

Evaluation of Serum and Urinary Neutrophil Gelatinase - Associated Lipocalin in Pediatric Patients with Chronic Nephrotic Syndrome

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ABSTRACT

Background: Chronic kidney disease (CKI) has recently assumed epidemic proportions, becoming a troubling emerging cause of morbidity, especially if it progresses to end stage renal disease (I:SRD).

Objectives: This study aimed at evaluating whether neutrophil gelatinase-associated lipocalin (NGAI), a novel specific biomarker of acute kidney injury, could predict the progression of CKI in children with chronic nephrotic syndrome.

Subjects and Methods: Serum NGAI. (sNGAI.) and urinary (uNGAI.) levels were evaluated in a study of 30 children affected by chronic nephrotic syndrome (NS). Their mean age was 8.7 t. 2.9 years. They were subdivided into two subgroups according to response to steroids therapy: 18 children with steroid dependent nephrotic syndrome (SINS) and 12 children with steroid sensitive nephrotic syndrome (SSNS). All patients were compared with control group of 15 age and sex matched healthy children.

Results: Both serum and urinary NGAI, showed significantly higher concentration in NS group vs. the control group (p - 0.001 and 0.024 respectively). sNGAI. was sigiiit'icantly higher in both SINS and SSNS groups versus the control group and SINS group versus SSNS group (p 0.001, 0.001 and 0.027 respectively). uNGAL was significantly higher in SINS group versus the control group while between SSNS group and control group. SINS group and SSNS group uNGAI. showed higher concentration but it didnt reach statistical signilicance (p . 0;.009, 0.055 and 0.637 respectively). There were also higher concentrations versus the control group of both sNGAI, and uNGAI, in proteinuric (p 0.001 and 0.011). and non- proteinuric (p -- 0.001 and 0.023) groups while there was no significant difference as regards sNGAI. and uNGAI. (p = 0.143 and 0.407 respectively) between proteinuric versus non-proteinuric groups. We round that sNGAL showed a significant positive correlation with serum creatinine in SINS group (p - 0.017). The estimated glomerular filtration rate (eGFR) showed an inverse correlation with sNGAI. in NS patients and with sNGAI. and UNCIAL in SANS group but yet, it didn't reach statistical significance (p 0.41 7, 0.062 and 0.996 respectively).

Conclusion: NGAI. closely reflects the degree of renal impairment and represents a strong and independent risk marker for progression of CKI.

INTRODUCTION

Nephrotic syndrome (NS) is a common chronic disorder. characterized by alterations of permeability at the glomerular capillary wall, resulting in its inability to restrict the urinary loss of protein¹ t.

Chronic kidney - disease (CKD) has recently assumed epidemic proportion becoming a troubling emerging cause of morbidity, especially if it progresses to terminal stage end stage renal disease^{1,t}.

The annual incidence of nephrotic

syndrome in most countries in the Western hemisphere is estimated to range from 2-7 new cases per 100,000 children, and the prevalence from 12-16 per 100,000 children⁽³⁾.

The reported prevalence of chronic renal failure in Egypt is 225 per million populations⁽⁴⁾.

The use of reliable biomarkers is becoming increasingly important for improved management of patient with acute and chronic kidney disease. Recent developments have identified a number of novel biomarkers in serum or urine that can determine the potential risk of kidney damage, distinguish different types of renal injury, predict the progression of disease and have the potential to assess the efficacy of therapeutic intervention".

Neutrophil gelatinase-associated lipocalin (NGAL), also named lipocalin-2, is a glycoprotein originally isolated from human neutrophils and localized in their specific granules. However, many other cells, like kidney tubular cells, may produce NGAL in response to various insults. NGAL has been found to have a role in kidney development and tubular regeneration after injury⁽⁵⁾.

Recent studies show that serum and urinary neutrophil gelatinase-associated lipocalin NGAL represents a novel, sensitive, specific biomarker for early detection of acute kidney injury. However, clinical significance of measuring serum and urinary NGAL in chronic renal diseases remains unclear".

AIM OF THE WORK

The aim of this work was to evaluate both serum and urine levels of NGAI, in

children with chronic nephrotic syndrome and to enlighten its role as sensitive marker of renal injury detecting the severity of renal impairment. We hypothesized that NGAI probably expresses the degree of active damage underlying the chronic condition of this disease.

