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PROXIMAL RENAL TUBULES ENZYMURIA AS A SCREENING TEST IN PATIENTS WITH SERONEGATIVE SPONDYLOARTHROPATIES

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Abstract

The aims of this study are: to compare diagnostic values and laboratory variables of alanine aminopeptidase (microsomal AAP), γ-glutamyl transferase (γ-GT), β2-microglobulin (β2-M), C-Reactive Protein (CRP), and the index for disease activity (PASI) in the early diagnosis of previously untreated psoriatic arthritis (Psa) and to see how untreated psoriatic arthritis affected tubular function and the sensitivity of the brush border region, together with the diagnostic value of enzymes originating from the proximal renal tubules.

From the standard methods of the International Federation for Clinical Chemistry (IFCC), we used the kinetic method for the determination of alanine aminopeptidase (microsomal AAP), γ-glutamyl transferase ((γ-G) and MEIA (Microparticles Enzyme Immunoassay (Abbott $A_x$sym system)) for determination of β2microglobulin in the urine. We examined samples (serum and urine) from 70 participants (35 Psa untreated, 35 healthy control group). RF and CRP were determined with the latex agglutination test in the same participants.

Of the 35 examined patients with Psa, 12 (34.28%) showed presence of APP enzymuria, 8 patients (22.85%) showed presence of γ-GT, and a low percentage (0% showed presence of β2-microglobulin in urine.

AAP has a higher sensitivity thanγ-GT and β2-M in asymptomatic renal lesions in untreated Psa.

***Keywords**:* alanine aminopeptidase (AAP),γ-GT (γ-glutamyl transferase),β2-microglobulin (β2-M), spondyloarthropathies.

Introduction

Brush epithelial cells can be found in two places in the human body. The initial location is in the intestine, where absorption occurs. The brush epithelium in the intestinal cover layer is the place of terminal carbohydrate digestion. The brush epithelium's microvilli contain enzymes for this final stage of digestion, which are identified as integral membrane proteins in the apical plasmatic membrane. These enzymes are located near the transporters, which allow the digested food to be absorbed [1].

Moreover, the brush epithelium in the kidneys can be used to differentiate proximal tubules (which have brush epithelium) from distal tubules (that do not). The brush border morphology of the brush epithelium increases cell surface, which is particularly beneficial for absorption. To be effective, cells that absorb substances require frequent contact with substance-containing surfaces. Under a light microscope, the luminal surface of the epithelial cells from this segment of the nephron is covered with densely packed microvilli that form a border. The microvilli significantly increase the luminal surface of the cells, supporting resorptive function [1].

Also, multiple classes of measurable proteins in urine are used for the evaluation of renal dysfunction: enzymes with a high molecular weight, which usually are not filtered in the glomerulus and originate mainly from the proximal tubules (microsomalalanine aminopeptidase, N-Acetyl-/β-glucosaminidase-NAG), intermediate proteins that normally are filtered in the glomerulus in small quantities and are reabsorbed in the tubules in great part (albumin, transferrin), and proteins with a low molecular weight that are filtered in the glomerulus and then reabsorbed in the tubules (β2-microglobulin) [2].

The aims of this study are:

- to compare diagnostic values and laboratory variables of alanine aminopeptidase (microsomal AAP), γ-glutamyl transferase (γ-GT), β2-microglobulin (β2-M), C-Reactive Protein (CRP), and the index for disease activity (PASI) in early diagnosis of previously untreated psoriatic arthritis (Psa).
- to see how untreated psoriatic arthritis affected tubular function and the sensitivity of the brush border region, together with the diagnostic value of enzymes originating from the proximal renal tubules.

Materials and methods

Sample description

As a patient sample group, this study included 35 patients (27 males and 8 females) suffering from Psa. The patients met the revised diagnostic criteria for psoriatic arthritis proposed by the American Association for Rheumatic Arthritis (ARA) in 2005 [2-3]. The healthy group sample included 35 people (13 males and 23 females). The two samples were collected over a two-year period.

Inclusion criteria

- Patients with psoriatic arthritis
- Age range: 18 to 65 years
- Newly diagnosed patients
- Previously untreated patients

Exclusion criteria

- A history of autoimmune diseases, spleen, thyroid, liver, kidney, hematological, cardiovascular, neurological, lung conditions, human immunodeficiency virus (HIV), diabetes mellitus, malignant disease, febrile conditions, arterial hypertension
- Under 18 years old and older than 65 years old
- The existence of diseases like vasculitis, mixed connective tissue disease, systemic lupus erythematosus (SLE), uric arthritis, and urine infections
- A history of blood transfusions and obesity
- Identification of baseline hyperglycemia or elevated degradation products such as serum and urine creatinine, serum urea, and arterial hypertension
- Patients who were treated with antibiotics and salicylates within six months of the study's start
- Patients who take drugs from the basic line
- Patients treated with antihypertensive, anti-diabetic, and cardiac therapies
- Patients who are hypersensitive to some drugs or their components
- Patients with acute or chronic renal failure

Ethical considerations

All participants voluntarily participated in the study; hence, the ethical criteria for conducting this study were fulfilled.

