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RESEARCH ARTICLE

Neutrophil gelatinase-associated lipocalin is a urine-based biomarker for diagnosing active lupus nephritis

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Abstract

Background: Lupus nephritis is one of the most serious complications of systemic lupus erythematosus (SLE). Urinary neutrophil gelatinase-associated lipocalin (NGAL) is a non-invasive biomarker that may aid in diagnosing active lupus nephritis. This study seeks to evaluate NGAL as a urine-based biomarker to diagnose active lupus nephritis.

Methods: This cross-sectional study was conducted in the Department of Laboratory Medicine, Rheumatology, and Nephrology at Bangabandhu Sheikh Mujib Medical University. Urine samples were collected to estimate NGAL levels using the ELISA method in the Department of Laboratory Medicine. Participants were divided into three groups: patients with SLE and active lupus nephritis, patients without nephritis, and healthy controls. Each group was comprised of 24 individuals. Patients with active lupus nephritis were classified into six categories (I to VI). ANOVA was performed to compare urinary NGAL values across the groups or categories. A receiver operating characteristic curve was created to establish the cut-off point for NGAL.

Results: The mean urinary NGAL level was 19.5 (SD 6.9), 7.2 (3.8), and 1.7 (6.5) ng/mL in SLE patients with active lupus nephritis, SLE patients without active lupus nephritis, and healthy individuals, respectively. Increasing mean levels were observed across the classes of lupus nephritis. The cut-off point for urinary NGAL in active lupus nephritis was 11.6 ng/mL, demonstrating a sensitivity of 83.3% and a specificity of 93.8%.

Conclusion: The level of urinary NGAL was elevated in SLE patients with active lupus nephritis. It could serve as a valuable biomarker for diagnosing active lupus nephritis.

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Key messages

Lupus nephritis is a major cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). We observed elevated levels of urinary neutrophil gelatinase-associated lipocalin (NGAL) in patients with SLE and active lupus nephritis compared to those without lupus nephritis and healthy individuals. Urinary NGAL serves as a valuable biomarker for identifying active lupus nephritis in SLE patients.

Introduction

SLE is a complex, multisystem autoimmune disease characterised by the production of numerous autoantibodies to cellular components and marked by complicated manifestations, ranging from detectable laboratory abnormalities to multi-organ inflammation and failure [1]. SLE predominantly affects women of childbearing age, particularly those between 15 and 44 years old [2]. It is one of the most gender-differentiated autoimmune disorders, with a female predominance of 9:1 [3].

The estimated global incidence of SLE is 5.14 per 100,000 population annually, and the estimated prevalence is 43.7 per 100,000 population [4]. In the Asia-Pacific region, the incidence of SLE ranges from 0.9 to 3.1 per 100,000 population annually, while the prevalence varies from 4.3 to 45.3 per 100,000 population annually [5]. Lupus nephritis is one of the most severe manifestations of SLE [6]. It affects up to 70% of children, and between 30% and 60% of adult SLE patients [7]. Approximately 40% of SLE patients in Bangladesh are affected by lupus nephritis [8]. It is a major cause of morbidity and mortality in SLE. It is an important predictor of poor outcomes and is often difficult to categorise [9].

Renal biopsy is considered the gold standard for diagnosing and grading lupus nephritis. This invasive procedure requires repeated testing to assess disease activity in lupus nephritis [7]. Urinary NGAL is a highly sensitive and early biomarker for kidney injury [10]. It is a 25 kDa lipocalin derived from human neutrophils [11]. As an acute-phase glycoprotein released in small amounts by neutrophils, renal tubular cells, epithelial cells, macrophages, hepatocytes, and neurons under physiological conditions, its production significantly increases in response to cellular stress [12].

Elevated urinary NGAL demonstrated a sensitivity of 95% and a specificity of 100% in diagnosing active lupus nephritis [13]. The urinary NGAL exhibited a sensitivity of 92% and a specificity of 75% when detecting active lupus nephritis in SLE patients [14]. Urinary NGAL has not been established as a global diagnostic marker for active lupus nephritis. No studies on urinary NGAL in lupus nephritis patients have been conducted in Bangladesh. This study aims to assess NGAL as a urine-based biomarker to diagnose active lupus nephritis.