SUBJECTS AND METHODS

Forty five children were enrolled in this study. They were subdivided into two groups:

Patients group: Included 30 pediatric patients with chronic nephrotic syndrome, 18 males (60%) and 12 females (40%). Their mean age was (8.7 -4- 2.9 years). Those patients were recruited from both the Pediatric Nephrology Clinic and the Inpatient Department of Beni-Suef University Hospital. The patients group was further subdivided into two subgroups according to response to steroids: 18 patients with steroid dependent nephrotic syndrome (SUNS) and 12 patients with steroid sensitive nephrotic syndrome (SSNS).

Steroid treatment was started with patients with NS in the form of prednisone 1-2 mg/Kg. Remission was expected to occur in the form of clinical improvement of generalized edema and regression of proteinuria within 4 weeks. Then steroid was withdrawn on alternate day therapy. Those patients who developed proteinuria on starting withdrawal of steroids were considered to be steroid dependent, while those who did not were considered to be steroid sensitive. None of the patients were hypertensive.

Control group: Included 15 healthy sex and age matched children. They had no

renal problems and were not receiving any medication.

All children were subjected to: Full history taking, thorough physical examination, and abdominal ultrasonography, related routine laboratory investigations including kidney function tests : urea and creatinine; Na; K; cholesterol; serum protein electrophoresis; urine analysis (mainly urine volume, hematuria and urine protein). Estimated glomerular filtration rate was calculated using Schwartz formula for children : $eGFR (ml/min/1.73 m^2) = \text{height (cm)} \times \text{constant}/\text{serum creatinine (mg/dL)}$, where height was expressed in "cm" and constants was 0.44 (for children < 2 years) and 0.55 (for children > 2 years). Renal dysfunction was defined as $eGFR < 90 mL/min/1.73 m^2$ (8).

Specific laboratory investigations included both serum and urinary level of NGAL using enzyme linked-immunosorbent assay (ELISA).

The Quantikine NGAL kit is a solid-phase sandwich enzyme linked-immunosorbent assay. A monoclonal antibody specific for NGA1. has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any NGAI, present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for NGAI, was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of NGAL bound in the initial step. The color development was stopped and the intensity of the color was measured.

The study was approved by the Ethical Committee of Faculty of Medicine, Beni-Suef University and informed consent was obtained from all the participants care givers.

Statistical methods:

For statistical analysis, Statistical Package for Social Science (SPSS) software version 17 was used. Data were statistically described in terms of range and mean :1, standard deviation (-t SD). Comparison of quantitative variables between the study groups was done using Kruskal Wallis analysis of variance (ANOVA) test with Mann Whitney U test for independent samples as multiple 2-group comparisons. In all tests, test results were considered statistically significant when the p-value was less than 0.05. Receiver Operator Characteristic curve (ROC) was plotted to get the best cut off of variables obtained in this study. -

RESULTS

This study was conducted on forty Live children (28 males and 17 females) their ages below 12 years. The groups were divided as follows:

Patients group: Thirty children with chronic nephrotic syndrome 18 males (60%) and 12 females (40%) their age below 12 years with mean (8.7 - 2.9 years) were selected from Pediatric Nephrology Clinic and Inpatient Pediatric Departments, Beni-Suef University Hospital. They were subdivided into two subgroups according to response to steroids: steroid dependent nephrotic syndrome (SDNS) including 18 patients and steroid sensitive nephrotic syndrome (SSNS) including 12 patients.

Control group: Oliguria and edema before treatment which improved after it.

Table (1) and figure (1) demonstrate that NS group showed significantly higher concentration versus control group as regards sNGAI, (86.8 ± 27.4 ng/ml) versus (45.6 ± 15.7 ng/ml) and uNGAL (6.1 ± 5.3 ng/ml) versus (2.9 ± 1.1 ng/ml) (p 0.001, 0.024 respectively).

Cholesterol, urea and K were significantly higher and albumin was significantly lower in NS patients versus control. There was no significant difference between two groups as regards Na, creatinine and eGFR.

Table (2) shows the descriptive laboratory data of patients' subgroups according to response to steroids : steroid dependent nephrotic syndrome (SDNS) and steroid sensitive nephrotic syndrome (SSNS) groups in comparison to each other and to norm comparison control.

