1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by the activation of T and B lymphocytes, production of auto-antibodies and formation of immune complexes causing wide spectrum of tissue and organ damage (Manson and Rahman, 2006).

The overall prevalence of SLE ranges from 1.4 to 21.9% cases per 100,000 people (Ortega et al., 2010). SLE can affect several organs and systems including the joints, skin, brain, heart, lungs, blood vessels, and kidneys.

N-Acetyl-β-d-glucosaminidase (NAG) is a lysosomal brush border enzyme of proximal renal tubular cells that is normally excreted in low amounts in urine. It has been proposed as a valuable marker for renal tubular dysfunction because its relatively large molecular weight(>130kD) precludes filtration by the glomerulus (Bosomworth et al., 1999).

Increased uNAG activity in patients with glomerulonephritis and proteinuria has been suggested as indicating functional changes within the kidney, rather than renal tubular damage (Bosomworth et al., 1999).

Renal biopsies have remained the "gold standard" of assessing lupus nephritis (LN) patients not only at diagnosis but also to assess the efficacy of treatment. But this may not always be feasible due to the invasive nature of the procedure. New laboratory tests are needed to identify renal involvement without renal biopsy (Li et al., 2006).

Hence, it is essential to identify noninvasive new biomarkers that are able to predict renal flares/relapses as well as reflect the severity of LN activity. This biomarkers could be followed serially and may enable timely institution of inflammatory injury in the kidney.

2 Rationale of the Study

The conventional laboratory markers such as serum complement levels, double-stranded DNA antibodies and serum creatinine are unreliable indicators of LN as they lack both sensitivity and specificity for prediction of active or relapsing LN.

Other laboratory tests such as proteinuria and urinary sediments are also non-specific markers. Renal biopsy is the gold standard for diagnosis of LN. But repeated biopsies are not always practical in real life practice as it is an invasive procedue with complications.

NAG is an enzyme of hydrolase class that is abundant in the kidney, predominantly in the lysosymes of proximal tubular cells. It is physiologically excreted in low amounts in urine as a consequence of the normal exocytosis process (Price, 1982).

The increased excretion of NAG is thought to be a specific marker of functional tubular impairment in many renal pathologies.

The aim of this study is to assess Urinary N-acetyl-beta-D-glucosaminidase as a marker of tubular dysfunction in lupus nephritis activity.

3. Research hypothesis

Urinary N-acetyl-beta-D-glucosaminidase is a marker for the diagnosis of activity of lupus nephritis.

4. Objectives

General objective:

To evaluate the role of urinary N-acetyl-beta-D-glutaminadese for the diagnosis of activity of lupus nephritis

Specific objectives:

- To measure the level of urinary N-acetyl-beta-D-glucosaminidase in patients with active and inactive lupus nephritis.
- To determine the sensitivity and specificity of urinary N-acetyl-beta-D-glucosaminidase for the diagnosis of activity of lupus nephritis
- To correlate urinary N-acetyl-beta-D-glucosaminidase with proteinuria, and SLEDAI-2K (renal).

6. Materials & Method

6.1 Study design: It was a cross sectional study.

6.2 Place of Study: This study was conducted in the Department of Nephrology, Dhaka Medical College Hospial, Dhaka.

6.3 Duration of study: This study was conducted from January 2017 to December 2017.

6.5 Study population: Diagnosed lupus nephritis patients (active and inactive) of indoor and outdoor of Dhaka Medical College hospital.

6.6 Sample size (n):

The sample size was determined by following the formula

$$n = \frac{z^2 \times \sigma^2}{d^2}$$

Where,

n = the desired sample size

z = the standard normal deviate, usually set at 1.96 at 5% level, which corresponds to 95% confidence level.

σ=4.64 (SD of uNAG in LN [Mohsen et al., 2012]),

d is the allowable error, which is considered 20.0% of mean value of uNAG in LN (0.20×6.54=1.308)[Mohsen et al., 2012]

Putting the values in the above equation the sample size n is estimates as

$$\frac{(1.96)^2 \times (4.64)^2}{(1.308)^2} = 48.342$$

n=48

So, the sample size was 48.

Finally, 60 samples were evaluated

6.7 Sampling technique:

Purposive sampling technique was used as per inclusion and exclusion criteria.

Selection criteria:

Inclusion criteria:

1. SLE patients with biopsy proven lupus nephritis.
2. Adult patients of both sexes.

Exclusion criteria:

- Patients with end stage renal disease (ESRD) and who had undergone renal transplantation.
- Pregnant patients.
- Patients with diabetes mellitus, Severe heart, lung, liver disease, urinary tract infection, chronic infection, e.g. tuberculosis, other immunological or inflammatory disorders, e.g. RA, vasculitis.
- Patients who are unwilling to participate in the study.

