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Study of Urinary Alpha Glutathione-S-Transferase in Children with Idiopathic Nephrotic Syndrome

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Abstract

Glomerulopathy associated with recurrent or persistent proteinuria may lead to progressive tubulointerstitial fibrosis. Early detection of tubulointerstitial fibrosis may result in a more favorable outcome of chronic kidney disease (CKD) because nephroprotective treatment may be instituted in due course. One of the early markers of tubulointerstitial fibrosis is glutathione S-transferase (GST). The aim of this study was to determine urinary alpha-GST in children with idiopathic nephrotic syndrome (INS), either in remission or relapse. This case–control study included 40 children with primary nephrotic syndrome (NS), either in remission or relapse. Also, 40 healthy children, age- and sex-matched as controls, were selected from the outpatients and the pediatric nephrology unit of Al-Zahraa Hospital, Al-Azhar University. Urinary alpha-GST was investigated in the study groups on the same lines as that of routine investigations of INS. Children with INS have significantly higher urinary GST either in remission or relapse, it was (5.23 ± 1.90) ng/mL, (5.32 ± 1.52) ng/mL respectively compared with healthy controls, it was (2.59 ± 1.12) ng/mL with (P = 0.001). A positive correlation between urinary alpha-GST and body weight BW, height, body mass index (BMI), white blood cells (WBCs) count, erythrocyte sedimentation rate, serum (cholesterol, triglyceride [TG]) level, blood urea nitrogen (BUN), and duration of the disease. Urinary alpha-GST was increased in children with NS even after remission, and it consequently led to oxidative stress and tubulointerstitial fibrosis. Nephroprotective treatment is recommended even in cases with INS, either in remission or relapse.

Keywords: children; nephrotic syndrome; urinary alpha-GST

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Introduction

Nephrotic syndrome (NS) is a common renal disorder with significant tubulointerstitial damage due to the combined effects of proteinuria and obstruction of efferent blood flow (1).

Tubulointerstitial fibrosis and tubular atrophy play a crucial role in the pathogenesis of chronic kidney disease (CKD). They are also major determinants in CKD development and progression in patients with primary renal diseases, characterized by persistent or recurrent proteinuria. Early

detection of tubulointerstitial fibrosis may result in a more favorable outcome of CKD because nephroprotective treatment may be instituted (2).

Numerous studies on novel early markers of tubulointerstitial fibrosis have been published. One of the early markers of tubulointerstitial fibrosis is glutathione S-transferase (GST). It is a cytosolic enzyme. There are many isoforms of GST. The alpha and pi isoforms of GST (alpha-GST and pi-GST) are typical of the human kidney. The alpha-GST is expressed in proximal tubular epithelial cells, whereas the pi-GST is specific to distal tubular epithelial cells (3, 4). Following tubular damage, GSTs are released into the urine (5).

Increased urinary GST excretions are reported in the early phase of acute tubular injury caused by various toxic substances and after cardiac surgery. Urinary GST excretions can be used as an early marker of tubular injury in glomerulopathies (3).

We aimed to assess the urinary GST level as a marker of oxidative stress and tubulointerstitial fibrosis in children with idiopathic nephrotic syndrome (INS), either in remission or relapse.

Subjects and Methods

This case–control study was carried out on 60 children, 40 males and 20 females, aged from 4 to 18 years, selected from those attending the outpatient pediatric clinic and the inpatients of the pediatric nephrology unit of Al-Zahraa Hospital, Al-Azhar University. Children included in the study were divided into the following two groups:

Group I: patients group. Forty children with INS, 33 males and seven females, aged between 4 and 18 years, formed the patient group. They were either in remission or relapse. The criteria of complete remission are proteinuria of <40 mg/m^2/hour, at least on 3 consecutive days within a period of 7 days, and serum albumin of ≥3.5 g/dL. The criteria of relapse are urinary protein excretion of >40 mg/m^2/hour or albustix of 3+ or more for three consecutive days and having previously been in remission (6). Twenty-nine (72.5%) of the patients were in remission and 11 (27.5%) were in relapse. Steroid-sensitive NS is the most common type (57.5%) of NS according to clinical classification of the study group. The minimal change is of the communist type according to the histological classification of 13 cases (59%), followed by focal segmental glomerulosclerosis in four cases (18%) and focal proliferative GN in three cases (13%). Meanwhile in 18 cases (45.0%) of the patient group, renal biopsy was not indicated.