Table (2) demonstrates that sNGAL was significantly higher in both SDNS and SSNS groups versus the control group and also SDNS group versus SSNS group (95.7 ± 29.9 ng/ml) and (73.4 ± 16.4 ng/ml) versus (45.6 ± 15.7 ng/ml) and also (95.7 ± 29.9 ng/ml) versus (73.4 ± 16.4 ng/ml) (p 0.001, 0.001 and , 0.027 respectively). uNGAL was significantly higher in SDNS group versus the control group (6.7 ± 5.2 ng/ml) versus (2.9 ± 1.1 ng/ml) while between SSNS group; versus control group and SDNS group versus SSNS group uNGAL showed higher concentration but it didn't reach statistical significance (p 0.009, 0.055, 0.637 respectively).

Patients group was subdivided into proteinuric (8 patients) and non proteinuric (22 patients) groups according to presence

and absence of urine protein respectively.

Table 3 demonstrates that the proteinuric group showed significantly higher concentration versus control group as regards sNGAL (99.0 ± 34.4 ng/ml) versus (45.6 ± 15.7 ng/ml) and uNGAL (4.8 ± 2.2 ng/ml) versus (2.9 ± 1.1 ng/ml) (p 0.001 and 0.011 respectively).

The non-proteinuric group showed significantly higher concentration versus control group as regards sNGAL (82.3 ± 23.7 ng/ml) versus (45.6 ± 15.7 ng/ml) and uNGAL (6.6 ± 6.0 ng/ml) versus (2.9 ± 1.1 ng/ml) (p = 0.001 and 0.023 respectively).

Although the proteinuric group showed higher concentration than the non-proteinuric group as regards sNGAL, the difference was not significant.

Figures 2, 3, 4 and 5 demonstrate that sNGAL showed significantly positive correlation with serum creatinine in SDNS group (r = 0.556, p 0.017). While eGFR showed inverse correlation with sNGAL, in NS patients and with sNGAL and uNGAL in SANS group, yet; it didn't reach statistical significance (r = -0.154, p 0.417), (r = -0.448, p = 0.062) and (r = -0.001, p 0.996) respectively.

Table (4) and figure (6) show that sNGAL at a concentration of 56.1 ng/ml showed the best cutoff value where the sensitivity was 86.7% and the specificity was 90% calculated by the ROC curves.

At a concentration of 3 ng/ml, uNGAL, had the best cutoff value where the sensitivity was 86.7% and the specificity was 80%.

Area under the ROC curve for sNGAL was 0.9 indicating best performance followed by 0.8 value for uNGAL (Fig. 6).

Table 1: Statistical comparison between NS patients and control group regarding different laboratory parameters.

Variable	NS Group n = 30	Control Group n = 15	p Value
Serum Albumin (g/dl)	3.4 ± 0.8	4.3 ± 0.3	0.0001*
Serum Cholesterol (mg/dl)	175 ± 51.7	116 ± 5.5	0.001*
Serum Na (mmol/L)	136 ± 4.1	135 ± 3.4	0.535
Serum K (mmol/L)	4.1 ± 0.4	3.7 ± 0.4	0.005*
Serum Urea (mg/dl)	23.6 ± 8.1	18.9 ± 1.8	0.031*
Serum Creatinine (mg/dl)	0.49 ± 0.16	0.44 ± 0.13	0.067
eGFR (ml/min/1.73 m²)	137.6 ± 46.1	143.6 ± 41.5	0.674
sNGAL (ng/ml)	86.8 ± 27.4	45.6 ± 15.7	0.001*
uNGAL (ng/ml)	6.1 ± 5.3	2.9 ± 1.1	0.024*

* p < 0.05 (significant)

Table 2: Descriptive statistical comparison between the laboratory parameters of SDNS, SSNS and control groups.

