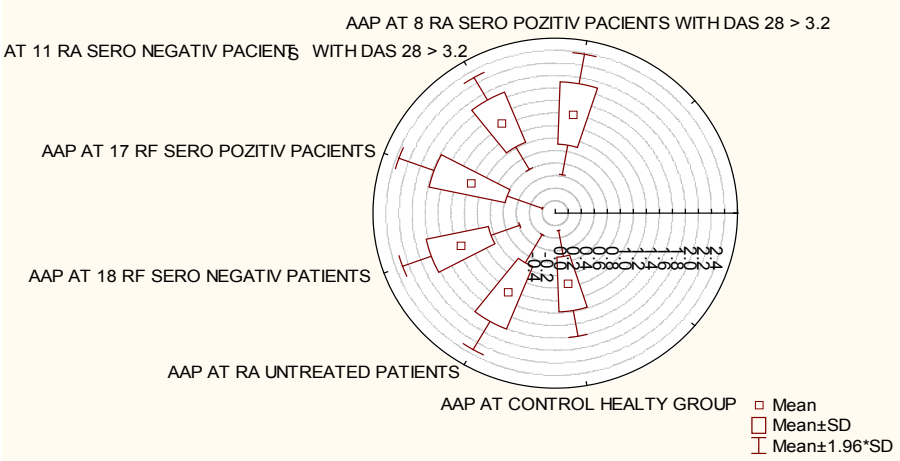

Figure 1: Distribution of alanine amino peptidase (AAP) (U/mmol creatinine) in all groups.

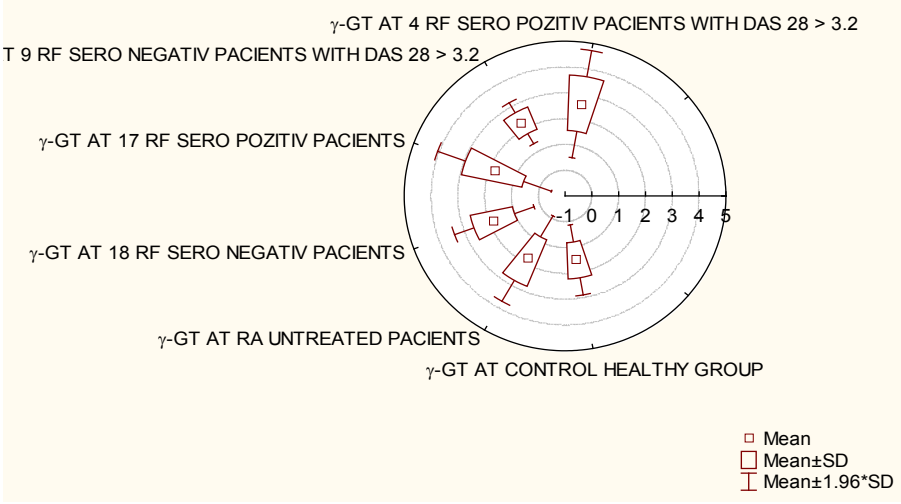

Figure 2: Distribution of -glutamyl transferase (γ-GT) (U/mmol creatinine) in all groups.