Group II: control group. Twenty healthy children age- and sex-matched with the group I. Children with secondary NS, autoimmune diseases, other causes of generalized edema, acute kidney disease or CKDs, and cardiac surgery were excluded from the study.

- All children included in the study were subjected to history taking, clinical examination, and laboratory investigations.
- ***Investigations:***
  A) Routine investigations: Urinary albumin-to-creatinine ratio: Nephrotic levels of proteinuria are associated with a ratio of urinary protein to urinary creatinine of >2 mg/dL (7). Complete blood count, erythrocyte sedimentation rate (ESR), serum urea, creatinine, Na, K, albumin, cholesterol and triglyceride, abdominal ultrasound, and renal biopsy results were taken from the patient's file.
  B) Specific investigations:
  Assessment of urinary alpha-GST of all studied children, enzyme-linked immunosorbent assay (ELISA) was performed.
- ***Urine sampling:*** The midstream of the first morning urine specimen was collected and preserved with stabilizing buffer at -20°C till the time of the test.
- ***Principle of the test:***
  Use the kit assay to assess the human alpha-GST level in the sample; use purified human alpha-GST to coat microtiter plate wells; make solid-phase antibody; add alpha-GST to wells; combine alpha-GST antibody with Horseradish Peroxidase (HRP) labeled to form antibody–antigen–enzyme–antibody complex; after washing completely, add Tetramethylbenzidine (TMB) substrate solution. The TMB substrate turns blue in color at HRP enzyme-catalyzed, and the reaction is terminated by the addition of sulfuric acid solution, with the color change measured spectrophotometrically at a wavelength of 450 nm. The concentration of alpha-GST in the samples is then determined by comparing the optical density (OD) of the samples to the standard curve (WKEA MED SUPPLIES CORP; www.wkeamedsupplies.com).

Statistical analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 20. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The P-value was considered significant as the following: $P > 0.05$: nonsignificant; $P < 0.05$: significant; and $P < 0.01$: highly significant.

Results

Table 1 revealed a significant increase in blood pressure, WBCs, ESR, and Hb levels in the patient group, compared to the control group, while there is no significant difference in the CRP level between both the groups. Table 2 revealed a significant increase in the serum level of cholesterol, triglycerides, and BUN in the patient group, compared to the

Table 1: Comparison between patient group and control group regarding demographic date, blood pressure, complete blood count, erythrocyte sedimentation rate, and C-reactive protein.

Variable	Patient group N = 40	Control group N = 20	Independent *t*-test	
	Mean ± SD	Mean ± SD	t	P
Age (years)	8.58 ± 3.90	6.70 ± 3.04	1.882	0.065
Females	7 (17.5%)	8 (40.0%)		
Males	33 (82.5%)	12 (60.0%)	2.602	0.012
Diastolic BP mmHg	66.88 ± 8.14	61.00 ± 4.47	3.001	0.004
WBCs (10^3/uL)	10.09 ± 3.76	7.56 ± 1.24	2.923	0.005
RBCs (10^6/uL)	4.58 ± 0.59	4.83 ± 0.42	–1.676	0.099
Hb gm/dL	13.31 ± 1.42	11.91 ± 0.75	4.130	0.001
Hct%	37.30 ± 3.03	36.63 ± 2.45	0.854	0.397
Platelet (10^3/uL)	313.73 ± 97.09	285.60 ± 54.49	1.201	0.235
ESR mm/1sth Median (IQR)	31.5 (23–53.5)	15 (12–19.5)	5.486	0.001
CRP mg/L <6>6	39 (97.5%)	20 (100.0%)	0.508	0.476

WBC, white blood cell; RBC, red blood cell, BP, blood pressure; ESR, erythrocyte sedimentation rate; IQR, interquartile range; CRP, C-reactive protein; Hct, hematocrit; Hb, hemoglobin.

Table 2: Comparison between patient group and the control group with regard to laboratory data.