Parameter	SDNS (n = 18)	SSNS (n = 12)	Control (n = 15)	p1-Value	p2-Value	p3-Value
Serum Albumin (g/dl)	2.9 ± 0.4	3.2 ± 0.6	4.3 ± 0.3	0.0001*	0.0001*	0.110
Serum Cholesterol (mg/dl)	178.3 ± 64.9	170.3 ± 22.3	116.5 ± 5.5	0.001*	0.001*	0.682
Serum Na (mmol/l)	136.9 ± 4.6	136.1 ± 3.3	135.8 ± 3.4	0.452	0.830	0.606
Serum K (mmol/l)	4.2 ± 0.5	4.0 ± 0.3	3.7 ± 0.4	0.003*	0.112	0.096
Serum Urea (mg/dl)	25.3 ± 9.7	21.2 ± 4.2	18.9 ± 1.8	0.017*	0.066	0.179
Serum Creatinine (mg/dl)	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.646	0.582	0.316
eGFR (ml/min/1.73 m ²)	141.9 ± 43.4	132.7 ± 50.1	143.6 ± 41.5			0.594
sNGAL (ng/ml)	95.7 ± 29.9	73.4 ± 16.4	45.6 ± 15.7	0.001*	0.001*	0.027*
uNGAL (ng/ml)	6.7 ± 5.2	5.8 ± 5.5	2.9 ± 1.1	0.009*	0.055	0.637

p1-value: between SDNS and control group.

p2-value: between SSNS and control group.

p3-value: between SDNS and SSNS.

* p < 0.05 (significant) from the control group.

Table 3: Descriptive statistical comparison between the laboratory parameters of proteinuric group, non-proteinuric and control groups.

Parameter	Proteinuric Group (n = 8)	Non-Proteinuric Group (n = 22)	Control Group (n = 15)	p1-Value	p2-Value	p3-Value
Serum Albumin (g/dl)	2.2 ± 0.6	3.3 ± 0.9	4.3 ± 0.3	0.0001*	0.0002*	0.002*
Serum Cholesterol (mg/dl)	171.9 ± 32.5	176.3 ± 57.7	116.5 ± 5.5	0.001*	0.001*	0.841
Serum Na (mmol/l)	135.4 ± 4.6	137.0 ± 3.9	135.8 ± 3.4	0.802	0.342	0.345
Serum K (mmol/l)	4.0 ± 0.4	4.1 ± 0.4	3.7 ± 0.4	0.077	0.007*	0.606
Serum Urea (mg/dl)	23.6 ± 4.2	23.6 ± 9.2	18.9 ± 1.8	0.001*	0.058	0.997
eGFR (ml/min/1.73 m ²)	132.9 ± 44	150 ± 52.1	143.6 ± 41.5			0.360
Serum Creatinine (mg/dl)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.932	0.902	0.854
sNGAL (ng/ml)	99.0 ± 34.4	82.3 ± 23.7	45.6 ± 15.7	0.001*	0.001*	0.143
uNGAL (ng/ml)	4.8 ± 2.2	6.6 ± 6.0	2.9 ± 1.1	0.011*	0.023*	0.407

p1-value: between proteinuric and control group.

p2-value: between non-proteinuric and control group.

p3-value: between proteinuric and non-proteinuric group.

* p < 0.05 (significant) from the control group.

Table 4: Operating characteristics of sNGAL and uNGAL in NS patients.

Variable	Cut-Off Value	Sensitivity %	Specificity %
sNGAL (ng/ml)	56.1	86.7%	90%
uNGAL (ng/ml)	3	86.7%	80%