Clinical evaluation of disease activity

Clinical evaluation for disease activity and disease diagnosis was based upon the diagnostic criteria of Moll-Wright for the classification of psoriatic arthritis. Patients were dermatologically

tested, including examination of the psoriatic changes of nails, psoriatic areas, and disease activity index (PASI), as well as evaluation of the peripheral and axial joints. Oligoarthritis was taken into consideration when 5 joints were involved, and polyarthritis when5 joints were involved. Symmetric arthritis was considered when bilateral joints were involved at > 50%.None of the patients included in the study had a previous or current history of renal disease. None of them had previously used non-steroidal anti-inflammatory drugs(NSAIDs). The patients negated the use of other drugs before entering the study, especially drugs from the baseline therapy such as methotrexate, antibiotics, or diuretics [1, 4].

Laboratory assessment

Every patient was evaluatedfor complete blood analysis (CBC with differential), reactants of the acute phase such as C-Reactive Protein (CRP), rheumatoid factor (RF), erythroid sedimentation rate (ESR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine kinase (CK), lactate dehydrogenase (LDH), serum urea and serum creatinine. The urine samples were taken not only for routine analyses but also to determine the levels of AAP, γ-GT, and β2-M. Due to the instability of β2-M<6 pH in urine, it was recommended that the urine be alkalized before testing [1].

Determination of the activity of alanine aminopeptidase (AAP): kinetic method

Alanine aminopeptidase (aryl amide amino acid, aminopeptidase, a-aminoacyl peptide hydrolase (microsomal), ANA, ES 3.4.11.2, former 3.4.1.2.) is hydrolized by peptides, amids, and p-nitroanilide. During the process of hydrolization of peptides, the N-terminal amino acid is separated (firstly by anilide). The activity of AAP is determined by methods similar to those for determining the activity of leucine aminopeptidase. This method uses L-alanine-4-nitroanilide as a substrate. The catalytic concentration of AAP is directly proportional to the absorption of p-nitroanilide measured at 405 nm. Reference values in the urine of AAP are between 0.25 and 0.75 U/mmol creatinine [1].

Determination of the activity of γ-glutamyltransferase (γ-GT): IFCC method

γ-glutamyltransferase (γ-glutamyl) – peptide amino acid γ-glutamyltransferase ES 2.3.2.2. (γ-GT) catalyzes transfer of γ-glutamyl groups with peptides (such as glutathione) to other peptides or aminoacids. γ-GT influences the release of glutamyl rest as glutamyn acid. With transpeptidation, glytamyl residues could be transferred again on a substrate (for example, γ-glutamyl-naphtylamide results in γ-glutamyl- γ-glutamyl-α-naphtylamide) or other suitable acceptor (amino acid, di-, or tri-peptide). The most suitable acceptor is glycylglycine.

Methods for measurements of the activity of this enzyme in serum use aromatic amids as substrates (γ-glutamyl-anilide and γ-glutamyl-naphtylamide). The superficial substrate peptide analogue, γ-glutamyl-p-nitroanilide is the most frequently used. It is suitable for determination of enzyme activity kinetically andcolorimetrically. γ-glutamyl--p-nitroanilide later substituted by L-γ-glytamyl-3-carboxy-4-nitroanilide (glucan) due to its high solubility. Glycylglycine is used as a substrate acceptor and buffer due to its high catalytic activity. This method is standardized by the International Federation of Clinical Chemistry (IFCC) and is considered a reference one [1, 4].

Determination of the concentration ofβ2-microglobulin (β2-M) in urine according to the MEIA (Microparticles Enzyme Immunoassay) method (Abbott $a_x$sym System)

Principles

The determination of $a_x$sym b2-microglobulin is based on MEIA technology (Microparticles Enzyme Immunoassay) and enables quantitative determination of β2-microglobulin in serum, plasma, and urine in patients with rheumatoid arthritis and renal impairment [1].

The reaction is based on the interaction of β2-M with anti- β2-M antibodies, forming a mutual complex. This complex reacts with the matrix cells and is bound to them. A conjugate of alkaline phosphatase is added; it binds to the complex, forming a sandwich complex. 4-methylumbelliferyl phosphate (4-MUP) is added to this complex, reacting with alkaline phosphatase from the complex, and a fluorescent product, methylumbelliferone, with a light blue colour is made. The concentration of β2-M depends proportionally on the degree of the optic fluorescence. It is determined automatically (the Abbott $A_x$sym system).

