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BIOMARKERS OF GLOMERULAR AND TUBULAR DAMAGE IN NON DIABETIC AND NON HYPERTENSIVE RHEUMATOID ARTHRITIS PATIENTS; A RETROSPECTIVE OBSERVATIONAL STUDY

Biochemistry

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ABSTRACT

Introduction: Etiology of Rheumatoid arthritis [RA] is place and before management. 'unknown, there is no cure, and management' is achieved by supportive measures. Renal disorders are a frequent cause of death in patients with Rheumatoid Arthritis. The present study is to identify the biomarkers that suggest renal pathology in non-diabetic and non-hypertensive individuals.

Material and Methods: The study was conducted in Osmania Medical College, Hyderabad. 17 RA patients' samples were collected from Dec 2000 – Feb 2001. 2hr timed urine [Ur] samples and blood was drawn simultaneously, the height and weights were measured for calculation of creatinine clearance [CrCl], which is an estimate of glomerular function. Aliquots of urine were used to estimate the urinary N-Acetyl B Glucosaminidase [NAG], and gamma glutamic transaminase [GGT].

Results: Proximal convoluted cell lysis was observed as the duration of the disease exceeds 24 months and also in patients who were aged less than 5 decades.

KEYWORDS:

RA, Ur, CrCl, NAG, GGT, HTN UTI.

INTRODUCTION:

Rheumatoid arthritis (1)(RA) is a chronic inflammatory disease marked by a symmetric, peripheral polyarthritis. It is the most common form of chronic inflammatory arthritis and often results in joint damage and physical disability1. Because it is a systemic disease, RA may result in a variety of extra articular manifestations, including fatigue, subcutaneous nodules, lung involvement, pericarditis, peripheral neuropathy, vasculitis, and hematologic abnormalities. The etiology of Rheumatoid Arthritis is unknown (2) and the pathogenesis speculative, and there is no cure. Good management is achieved by judicious choice of drugs and supportive measures by a wise and caring Physician.

Insights gained by the wealth of basic and clinical research over the past two decades have revolutionized contemporary paradigms for the diagnosis and management of RA. The science of RA has taken a major leap forward with the identification of new disease-related genes and further deciphering of the molecular pathways of disease pathogenesis. Serum antibodies to cyclic citrullinated peptides (anti-CCPs) are now recognized to be valuable biomarkers of diagnostic and prognostic significance.

Renal disorders (3) are a frequent cause of death in patients with Rheumatoid Arthritis, but are less apparent in living Rheumatoid Arthritis patients. Our study of Rheumatoid Arthritis patients aims to prevent further renal insults leading to renal failure and death. With this in view, The Department of Bio Chemistry, Osmania Medical College, took up this area for the purpose of researching and studying the Urinary enzymes NAG, GGT (4) and Creatinine Clearance (5) so as to monitor consequences of disease on the kidney of RA patients; for better treatment schedules.

MATERIALS AND METHODS;

Selection of subjects was done in Rheumatology OP and in Medical Wards of Rheumatoid Arthritis patients between age group of 16 – 65 years, fulfilling the 1988 revised criteria for the classification of rheumatoid arthritis. Patients with known hypertension, diabetes, Urinary Tract Infection and Proteinuria were excluded.

The sample collection of all patients and controls, was done between 12 noon to 6 PM from December, 2000 to February, 2001. 2 hour timed urine sample was taken, for which patients were given non-aerated water of 20 ml/kg/body weight (5) and were advised to drink within ½ hour from the time of voiding. Time was noted at the beginning of water consumption and subjects were given a clean bottle to void urine after 1 hour, and at the end of 2 hours. A midpoint Blood sample was drawn and serum separated.

Urine was measured with a graduated measuring jar and aliquots of 20 ml samples were screw capped with 1 ml of Toluene as preservative and stored at – 20o C after labeling.

NAG(6) is separated from urine inhibitors by gel filtration on chromatographic columns of Sephadex G-25. The NAG containing eluate is added to an enzyme reaction mixture that consists of the substrate (p-nitro phenyl-N-acetyl-B-D glucosamine) dissolved in sodium citrate buffer pH 4.4. During incubation at 37o C for 15 min, enzyme hydrolysis of the substrate liberates p-nitrophenylate ion. The reaction is stopped by adding 2-amino-2-methyl-1-propanol (AMP) buffer (pH 10.25), and the reaction product is measured by spectrophotometry at 405 nm (7). Urine NAG activity is proportional to the absorbance of the liberated p-nitrophenylate ion, after correction for absorbance of a urine 'blank' sample(8). NAG activity of urine is expressed as U/g of creatinine (8, 9). Reference values: 3.2 ± 1.3 U/g Cr (8), and increased levels are mark of renal tubular damage(10).