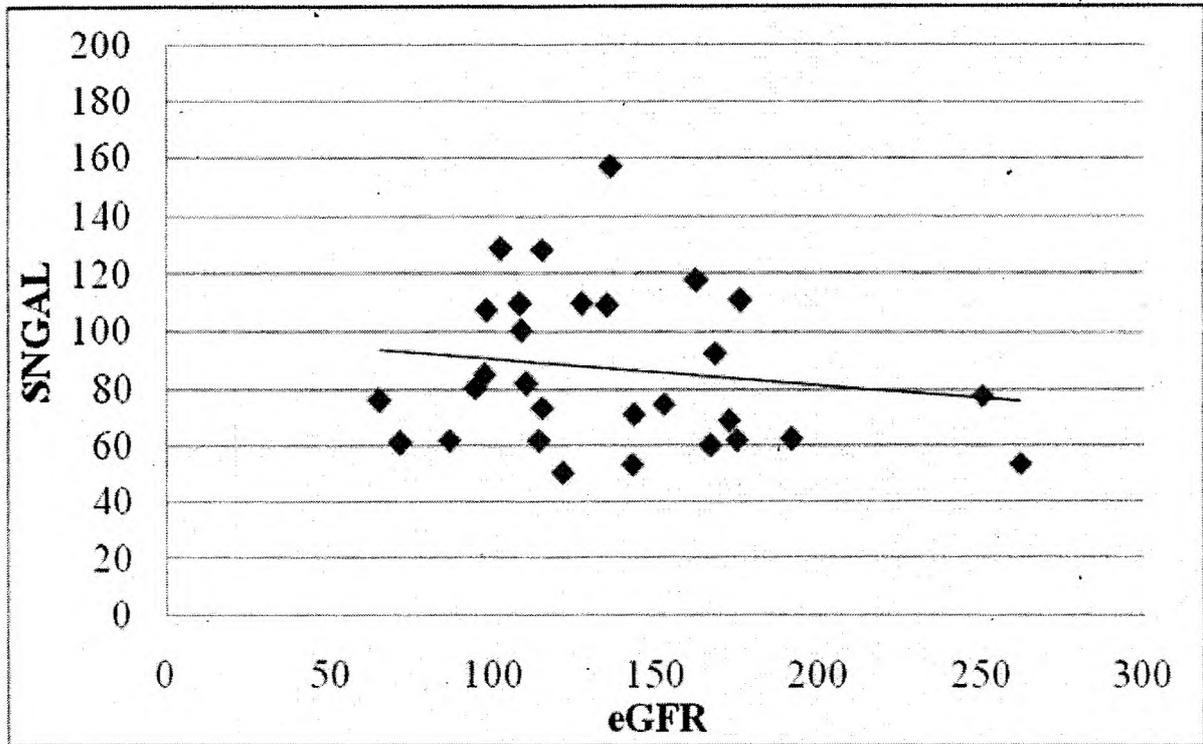


Fig. 3: Correlation between eGFR (ml/min/1.73 m²) and sNGAL (ng/ml) in NS patients (r = -0.154, p = 0.417).