Variables	Patient group N = 40	Control group N = 20	Independent *t*-test	
	Mean ± SD	Mean ± SD	t	P
Cholesterol mg/dL	288.48 ± 107.99	64.95 ± 16.23	9.167	0.001
TG mg/dL	153.88 ± 50.85	45.05 ± 6.58	9.492	0.001
BUN mg/dL	27.73 ± 9.67	13.60 ± 4.22	6.221	0.001
Creat mg/dL	0.44 ± 0.11	0.43 ± 0.07	0.460	0.647
Serum Alb mg/dL	3.34 ± 1.02	4.14 ± 0.39	–3.371	0.001
GFRml/min/1.73m^2	155.93 ± 28.17	147.70 ± 28.91	1.057	0.295
Urinary alpha-GST ng/mL	5.26 ± 1.79	2.59 ± 1.12	6.085	0.001

TG, triglyceride; Creat, creatinine; BUN, blood urea nitrogen; Alb, albumin; GFR, glomerular filtration rate; GST, glutathione S-transferase.

control group, while there is a significant decrease in the serum albumen and urinary alpha-GST levels in the patient group, compared to the control group. Table 3 revealed that there is no significant difference between clinical types with regard to urinary alpha-GST levels.

Figure 1 shows a significant positive correlation between urinary alpha-GST and systolic blood pressure. Figure 2 shows a significant positive correlation between urinary alpha-GST and WBCs. Figure 3 shows a significant positive correlation between urinary alpha-GST and the level of triglyceride serum.

Table 3: Comparison between nephrotic syndrome clinical types with regard to urinary alpha glutathione S-transferase level.

Variable	Urinary alpha-GST ng/mL		Independent *t*-test	
	Mean ± SD	Range	t	P
SSNS (n = 23)	5.18 ± 1.96	2 – 8.4	0.384	0.703
SRNS (n = 16)	5.41 ± 1.47	2.1 – 7.3		
Remission (n = 29)	5.23 ± 1.90	2 – 8.4	0.131	0.897
Relapse (n = 11)	5.32 ± 1.52	2 – 7		

SSNS, steroid sensitive nephrotic syndrome; GST, glutathione-S-Transferase; SRNS: steroid resistant nephrotic syndrome.

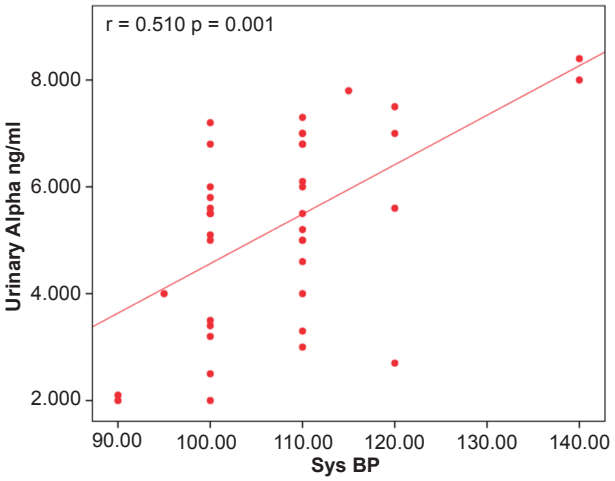


Figure 1: Correlation between urinary alpha glutathione S-transferase and systolic blood pressure.