For urinary GGT The 911 Hitachi Auto analyzer at Osmania General Hospital, Hyderabad was programmed for urinary GGT estimation with reagent volume of 0.3 ml and sample volume (urine) of 0.05 ml. The value was expressed as U/L. GGT activity in spot urine specimen was expressed in U/g Creatinine., If Urinary creatinine when measured in mg/dl GGT activity in U/gm Creatinine = GGT U/L X 100 / Urinary creatinine in mg/dl Ref Value: 25.75 - 2.92 U/g Cr (10).

Creatinine clearance (1) is based on the Jaff'e reaction first described in 1887 using the reaction of creatinine with alkaline picrate to form an orange red Janovsky complex. Ref ranges Males 85 - 125 ml/mint/ 1.73 m2 ; Females 75 – 115 ml/mint 1.73 m2.

The data was compiled in an excel sheet and analyzed.

RESULTS:

The youngest patient enrolled for the study was 16 years old and the oldest 65, the mean age being 42 years. Females were 59% [10/17] compared against males 41% [7/17]. There was a Male to Female preponderance of 41:35, when considering those whose age was below the 5th decade.

The creatinine clearance was least at 5ml/min and the maximum being 134ml/min with mean of 86 ml/min. 24% females and 11% males had decreased creatinine clearance ranging from 5ml/min to 73 ml/min in females and 17.8 to 52 ml/min in males.

The urinary NAG levels was between 0.3 to 86.95 U/g Cr Mean being 21.

NAG enzymuria was observed in 29% of females and 17.6 % Males , ranging from 4.97 to 86.95 U/gCr in females and 5.15 to 6.59 U/g Cr in males. Urinary GGT levels were between 7.9 to 94 U/g Cr with a mean of 36.Urinary GGT values were raised more in males compared to females at 30% males and 26% in females, The Ur GGT values ranged from 29.91 to 93.75 U/g Cr in males.35.3 to 62.8 U/g Cr in females.

41% of RA patients below the 5th decade had decreased CrCl ranging from 4.8 to 72.6 U/g Cr suggesting glomerular dysfunction of which , 71% of them were Rh sero positive.

46 % of Patients below the 5th decade had increased Ur NAG ranging from 4.9 to 86.9 U/g Cr with 38 % being sero positive suggesting renal proximal convoluted tubule cell lysis.

The Ur GGT ranged from 39.02 to 93.75 U/g Cr.was seen in 35 % total patient all of whom were below the 5th decade.

The duration of disease among the subjects was between 2 to 114 months, with mean of 37 months. 64% subjects had disease duration < 24 months, with 72 % of them being Rh sero positive. 46% had decreased Creatinine clearance 27%, had raised Ur NAG, 36% of those with disease duration < 24 months raised Ur GGT levels.

83 % who had disease duration > 24 months had raised Ur NAG., 82% of patients were Rh Sero positive.

Table 1. Clinico demographic data

Gender	Males 7/17[41 %]	Females 10/17 [59%]
Age	< 20 - 1/7 [14%] <21 to 30 - 1/7 [14 %] <31 to 40 - 3/7 [44 %] <41 to 50 - 1/7 [14%] <51to 60 -0/7[0%] < 61to 70 -1/7[14%]	< 20 -0/10 [0%] <21 to 30 - 3/10 [30%] <31 to 40 - 2/10 [20%] <41 to 50 - 2/10 [20%] <51 to 60 -2/10 [20%] <61 to 70 - 1/10 [10%]
Patients disease duration 6	< 12 months-3/7 < 24 months- 4/7	<12 months 1/10 <24 months 3/10 < 36/<48 months 0/10 <60 months [5 years] 2/10 <72 months [6 years] 2/10 <106 months [8.3 years] 1/10 <114 months [9.5 years] 1/10,
Sero Positive/ Negative RA patients	Sero Positive RA patients 10/17 (58.8%)	Sero negative RA patients 7/17 (41.17%)
Patients on Methotrexate [MTX] treatment	On treatment 8/17 (47.05%)	Not on treatment 9/17 (52.9%)
Patients on other supportive therapy	Patients on Cox-2 treatment 4/17 (23.5%)	Patients on other NSAID's 13/17 (76.4%)