Methods

Study design

This cross-sectional study was conducted from September 2023 to August 2024 in the Department of Laboratory Medicine in collaboration with the Department of Rheumatology and Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

Sample and sampling of subjects

Seventy-two patients were purposively selected from the inpatient and outpatient departments of Rheumatology and Nephrology at BSMMU. They were grouped as Group I: SLE patients with active lupus nephritis, Group II: SLE patients without active lupus nephritis, and Group III: healthy individuals. SLE patients were diagnosed according to the 2019 EULAR and American College of Rheumatology criteria. Patients with diabetes mellitus, metabolic disorders, malignancies, drug-induced lupus, overlap syndrome, renal stones, urinary tract infections, those undergoing haemodialysis, or with a history of renal transplantation were excluded from this study.

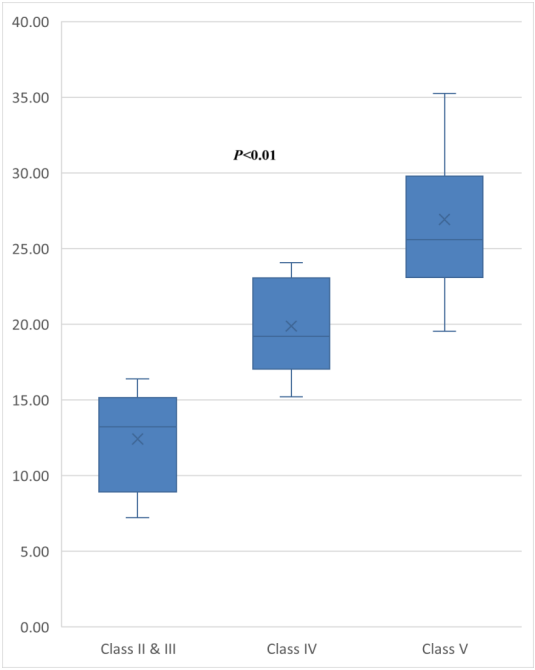

Figure 1 Comparison Urinary neutrophil gelatinase associated lipocalin according to International Society of Nephrology (ISN)/Renal Pathology Society (RPS) (2003) classification of lupus nephritis (n=24)

Class I: Minimal mesangial lupus nephritis; Class II: Mesangial proliferative lupus nephritis; Class III: Focal lupus nephritis; Class IV: Diffuse lupus nephritis; Class V: Membranous lupus nephritis; Class VI: Advanced sclerotic lupus nephritis.

The ethical standards of the Helsinki Declaration were upheld. A structured questionnaire was developed in both English and Bangla. A comprehensive investigation was carried out, and written consent was obtained from each patient. Subsequently, clinically suspected patients of active lupus nephritis were selected for renal biopsy by the nephrologists. Renal tissue was preserved in 10% formalin and sent to the Department of Pathology at BSMMU. The renal tissue was stained using hematoxylin and eosin. A histopathologist prepared the biopsy reports for lupus nephritis.

Measurement of urinary NGAL

A Random urine sample (10 mL) was collected from each participant at the Department of Laboratory

Table 1 Comparison of age and urinary neutrophil gelatinase-associated lipocalin (NGAL) among the three groups[a] of participants

Variables	Group I (n=24)	Group II (n=24)	Group III (n=24)	*P*
Age (years), mean (SD)[b]	30.2 (13.1)	28.0 (7.6)	33.3 (9.8)	0.10
Urinary NGAL in ng/ml, median (IQR)[c]	19.2 (14.5–24.1)	6.5 (4.4–10.0)	3.98 (2.9–5.1)	<0.01

[a]Group I: SLE patients with active lupus nephritis; Group II: SLE patients without lupus nephritis; Group III: Healthy Individuals; [b]SD indicates standard deviation; [c]IQR indicates interquartile range.