6.8 Variables:

Demographic and clinical variables:

Age of the patient

Gender

Weight (kg)

SBP (mmHg)

DBP (mmHg)

Laboratory variables:

- Urine R/M/E
- Serum creatinine
- Calculation of eGFR by MDRD equation.
- 24 hours UTP
- S Albumin
- Complete blood count
- Serum C3
- Serum C4
- Anti dsDNA Ab titres
- Urinary NAG level
- SLEDAI-2K(renal)

6.9 Research Instruments: A pre-tested data collection sheet.

6.10 Procedures:

All the patients were recruited as per inclusion and exclusion criteria. All patients were subjected to full history taking, with special emphasis on disease activity in SLE patients measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Gladman et al., 2000).Thorough clinical examination was performed for all patients.

Laboratory tests were carried out in the form of urine analysis, serum creatinine, complete blood count, 24 hours UTP, anti-ds DNA Ab titre and complement 3 and 4 levels. Urinary N-acetyl-beta-D-glutaminadase was determined using an enzyme-linked immunosorbent assay (ELISA) kit (15) of R&D Systems. Assessment of disease activity of SLE (renal) was carried out by the SLEDAI-2K(renal). Renal histopathology was classified according to the World Health Organization criteria for LN.

6.11 Data collection:

A questionnaire was prepared considering key variables like demographic data, clinical presentation, clinical findings, predisposing factors, investigations were collected which was verified by the guide and the data was collected by the researcher himself (Appendix-I). Informed written consent was taken from each patient (Appendix II).

6.12 Statistical analysis:

Statistical analysis of the study was done by the Statistical Package for Social Science (SPSS-22). The results were presented in tables, figures and diagrams. Categorical data were presented as frequency & percentage and numerical data as mean & standard deviation.

Confidence interval was considered at 95% level. Receiver-operating characteristics (ROC) analysis was used to calculate the area under curve (AUC) for uNAG and to find out the best cut-off value for identifying lupus nephritis activity. uNAG was compared with serum C3, C4 and anti dsDNA Ab titres. A p value of < 0.05 was considered statistically significant.

Operational definition:

Active LN : defined by the presence of one or more of the following criteria:

I. Proteinuria with or without any of the following features [Kong N et al., (2011)].

a) Presence of haematuria and/or red cell casts.

Proteinuria will be measured as 24 hours UTP and positive if the value is >500 mg/24 hours

II. Renal SLEDAI score ≥ 4 (Gladman et al., 2000).

Inactive LN: defined by the presence of one or more of the following criteria:

I. Proteinuria (24 hours UTP) <500 mg/ 24 hours with/ without any of the following features:

a) Inactive urine sediments (<5 red cells/HPF and no red cell casts and no leucocyturia (<5 white cells/HPF)

II. Renal SLEDAI score 0 or <4

6.13 Ethical implication:

Prior to commencement of this study, the research protocol was approved by the “Ethical Committee” of DMCH, Dhaka. All the patients included in this study were informed about the nature, risk and benefit of the study. Proper permission was taken from the department and institution concerned for this study.

7. Results and observations:

This cross sectional study was conducted in the Department of Nephrology, Dhaka Medical College, Dhaka over a period of one year from January 2017 – December 2017 to evaluate the role of urinary N-acetyl-beta-D-glutaminadese for the diagnosis of activity of lupus nephritis. The results are as follows:

	Lupus nephritis		Total	p value
	Active	Inactive		
Age (years)				
≤20	9 (31.0)	7 (22.6)	16 (26.7)	
21 - 30	13 (44.8)	13 (41.9)	26 (43.3)	
31 - 40	5 (17.2)	6 (19.4)	11 (18.3)	
>40	2 (6.9)	5 (16.1)	7 (11.7)	
Total	29 (100.0)	31 (100.0)	60 (100%)	
Mean SD	25.40 ± 8.07	30.13 ± 10.81	27.67 ± 9.75	0.060
Gender				
Male	0 (0.0)	4 (12.9)	4 (6.7)	0.113
Female	29 (100.0)	27 (87.1)	56 (93.3)	

Table II: General examination findings of the patients (n=60)

General examination	Lupus nephritis		p value
	Active	Inactive	
Anaemia	25 (86.2)	2 (6.5)	<0.001
Edema	19 (65.5)	0 (0.0)	<0.001

Table II shows general examination findings of the patients. Anaemia and edema was observed significantly higher in active than that of inactive lupus nephritis.

Table III: Clinical findings of the patients (n=60)

Clinical findings	Lupus nephritis		p value
	Active	Inactive	
Systolic BP	142.83 ± 17.89	121.17 ± 14.48	<0.001
Diastolic BP	84.67 ± 8.60	73.00 ± 6.51	<0.001
BMI	26.01 ± 3.00	24.71 ± 3.47	0.126

Table III shows clinical findings of the lupus nephritis patients. Systolic and diastolic blood pressure was significantly higher in active lupus nephritis than that of inactive lupus nephritis patients. Though BMI was higher in active LN than inactive LN, no significant difference was found between two groups.