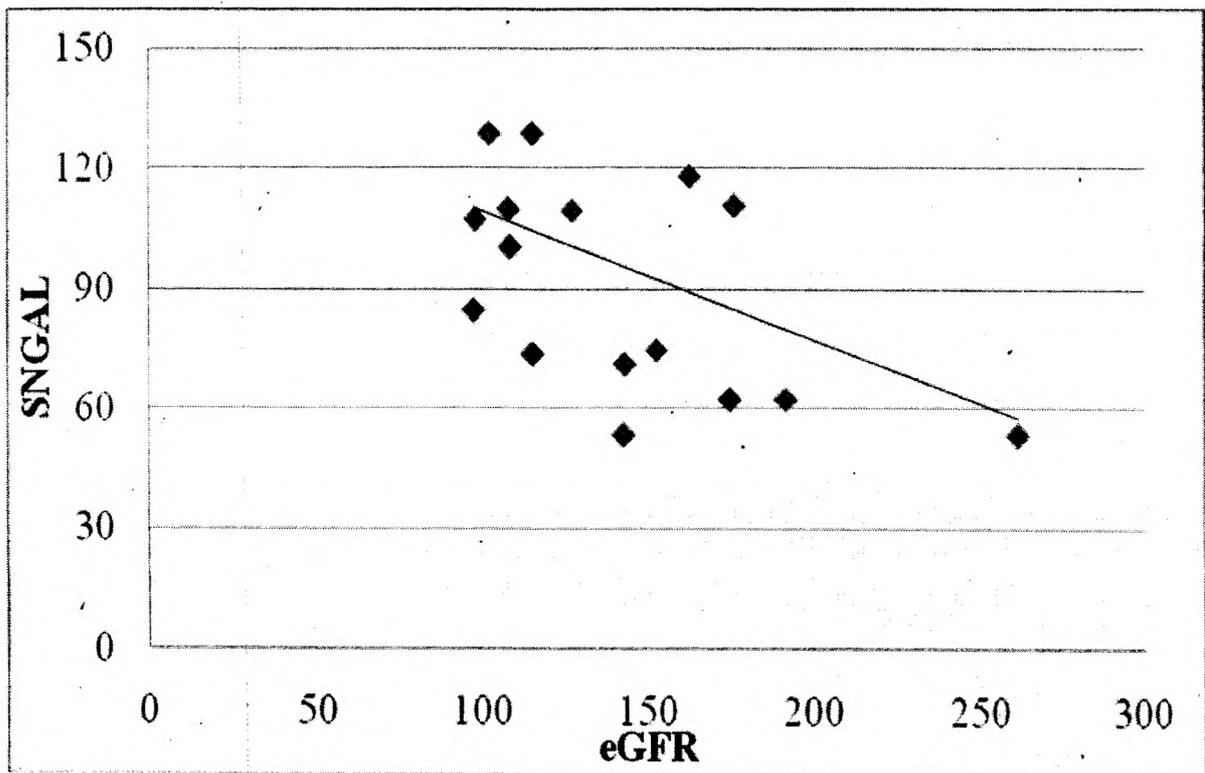


Fig. 4: Correlation between eGFR (ml/min/1.73 m²) and sNGAL (ng/ml) in SDNS (r = -0.448, p = 0.062).

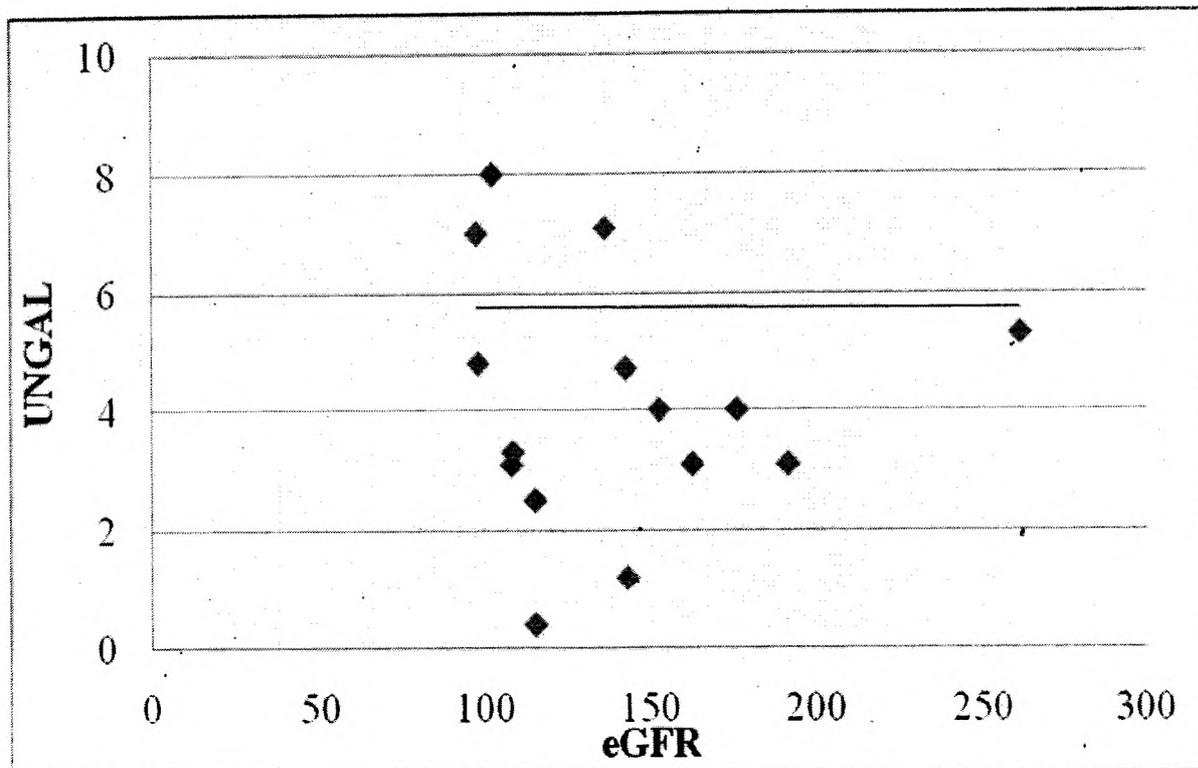


Fig. 5: Correlation between eGFR (ml/min/1.73 m²) and uNGAL (ng/ml) in SDNS ($r = -0.001$, $p = 0.996$).

