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Original Research Paper

Urology

A New early diagnostic marker for Adenine Phosphoribosyl Transferase and Xanthine Oxidase deficiency cause uric acid stone formation

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ABSTRACT

Background:
The prevalence and incidence of urolithiasis is increasing worldwide. Adenine phosphoribosyl transferase and xanthine oxidase well known to be associated and increases the risk of urolithiasis by altering the composition of urine. The aim of the study was to assess the serum levels of enzymatic markers i.e. Adenine phosphoribosyl transferase and xanthine oxidase deficiency to cause specifically uric acid stone formation and also compared to normal subjects.

Materials and Methods:
The study involved two groups. Group A consisted of control (n=30) and Group B consisted of kidney stone patients (n=30). Blood samples were obtained and analysed for Urea, Uric acid, Total Protein, Adenine phosphoribosyl transferase and xanthine oxidase were measured.

Results:
We found a significant increase in Adenine phosphoribosyl transferase and xanthine oxidase enzyme activity as compared to normal control subjects.

Conclusion:
The data from the present study shows that Adenine phosphoribosyl transferase and xanthine oxidase is altered, and also significantly decreased in uric acid stone patients when compared to normal subjects. This evidence also proves both enzymes are diagnostic marker for uric acid stone patients.

KEYWORDS : Adenine phosphoribosyl transferase, xanthine oxidase and urolithiasis

Introduction:

A kidney stone is a small particles, generally made up of calcium crystals, that formed inside the kidney wherever urine collects. Uric acid nephrolithiasis can result from different etiologies [1, 2]. Patients can present with uric acid and/or calcium oxalate kidney stones [3]. Biochemically, patients with pure uric acid stones can have normal or high plasma uric acid levels with normal or high urinary uric acid [4]. Adenine phosphoribosyltransferase deficiency (APRTD) is a rare autosomal recessive metabolic disorder due to a mutation of the APRT gene [5]. APRT is a purinemetabolism enzyme that catalyzes the formation of 5 - adenosine monophosphate (5 - AMP) and pyrophosphate (PP) from adenine and 5-phosphoribosyl-1-pyrophosphate [6]. In patients with complete APRT deficiency, adenine is oxidized by xanthine oxidase (XO) to the highly insoluble and nephrotoxic derivative 2,8-dihydroxyadenine (2,8-DHA) [7], leading to urolithiasis and renal failure caused by intratubular crystalline precipitation [8].

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid, which performs as a secondary product in a superoxide molecule [9]. The metabolic reactions of xanthine oxidase have a broad effect cellular protection from toxic complexes and also general protection like innate immunity [10].

Uric acid is the finale product of purine metabolism in human. Hyperuricemia can be the result of increased uric acid production or reduced excretion. At all cause for reduction glomerular filtration, tubular excretion or increased reabsorption would result in an elevated serum uric acid

In the present study, we report the identification of deficiency of Adenine phosphoribosyl transferase and Xanthine oxidase in serum uric acid stone formation patients.

MATERIALS AND METHODS:

Experimental Design

Out of 100 Patients were divided in to two groups. Group I – Normal Control (50 Subjects) and Group II – Uric acid renal stone patients (50 Subjects). The study was conducted during the period of May 2016 to July 2017 in department of Urology, Meenakshi Medical College Hospital And Research Institute, Kanchipuram, and Tamil Nadu.

Exclusion criteria

Patients suffering from major infections like HIV, type 2 diabetes mellitus, diabetic nephropathy, heart disease, history of alcohol intake, taking potent antioxidant, and pregnant females were excluded from the current study.

Inclusion criteria

Patients of kidney stones only attending outpatient department and admitted in the ward of urology, Meenakshi Medical College and Research Institute, who agreed to participate in the study were included. Medical history, standard physical examination, and test of biochemical parameters (Urea, Total Protein and Uric acid) listed in Table 1. Determination of Adenine Phosphoribosyl Transferase (Table.2) and xanthine oxidase Table.3).

Collection of Blood Sample

The blood drawn was allowed to coagulate and the serum was separated by centrifuged and the samples were transported to the laboratory within 30minutes for biochemical analyses.

BIOCHEMICAL PARAMETERS:

Measurement of Xanthine Oxidase Activity

Xanthine oxidase (XO) activity was assayed as described by Prajda and Weber (1975) [11].. The reaction mixture containing 0.2mL sample was diluted to 1mL with phosphate buffer and incubated for 5min at 37˚C. The reaction was started by adding 0.1mL xanthine and kept at 37˚C for 20min.The reaction was terminated by the addition of 0.5mL ice-cold perchloric acid (10%). After 10min, 2.5mL distilled water was added to it and the mixture was centrifuged at 4000 rpm for 10 min. The absorbance of the urine samples was read at 290 nm. The activity of XO is expressed as U/L.

Measurement of Adenine Phosphoribosyl transferase activity

High performance liquid chromatography (HPLC) analysis was used to measure APRT enzymatic activity in serum (Di Pietro et al., 2007)

[12].All other biochemical parameters like Urea, Uric acid and Total Protein levels were estimated by using standard kit methods.

Statistical Analysis:
Statistical analysis was performed by SPSS, a commercially available statistics software package. Group comparisons (between control and patient groups) were performed using the -test. A < 0.05 was considered to be statistically significant.