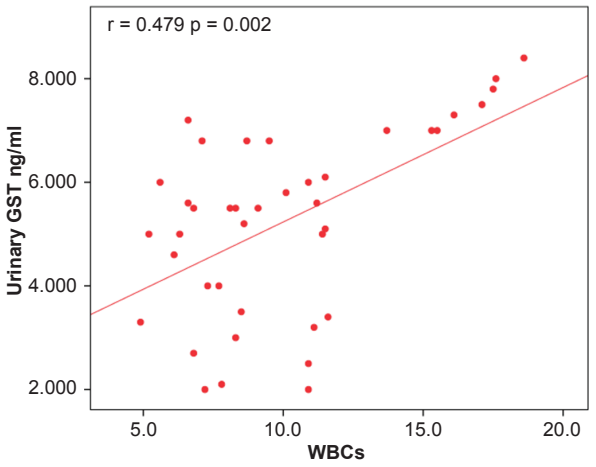
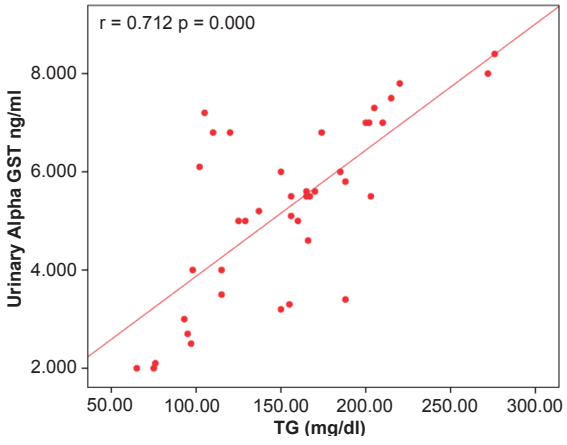


Figure 2: Correlation between urinary alpha glutathione S-transferase and white blood cells.



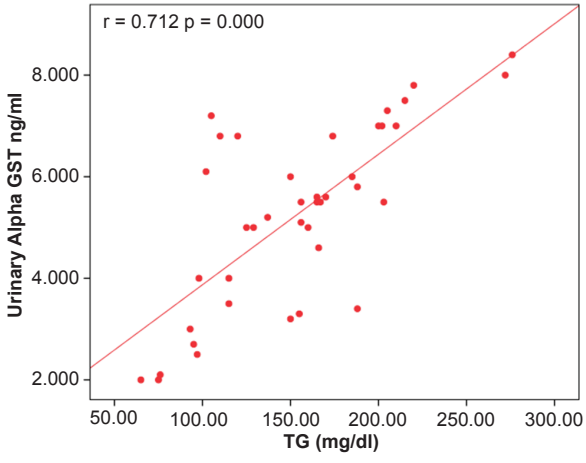


Figure 3: Correlation between urinary alpha glutathione S-transferase and triglyceride serum level.

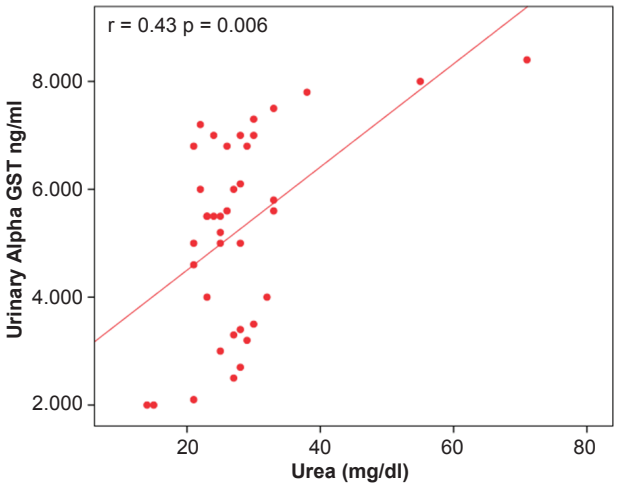


Figure 4: Correlation between urinary alpha glutathione S-transferase and blood urea nitrogen.

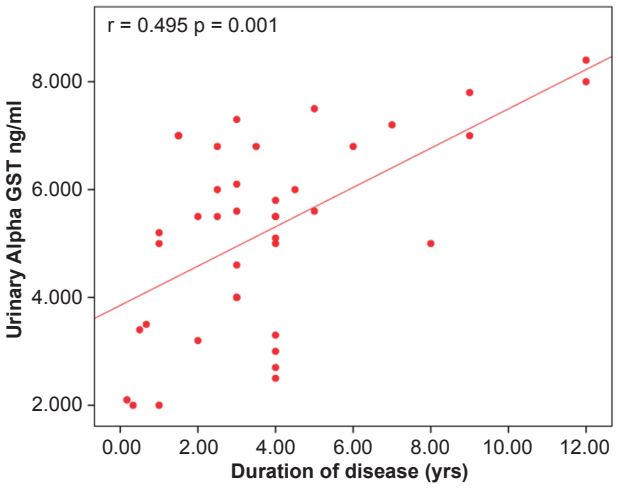


Figure 5. Correlation between urinary alpha glutathione S-transferase and duration of nephrotic syndrome.