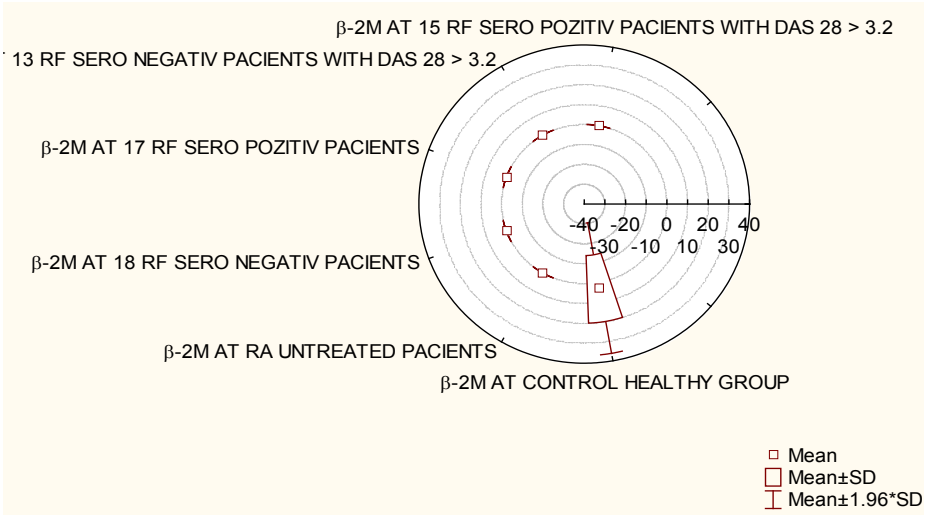

Figure 3: Distribution of β2 microglobuline (β2M) in urine(mg/L) in all groups.

γ-GT vs serum creatinine (p=0,00); γ-GT vs urine creatinine (p=0,00); γ-GT vs serum urea (p=0,00).

8. There was not statistical correlation using Wilcoxon-matched test between β2M, age, duration of disease in month, DAS 28 index, RF, CRP, ESR, anti CCP 2, morning stiffness, creatinine in serum and urine and urea serum in the same group: *β* 2M vs age (p=0.00); *β*2M vs duration of disease in months (p=0.00); *β*2M vs DAS 28 (p=0,00); *β*2M vs RF (p=0,018345); *β*2M vs CRP (p=0,04); *β*2M vs ESR (p=0,00); *β*2M vs anti CCP 2 (p=0,00); *β*2M vs morning stiffness (p=0,00); 2M vs serum creatinine (p=0,00); *β*2M vs urine creatinine (p=0,00); *β*2M vs serum urea (p=0,00).

9. There was not statistical correlation using Wilcoxon-matched test between γ-GT and anti CCP 2 in the same group (γ-GT vs anti CCP 2 (p=0,49) .

Discussion

Elevation in urine enzymes' activity could indicate primary renal tubular damage, because of their location in brush border area, such as microsomal AAP (E.C 3.4.11.2) and tubular lysozyme (NAG E.C.3.2.1.30). They could be used in early diagnosis of acute renal failure caused by immunosuppressive drugs, contrast materials, antibiotics and cadmium exposition [46-52]. Urine enzymatic activity normally is low and is increased in case of the renal tubular damage [53]. Urine enzymes, especially NAG, AAP, AP are very sensitive indicators of renal parenchymal damage in comparison with functional measurements such as glomerular filtration rate (GFR), creatinine and inulin clearance. Relatively low sensitivity of GFR can be explained with large functional kidney and its great compensatory ability [53]. Sensitivity of AAP is higher compared with γ-GT and *β* 2M (68,57% vs 45,71% vs 0%) with approximately equal specificity (80% vs 82,85% vs 97,14%). The other standard routine analyses used for evaluation of renal function showed low sensitivity (creatinine in serum and urine, serum urea (8.57% vs 25.71% vs 11.42%). RF negativity had influence on the presence of AAP enzymuria. It was presented in RF negative patients with DAS 28>3.2 with much higher β2M than in RF negative patients with DAS 28>3.2 (0.07 ± 0.04) (0.01-0.15) vs (0.047 ± 0.039) (0.01-0.13), but that was not the case with the intensity of γ-GT, where RF positivity had different meaning. The duration of the disease in months and AAP, γ-GT and *β*2M enzymuria (p=0.00) showed that untreated RA had an influence on renal tissue as one of visceral manifestation of the disease. Enzymes originating from primary damaged proximal tubular brush border area in untreated RA had higher sensitivity.

Conclusion

AAP has higher sensitivity then γ-GT and *β*2M in detection of asymptomatic renal damage in untreated RA. AAP and γ-GT can be used in everyday clinical practice in diagnose of early asymptomatic renal damage.

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