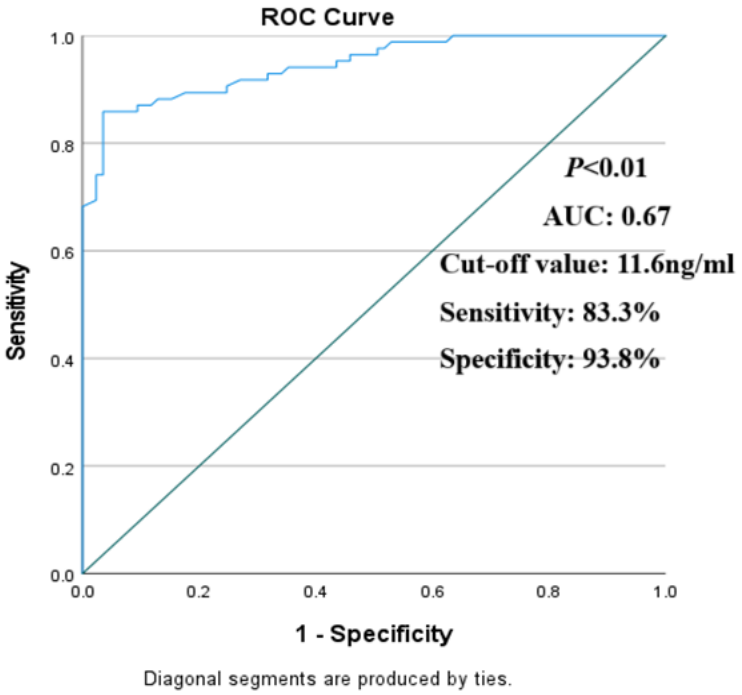

Figure 2 Receiver operating characteristic curve of urinary neutrophil gelatinase-associated lipocalin for detecting active lupus nephritis

Medicine, BSMMU. The samples were then centrifuged at 3000 rpm for 5 minutes. One millilitre of the urine supernatant was placed in an Eppendorf tube and stored at -20°C until the analysis was conducted. The urinary NGAL level was measured using the direct Sandwich-ELISA principle. The optical density of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes.

Statistical analysis

Data were processed and analysed using SPSS version 27. Means, medians, and standard deviations were employed to express quantitative data. The NGAL values among three groups of participants and six classes of active lupus nephritis (I to VI) were compared using ANOVA. Urinary NGAL sensitivity and specificity for identifying biopsy-proven nephritis were calculated, and a receiver operating characteristic (ROC) curve was constructed. A $P<0.05$ was deemed statistically significant.

Results

The mean age of the participants was 30, 28 and 33 years in groups I, II and III, respectively ($P=0.10$). Median of urinary NGAL was 19.2 ng/ml, 6.5 and 3.9 ng/mL in groups I, II and III, respectively ($P<0.01$) (Table 1). There were no classes I and VI in the active SLE patients. There was one patient in class II. Therefore, they were merged with class III before they were compared with classes IV and V. The NGAL showed an increasing level with the classes ($P<0.01$) (Figure 1).

The area under the receiver operating characteristic curve of urinary NGAL was 0.67 (95% confidence interval, 0.28–0.99) ($P<0.01$). The best cut-off point of urinary NGAL level for detecting active lupus nephritis in SLE patients was 11.6 ng/mL. This cut-off point showed a sensitivity of 83.3% and a specificity of 93.8% (Figure 2).

Discussion

We report that urinary NGAL levels remain elevated in patients with active lupus nephritis compared to those without lupus nephritis and healthy controls. These findings provide an opportunity for the early detection of lupus nephritis, helping to prevent its progression to end-stage renal disease. NGAL demonstrates high sensitivity and specificity in predicting lupus nephritis in SLE. It is well known that early detection and treatment of lupus nephritis can enhance long-term survival in SLE [9]. The confirmatory test for lupus nephritis is a renal biopsy, which may pose risks to patients [7]. Therefore, a urinary biomarker like NGAL is easily obtainable without the risk of invasion to the patient. It can even be conducted in primary care settings of low-resource countries such as Bangladesh because it is cheaper [10]. NGAL has high potential for detecting renal injury from various aetiologies [10].

It is well established that SLE is predominantly found in females [15], much like our findings. The current study demonstrated significantly higher levels of NGAL in SLE patients with active lupus nephritis than those without. This result aligns with other studies [14]. We noted the best performance of the NGAL test at cut-off points of 11.6 ng/mL. However, a similar study indicated a cut-off point of 13.7 ng/mL [14]. Their sensitivity (92%) and specificity (75%) were comparable to our results.

The present study has inherent limitations due to a small sample size. We obtained the NGAL measurement at a single point in time. Serial measurements, along with indicators of disease activity, could provide greater insight. Understanding its response to treatment(s) would be beneficial.

Conclusion

Urinary NGAL levels are elevated in patients with SLE and active lupus nephritis compared to patients without active lupus nephritis and healthy individuals. Therefore, it may be considered a potential candidate biomarker for detecting active lupus nephritis in patients with SLE, serving as an adjuvant to renal biopsy.

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Author contributions

Conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: AC, DP, MRC, SF. Drafting the work or reviewing it critically for important intellectual content: AC, DP, SF, MSI. Final approval of the version to be published: DP, SF. Accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: AC.

Conflict of interest

We do not have any conflict of interest.

Data availability statement

We confirm that the data supporting the findings of the study will be shared upon reasonable request.

Supplementary file

None

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