Figure 4 shows a significant positive correlation between urinary alpha-GST and urea. Figure 5 shows a correlation between urinary alpha-GST and the duration of NS.

Discussion

Different studies have successfully investigated the role of several markers for detecting tubulointerstitial fibrosis in renal diseases. Regarding GST and NS, only one study was conducted on urinary alpha-GST level in NS; hence, not much data were available on this issue as per the comprehensive electronic survey.

In the current study, we found a significant increase in urinary alpha-GST excretion in all the participants with NS in comparison to the control group. The number of patients with remission and relapse was 29 (72.5%) and 11 (27.5%), respectively; surprisingly we found no significant difference between children with NS, either in remission or relapse, with regard to urinary alpha-GST level.

Urinary GST increases might represent subclinical renal tubular injury and/or a tubular response to oxidant stress; oxidative stress persisted in NS even after remission, and deoxyribonucleic (DNA) damage can occur as a result of persistent oxidant stress. GST can be used as a marker in NS and as an indicator of oxidative stress (8).

Isoforms of GST and glutaredoxin (Grx) play a significant role in the regulation of cell signaling by protein-to-protein interactions, with regulatory kinases controlling the cell response to stress, proliferation, and induction of apoptosis (9–11). GST enzymes protect cells against reactive oxygen species (ROS), which is important for preventing DNA damage (12). The expression of GSTs can be regulated by different stressors (e.g., variation in temperature, oxidative damage, and exposure to toxins), and cytosolic glutathione transferases (cGSTs) promoters contain antioxidant response elements (13, 14).

Fan et al. (15) reported a significant increase in oxidative stress markers, malondialdehyde (MDA) and superoxide dismutase (SOD), in children at first presentation and after 4 weeks of steroid treatment.

From my point of view, the current research, and others, children with NS are exposed to oxidative stress even after remission.

We reported a significant positive correlation between urinary alpha-GST and the prolonged disease length. *This association may relate to the prolonged generation of oxidative stress*; also, prolonged exposure to proteinuria can cause tubulointerstitial fibrosis through the induction of tubular chemokines expression and complement activation that leads to inflammatory cell infiltration in the interstitium and sustained fibrogenesis. Chemoattractants and adhesive molecules for inflammatory cells are upregulated by an excessive ultrafiltrate protein load of proximal tubular cells (16).

A significant positive correlation between urinary alpha-GST level and body weight & BMI was reported in the present study; obesity is a state of mild inflammation and oxidative stress that increases the release of GST (17, 18). Also, cholesterol and TG were positively correlated with urinary alpha-GST; abnormal lipid homeostasis may contribute to the pathogenesis of renal fibrosis and vascular calcification (19–21). Lipids are susceptible to oxidation, and lipid peroxidation products are potential biomarkers for oxidative stress status *in vivo* and its related diseases (22).

There was no correlation between urinary alpha-GST excretion and the magnitude of proteinuria. This observation suggests that renal tubular injury due to proteinuria seems to be independent of its magnitude. In the study by Bashir et al. (23), urinary alpha-GST and pi-GST excretions were suggested to be useful markers of proteinuria-induced renal tubular injury. They also revealed a positive correlation between urinary alpha-GST excretion and the severity of proximal tubular histopathologic changes. They proved that in proteinuric patients, the initial injury was localized in proximal tubules. Our results also confirmed this observation. This might indicate that in children with NS, regardless of clinical types, renal tubular injury begins in the region of proximal tubules.

Cawood et al. (24) observed that in diabetic proteinuric patients, urinary alpha- and pi-GST markers appeared to identify renal damage, which was related to but distinct from albuminuria.

A significant correlation between alpha-GST and hypertension was detected, which decreased renal blood flow, resulting in increased mitochondrial oxygen use, shunting of blood from arterial to venous blood in preglomerular vessels, the release of cytokines, presence of oxidative stress, and the release of ROS (25).

In conclusion, oxidative stress persists in children with INS even after remission, which is evidenced by elevated GST; thus, nephroprotective treatment should be intensified. In the future, larger studies will be required to further validate these findings.

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