RA Patients	Decreased Creatinine Clearance [Total 6/17 =35 %]	Increased Ur NAG [Total 8/17= 47%]	Increased Ur GGT Total 9/17= 53 %
Gender	Females 24%[4/17] Males 11% [2/17]	Females 29% [5/17] Males 17.6 % [3/17]	Males 30% [5/17] Females 26 % [4/17]
Age <5th Decade	35 % [6/17]	Total 35 % [6/17]	53 % [9/17]
Duration Of Disease <24 Months	24% [4/17]	17% [3/17]	35 % [6/17]
Duration Of Disease >24 Months	11% [2/17]	30 % [5/17]	17.6 % [3/17]
On Methotrexate	17.6 % [3/17]	11% [2/17]	17.6 % [3/17]

DISCUSSION.

Our study has shown that glomerular and renal proximal convoluted tubular cell(10) as well as brush border cell lysis [γ-glutamyl transferase, γ-GT(10)] is occurring in Rheumatoid arthritis patients suggesting renal papillary damage (10, 11, and 12). In the present study group, the test subjects were non Hypertensive and non-Diabetic but the kidney injury biomarkers were similar to Hypertensive and Diabetic nephropathy (4, 7).

The decrease in Cr Cl in patients less than 30 yrs. may be indicative of more severe glomerular damage in younger RA patients. In our study the Cr Cl was decreased markedly in two patients < 55ml/min/1.73A2 out of which one of them was a patient with Juvenile RA.

It is observed in this study that the NAG in urine was raised with decrease creatinine clearance in patients of rheumatoid arthritis similarly seen by Wiland, P.Wiela et al (13). Wiland and Wiela comment that the Tubular Enzymuria and Cr Cl reduction are reversible and concluded that there are no significant renal changes of patients on supportive pharmacotherapy.

Ur NAG excretion did reflect sex related differences which was unlike the findings of Dieter Maruhn and I.F et al, Horak et al (8, 9).

In our study 24% of females and 11% of males patients had decreased Cr Cl, 29.4% females and 17.6% males had raised Ur NAG, 23% Females and 30% males have raised Ur GGT.

Cr Cl was very significantly decreased in patients with disease duration less than 24 months maybe due to glomerular involvement in the initial phases of treatment with anti-rheumatic drugs as suggested by Burry et al(14).

In our study the patients on Methotrexate had no apparent proximal renal lesions, (15) though the present sample size is small to conclude the same. However, GGT was raised in 3 of 7 patients on methotrexate treatment suggesting brush border epithelial (10) cell lysis.

From this study we conclude that Ur NAG, Ur GGT and CrCl can be used as non-invasive screening tests for patients with RA so that it will help alleviate their suffering whilst being under treatment for their chronic ailment.

The results thus obtained provide safety in treatment of RA patients. Measures can be taken for prevention of nephrotoxicity such as follow up of the renal function by regular checkup of the enzyme activity in the urine, estimation of the effectiveness of the exfoliative turn over on tubular cells, avoidance of frequent use of drugs and individual adjustment of drug dosage. It is ideal to determine urine NAG /GGT together with CrCl to assess renal damage in patients with RA(16). These tests are a good alternative to antibodies against cyclic citrullinated peptides [ACCP] which are being used, but not easily available in small peripheral centers in countries like India, when assessing patients with rheumatoid arthritis and identifying their renal condition as the disease progresses.

In this study the patients who were Rh +ve had GGT enzymuria which was unlike Helin et al (17) who did not find any renal damage in Rh +ve factor RA patients. Hence this area requires more research to make patient care more affordable with simple markers for renal tubular cell lysis.

CONCLUSION

Enzymuria in RA patients who are non-diabetic and non-hypertensive, with sero positive Rh factor and age below 5th decade had bearing on the renal damage such as Glomerular and brush border cell lysis. Proximal convoluted cell lysis is observed as the duration of the disease exceeds 24 months. Our study makes an attempt to emphasize the need in screening RA patients with inexpensive Urinary NAG, GGT and Creatinine Clearance as early, non-invasive biomarkers of renal damage, to titrate the supportive pharmacotherapy.

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