β2-M is very sensitive to changes in urine pH, i.e., it is degraded very quickly into low pH levels. If pH is below 6.0, it is monitored, and if it is acidic, it should be alkalized. The reference values of the b2-microglobulin in the urine are from 0.02 to 0.19 mg/L [1, 4].

Statistical analysis

Data analysis was done with the statistical package Statistica7.0. For testing the significance of differences between two arithmetical means, i.e., proportions, the Student's t-test was used to compare the mean parameters of certain numerical parameters between groups, as well as the Willcoxon-matched test for independent samples. The sensitivity and predictivity for positive and negative tests of the examined markers were determined with the test for sensitivity and specificity. A P-value between 0.05 and 0.1 was considered statistically significant.

Results

Socio-demographic indicators of the study

The median age was 50.18 years (SD±8.09) (35-65 years) in Psa (patient) group, while it was 48.2 years (SD±10.19) (29-65 years) in the healthy control group. The median disease duration was 30.17 months (SD±40.13) in the interval of 1-60 months.

Diagnostic value of alanine aminopeptidase (microsomal AAP), γ-glutamyl transferase (γ-GT), β2 microglobulin (β2M) in Psa patients

Of the 35 examined patients with Psa, 12 (34.28%) showed presence of APP enzymuria, 8 patients (22.85%) showed presence of γ-GT, and a low percentage (0% showed presence of β2-microglobulin in urine. No presence of RF was observed.APP sensitivity was 34.28%, γ-GT sensitivity was 42.85%, β2-microglobulin sensitivity was 0%, and RF sensitivity was 0% in the 35 patients with Psa (*Table 1 and Figure 1*).

Table 1.AAP,□□-GT□□□2-microglobulin and other laboratory variables in Psa and heathy control group

	Psa untreated group (N=35) Value (M ± SD)	Heathy control group (N=35) Value (M ± SD)
	Positive/negative	Positive/negative
AAP + > 0,75 (U/mmol/creatinine)	12/23	1/34
□-GT + >1,80 (U/mmol/creatinine)	8/27	0/35
□□2 M +> 0.19 (mg/)	0/35	0/35
RF +30 ≥ IU/ml	0/35	0/35
CRP +12 ≥ mg/L	13/22	1/34

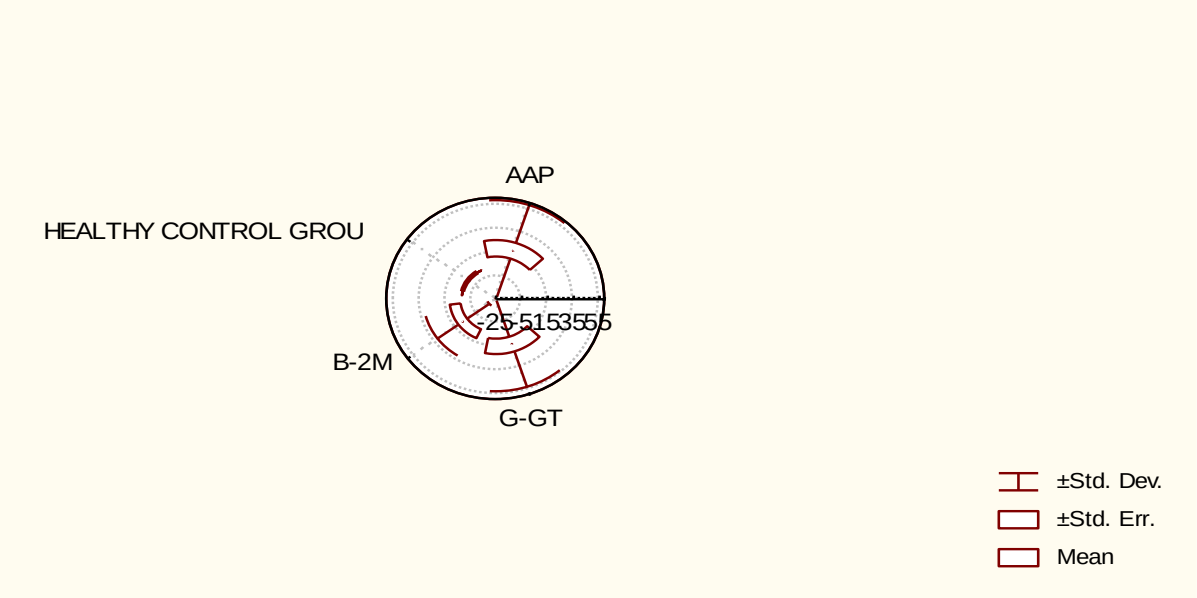

Figure 1. Distribution of alanine aminopeptidase (AAP), γ-glutamyl transferase (γ-GT), □2-microglobulin(□2M) (urine)

AAP had better diagnostic performances than γ-GT and β2M taking into consideration sensitivity and specificity (sensitivity 34.28% vs. 22.86% vs. 0%) and almost equal specificity (specificity 75.6% vs. 100% vs. 100%) in the detection of renal impairment in untreated Psa.