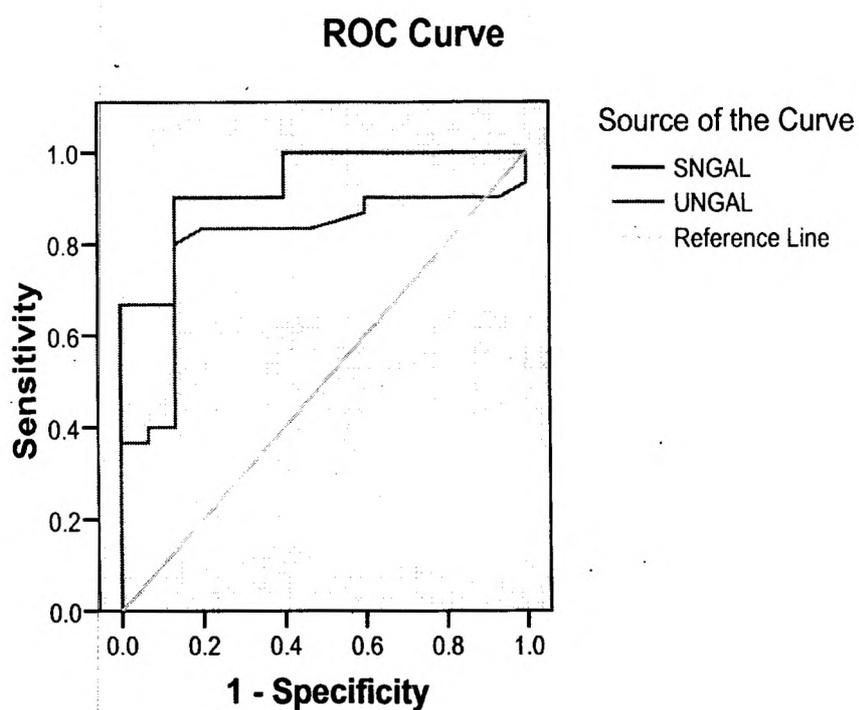


Fig. 6: Receiver operator characteristic curve (ROC) of sNGAL and uNGAL.

DISCUSSION

Neutrophil gelatinase-associated lipocalin expression is rapidly induced in the nephron in response to renal epithelial injury. NGAL levels are predictive of the onset of acute renal injury and also the serious exacerbation of unstable nephropathy. Also, NGAL may be involved in the pathophysiological process of chronic renal conditionst 10).

In our study, sNGAL and uNGAL showed significantly higher concentration in chronic relapsing NS group versus control group ($p = 0.001, 0.024$ respectively). Although, there was no significant difference between NS patients and the normal control regarding eGFR or creatinine.

This shows that with normal eGFR in chronic kidney diseases such as chronic nephrotic syndrome, the ongoing renal pathology needs to be monitored with more sensitive renal markers.

NGAL is one of the most promising biomarkers that are able to reveal the onset of acute kidney injury. However, in certain studies on chronic kidney diseases like ours, it showed that it would be beneficial to predict CKD progression.

Bolignano et al., reported that in subjects affected by non-terminal CKD of various etiology the mean uNGAL concentrations were higher in CKD patients having polycystic kidney, disease and glomerulonephritis than normal control ($p = 0.01$)(11).

Another study confirmed these results and reported that subjects with CKD due to chronic glomerulonephritis had higher mean uNGAL concentrations than normal controls ($p = 0.01$)⁽¹⁾.

More recently, Malys/ko, in 2010 and

Shavit et al, in 2011 reported that sNGAL was significantly higher in patients with CKD and stated that the NGAL is a highly sensitive marker of renal injury as it is also elevated in CKD and it might perform better in children than in adults, probably because they have less or even no comorbidities^{3,14)}

Soni et al., reported that uNGAL measurement is probably more reflective of local renal injury and is non-invasive in nature reducing the need for frequent blood sampling and handling of blood lines in critically ill patients⁽⁶⁾.

Kuwabara et al., reported that patients with nephrotic syndrome or interstitial nephritis had markedly elevated uNGAL levels that decreased in response to successful treatment(15).

According to our findings concerning sNGAL and uNGAL which showed significantly higher concentrations than control, and supported by similar results in previously mentioned research, we can assume that in patients with CKD, NGAL closely reflects the entity of renal impairment and represents a strong and independent risk marker for progression of CKD and that NGAL may become one of the most promising next generation biomarkers in clinical nephrology. Whether one measurement of sNGAL or uNGAL is useful over the other can be known after wider clinical application of these tests. It is also possible that together they provide complementary information.

Our study reported that sNGAL showed significantly higher concentration in the SDNS group versus the control group. SSNS group versus control group and

SDNS group versus SSNS group (p 0.001, 0.001 and 0.027 respectively). So sNGAL might give information about the degree of response to steroid treatment.