Results:

Comparison of biochemical parameters in studied groups
Two groups were included in this study: Group 1 consisted of fifty men suffering from kidney stones (The mean age of the patients was 55.62 ± 5.19 years), and the second group was the control fifty men with mean age (49.27 ± 5.21) years. The serum urea level, protein, albumin, globulin and albumin/globulin ratio non-significantly changed (P > 0.05) in patients group, when they were compared with control group.

Table.1 Comparison of biochemical parameters in studied groups

Parameters	Control	Stone Patients
Serum Urea (mg/dL)	35.81±3.82	41.35±4.11
Serum Protein (g/dL)	7.84±0.81	7.61±0.75
Serum uric acid (mg/dL)	4.93.±0.33	6.92±0.94

Adenine Phosphoribosyl transferase activity :
Table.1 shows that the APRT activity in serum and urine sample. APRT activity was determined by calculating the Adenine monophosphate produced by hemolysates during the incubation with substrates over the basal hemolysate value, measured in a Phospho ribosyl pyrophosphate starved reaction.

The APRT activity in the patient was about 1%: residual APRT activity was 0.78 μmol/min/mg of hemoglobin while in control individuals the mean value was 34.3 ± 3.5 μmol/min/mg. The concentration of adenine in the patient`s urine was 12.42± 1.1 mmol per mol of creatinine while in the control range it is 0.18-0.20 mmol per mol of creatinine.

In the present study shows that the serum and urine Adenine phsosphoribosyl transferase activity were significantly (P <0.001) increased in Uric acid stone formation patients when compared with normal subjects.

Table.2. Adenine Phosphoribosyl transferase activity in serum and urine

Samples	APRT Enzyme activity
Hemolysate	Control - 0.78± 0.08 μmol/min/mg
	Patients - 34.3 ± 3.5 μmol/min/mg
Urine	Control - 0.18-0.20 mmol/mol of creatinine
	Patients - 12.42 ± 1.1mmol/mol of creatinine

Xanthine Oxidase Activity
Table 3. Shows that the xanthine oxidase enzyme activity were significantly increased (P<0.001) in serum and urine in uric acid stone patients when compared with normal control group

Table 3. Xanthine Oxidase Activity

Samples	Xanthine Oxidase Enzyme activity
Serum	Control - 19.05± 2.8 U/L
	Patients - 42.97 ± 5.67 U/L
Urine	Control - 94.67 ± 10.28 U/L
	Patients - 6350.00 ± 655.00 U/L

Discussion :
In this study, we showed significantly elevated levels of Adenine phosphoribosyl transferase and Xanthine oxidase in patients compared with healthy control. Xanthine Oxidase enzyme is confined in man mainly to the liver; a significant but lower activity has been found also in jejuna mucosa and colostrum; on the other hand, the kidney, prostate, and blood elements, as well as normal serum, are virtually devoid of Xanthine oxidase activity [13]. Xanthine oxidase is a ubiquitous complex cytosolic molybdo-flavoprotein which controls the rate limiting step of purine catabolism by converting xanthine to uric acid [14]. Serum xanthine oxidase levels are increased in several pathological states: inflammation, ischemia-reperfusion, aging and atherosclerosis. The reactive oxygen species are involved in oxidative damage. The inhibition of this enzymatic pathway might be beneficial. Excess of uric acid, the metabolic product of xanthine oxidase, can lead to gout [15]. Increased in xanthine oxidase may be leads to increase formation of uric acid and decrease of xanthine thus is present at low concentration in the ureters and urine, and because of its poor solubility precipitates out readily, especially in hot climates where urine volumes are small and urine very concentrated which leads to increased uric acid in blood.

The normal human APRT is a protein of 180 amino acids, composed of 9 β-strands and 6 α-helices, which can be divided into the "core" (residues 33–169), the "hood" (residues 5–34), and the "flexible loop" (residues 95–113) domains. On the basis of the APRT structure which has been widely described [16], it is evident that Ala131 and Leu159 are essential residues for the specific recognition of adenine among different purines through hydrophobic interactions. Moreover, the importance of the Leu159 residue is also confirmed by the presence in the same position of a lysine residue in the PRTases that binds hypoxanthine, xanthine, or guanine. This difference between residues located at the amino acid 159 position explains the specificity of type I PRTases for their respective purines [17].

The deficiency of adenine phosphoribosyl transferase (APRT) prevents the conversion of the adenine nucleotide to adenylic acid via the scavenger pathway. The result is increased production of the precursors 8- hydroxyadenine and 2,8-dihyrdroxyadenine (8-DHA and 2,8-DHA). This is a genetic disorder of metabolism localized to the long arm of chromosome 16. There currently is no evidence to suggest that heterozygotes get calculi or significantly excrete adenine, 8-DHA or 2,8- DHA. There are reported patients with these calculi that have only partial APRT deficiency. 2,8-dihydroxyadenine stone give a false-positive reaction to the colorimetric analysis for uric acid stones[18].

Conclusion:
We conclude that xanthine oxidase and ecto-5 -nucleotidase activities can be used as biomarkers for diagnosis of kidney damage in patients with kidney stone specially Uric acid stone.

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