Using the Wilcoxon-matched test, it was observed a statistical relation between AAP in Psa and the healthy control group for $p<0.05$ ($p=0.01$). In the Psa group, there was a statistical relation between AAP and γ-GT for $p< 0.05$ ($p=0.00$); AAP and β2M ($p=0.00$).

Also, there was a statistical relation using the Wilcoxon-matched test between γ-GT in the Psa and the healthy control group for $p<0.05$ ($p=0.40$); β2Min in the Psa and the healthy control group for $p<0.05$ ($p=0.06$). Furthermore, there was a statistical relation using Wilcoxon-matched test between AAP in Psa and age, disease duration (in months); PASI index, RF and CRP, serum creatinine, serum urea in the same group for $p< 0.05$: AAP vs. age for $p=0.00$; AAP vs. disease duration (in months) for $p=0.00$; AAP vs. PASI for $p=0.00$; AAP vs. RF for $p=0.02$; AAP vs. CRP for $p=0.041$; AAP vs. ESR for $p=0.00$; AAP vs. serum creatinine for $p=0.00$; AAP vs. serum urea ($p=0.00$).

Furthermore, there was a statistical relation using Wilcoxon-matched test between γ-GT in Psa and age, disease duration (in months), PASI index, RF, CRP, ESR, serum creatinine and serum urea in the same group for $p< 0.05$: (γ-GT vs. age $p=0.00$; γ-GT vs. disease duration (in months) for $p=0.00$; γ-GT vs. PASI index for $p=0.00$; γ-GT vs. RF for $p=0.02$; γ-GT vs. CRP for $p=0.042$; γ-GT vs. ESR for $p=0.00$; γ-GT vs. serum creatinine for $p=0.00$; γ-GT serum urea for $p=0.00$. As well, there was a statistical relationbetween β2M in Psa and age, disease duration (in months); PASI index, RF and CRP, ESR, serum creatinine and serum urea in the same group for $p<0.05$: β2M vs. age for $p=0.00$; β2M vs. disease duration (in months) for $p=0.00$; β2M vs. PASI index for $p=0.00$; β2M vs. RF for $p=0.02$; β2M vs. CRP $p=0.044$; β2M vs. ESR for $p=0.00$; β2M vs. serum creatinine for $p=0.00$; β2M vs. serum urea for $p=0.00$.

Discussion

In the field of standard medical rheumatology, the most emphasis is placed on rheumatoid arthritis as the most exposed disease, neglecting other diseases, particularly seronegative arthropathies, owing to their lesser prevalence. The renal tubular enzymes are explained by increased exfoliative turnover of the epithelial cells in Psa, which is also present in proximal tubular epithelial cells [5].

Of all enzymes, the greatest emphasis is put on NAG as a dominant lysosomal tubular enzyme. Traditional treatment of Psa and RA includes non-steroid anti-inflammatory drugs (NSAIDs), disease-modifying drugs (DMARDs), steroids, and immunosuppressive cytotoxic drugs.

Methotrexate in a low-dose regime is the most frequently prescribed drug from DMARDs, while Ketoprofenand Paracetamol are from NSAIDs.

Enzymes in urine could originate from plasma, glands from the urogenital tract, epithelial cells of the urinary tract, white blood cells, erythrocytes, and the kidneys. There are 40 different enzymes in the urine belonging to different groups:oxydo-reductases, transferases, hydrolases, and lyases, while isomerases and ligases are not found in urine. The presence of so many enzymes in urine indicates the dominant role of the kidneys in their excretion [6].

The enzyme activity in urine is normally low and increases if there is renal tubular cell damage. Urinary enzymes, especially NAG, AAP, and AF, are very sensitive indicators of renal parenchymal damage in comparison to functional measurements such as glomerular filtration rate and creatinine clearance. The relatively low sensitivity of glomerular filtration rate (GFR) could be explained by the great functional reserves of the kidneys and their great compensatory ability [7–11].

AAP sensitivity is greater in comparison toγ-GT and β2M (34.28% vs. 22.85% vs. 0%), with approximately equal specificity (75.6% vs. 100 % vs. 100%).The statistical relation between disease duration (in months) and AAP, γ-GT and β2M enzymuria (p=0.00) pointed out that untreated Psa damaged the renal tissue as one of the visceral manifestations of the disease.Untreated Psa primarily damages the tubular-brush border region, and enzymes originating from it have greater sensitivity.

Conclusions

AAP has a higher sensitivity thanγ -GT and β2M in asymptomatic renal lesions in untreated Psa. AAP and γ-GT could be used in everyday clinical practice in the diagnosis of early, asymptomatic renal lesions.

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