We found that uNGAL showed significantly higher concentration in the SDNS group versus the control group while between SSNS group and the control group, SDNS group and the SSNS group uNGAL, showed a higher concentration but it didn't reach statistical significance (p = 0.009, 0.055, 0.637 respectively).

In the proteinuric group versus the control group, higher concentrations of both sNGAL and uNGAL were found (p 0.001, 0.011 respectively).

In agreement with our study is Bolignano et al., who reported that in patients with proteinuria, uNGAL concentrations were significantly higher than in controls (P=0.0¹)¹⁷

The authors suggested that correlation between uNGAL and the degree of proteinuria can be attributed to a response of such tissue to the condition of chronic tubular stress. They reported that it is well-known, in fact, that a persistent macroproteinuria itself constitutes a factor of progressive renal damage caused by the toxicity of the plasmatic protein on tubular renal cells, particularly proximal ones. Such toxicity promotes apoptosis. The increased production of NGAL by tubular cells could in this context constitute a compensatory mechanism, with defensive intent, based on the inhibition of apoptosis processes by this protein, but it should not be excluded that the same cell damage from chronic tubular injury causes increased urinary excretion of NGAL¹⁸.

In our study, sNGAL showed significance positive correlation with Serum creatinine (r = 0.556, p = 0.017) in SDNS group.

We found that eGFR showed inverse correlation with sNGAL in NS patients and with sNGAL and uNGAL in SDNS group. Yet, it didn't reach statistical significance (r = -0.154, p = 0.417), (r = -0.448, p 0.062) and (r = -0.001, p = 0.996) respectively.

Several studies were in agreement with ours and reported that uNGAL or sNGAL concentrations were significantly correlated with either serum creatinine concentrations, eGFR or proteinuria^{7, 16, 17, 18}

Wasilewska et al., confirmed these results and stated that most studies that have investigated the correlations of NGAL and parameters of kidney function have found a positive correlation between sNGAL and creatinine and a negative correlation between sNGAL and eGFR⁹.

An interesting theory was proposed by Mori and Nakao and might explain the relationship between NGAL and GFR, suggesting that the increase in NGAL, is not just the passive consequence of a reduced renal clearance. This hypothesis, called the "forest Fire theory", assumes that the increase in NGAL in chronic kidney disease "forest fire" is the consequence of a sustained production by "inflamed" but vital tubular cells, whereas the rise in serum creatinine and the contraction of GFR are the mere passive result of a general loss of functional cells or nephrons¹⁹.

Our findings of a significantly higher frequency as regards urine protein in NS compared with the control group, positive

correlation between sNGAL and creatinine and negative correlation between both sNGAL and uNGAL, versus eGFR among patients which were documented by similar results in other studies suggest that NGAL levels clearly correlated with severity of renal impairment probably expressing the degree of active damage underlying the chronic condition and that NGAL should be investigated as an early marker of kidney function impairment in patients with CKD. From this point of view, NGAL would represent a real-time indicator of how much active kidney damage exists within the overall condition of chronic renal impairment.

Arena et al., reported that NGAL levels, clearly correlated with the severity of renal impairment and probably expresses the degree of active damage underlying the chronic condition¹⁰.

In our study, we found that sNGAL at a concentration of 56.1 ng/ml showed the best cutoff value where the sensitivity was 86.7% and the specificity was 90%

calculated by the ROC curve. While at a concentration of 3 ng/ml, uNGAL, had the best cutoff value where the sensitivity was 86.7% and the specificity was 80%. Bolignano et al., reported that for sNGAL the best cut-off level was found to be 435 mg/ml (sensitivity 83.9%, specificity 53.8%), whereas for uNGAL it was 231 mg/ml (sensitivity 80.6%, specificity 73.8%)⁽²⁾.

Conclusion and Recommendations:

Serum and urinary NGAL are useful markers of progression of CKD in chronic nephrotic syndrome. They could provide a good reflection of the severity of the renal disease as sNGAL was found to be significantly higher in SDNS versus SSNS. It might reflect also the degree of response to steroid treatment.

Further studies are needed especially for a longer observation period to confirm our findings and to determine whether therapeutic measures targeting NGAL balance would be helpful in delaying the progression of CKD.

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