Table IV: Laboratory findings of the patients (n=60)

Laboratory findings	Lupus nephritis		p value
	Active	Inactive	
Hb	9.34 ± 1.54	10.72 ± 0.96	<0.001
RBC	3.81 ± 0.61	6.03 ± 7.02	0.096
WBC	7.45 ± 2.75	13.98 ± 19.65	0.077
Platelet	197.97 ± 105.70	222.07 ± 62.68	0.289
ESR	49.92 ± 29.18	22.25 ± 16.84	<0.001
Serum creatinine	1.26 ± 0.68	0.97 ± 0.35	0.038
Proteinurea	2.43 ± 1.05	0.34 ± 0.41	<0.001
Serum C_3	0.79 ± 0.38	1.21 ± 0.26	<0.001
Serum C_4	0.21 ± 0.25	0.20 ± 0.12	0.755
Anti ds DNA Ab titre	103.00 ± 66.64	54.23 ± 78.16	0.012
uNAG	104.58 ± 32.76	47.25 ± 14.73	<0.001

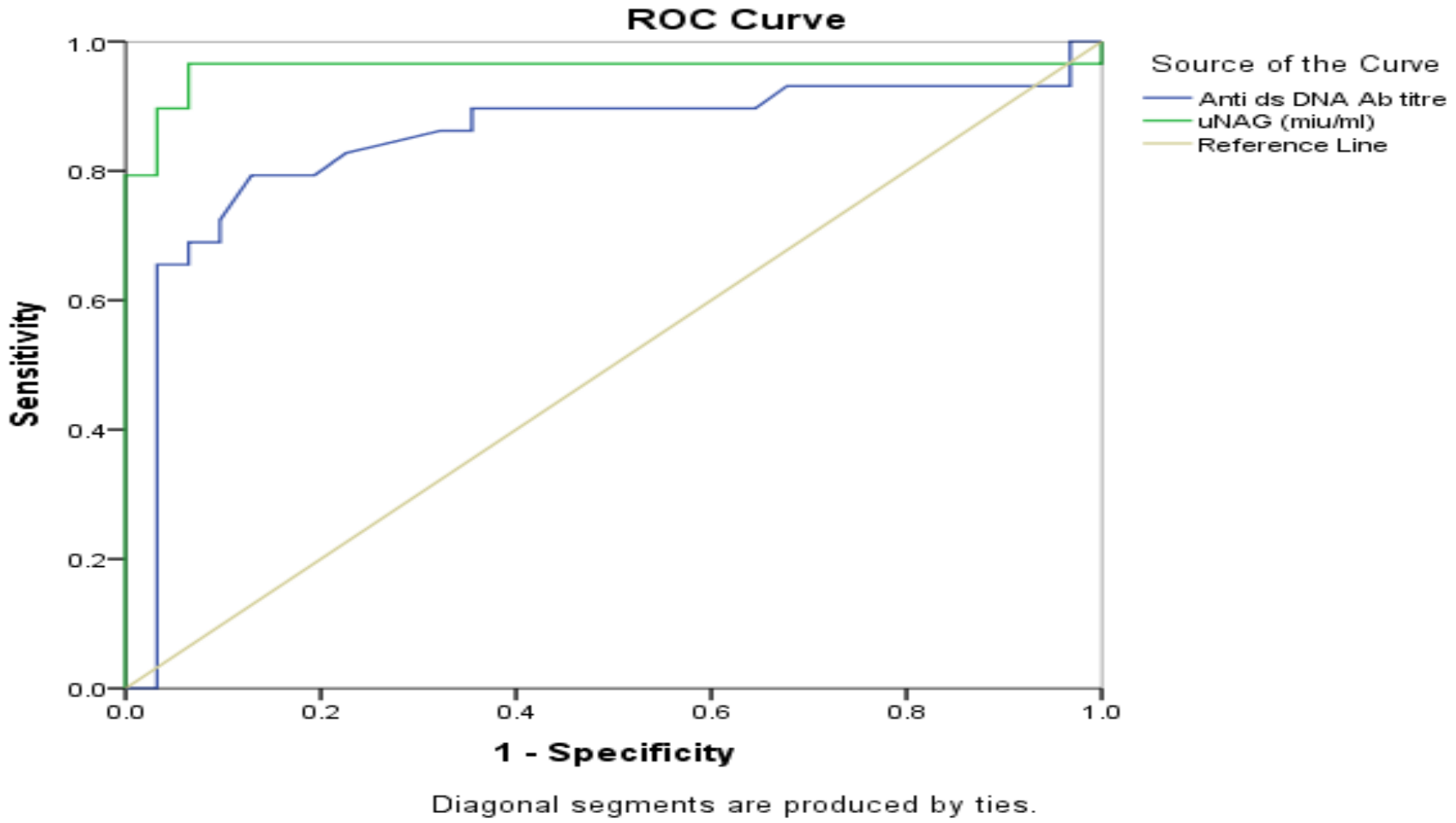

Figure 1: ROC curve of uNAG and Anti ds DNA Ab titre in diagnosis of lupus nephritis activity. Area under curve (AUC) of uNAG=0.958 and Anti ds DNA Ab titre = 0.847.

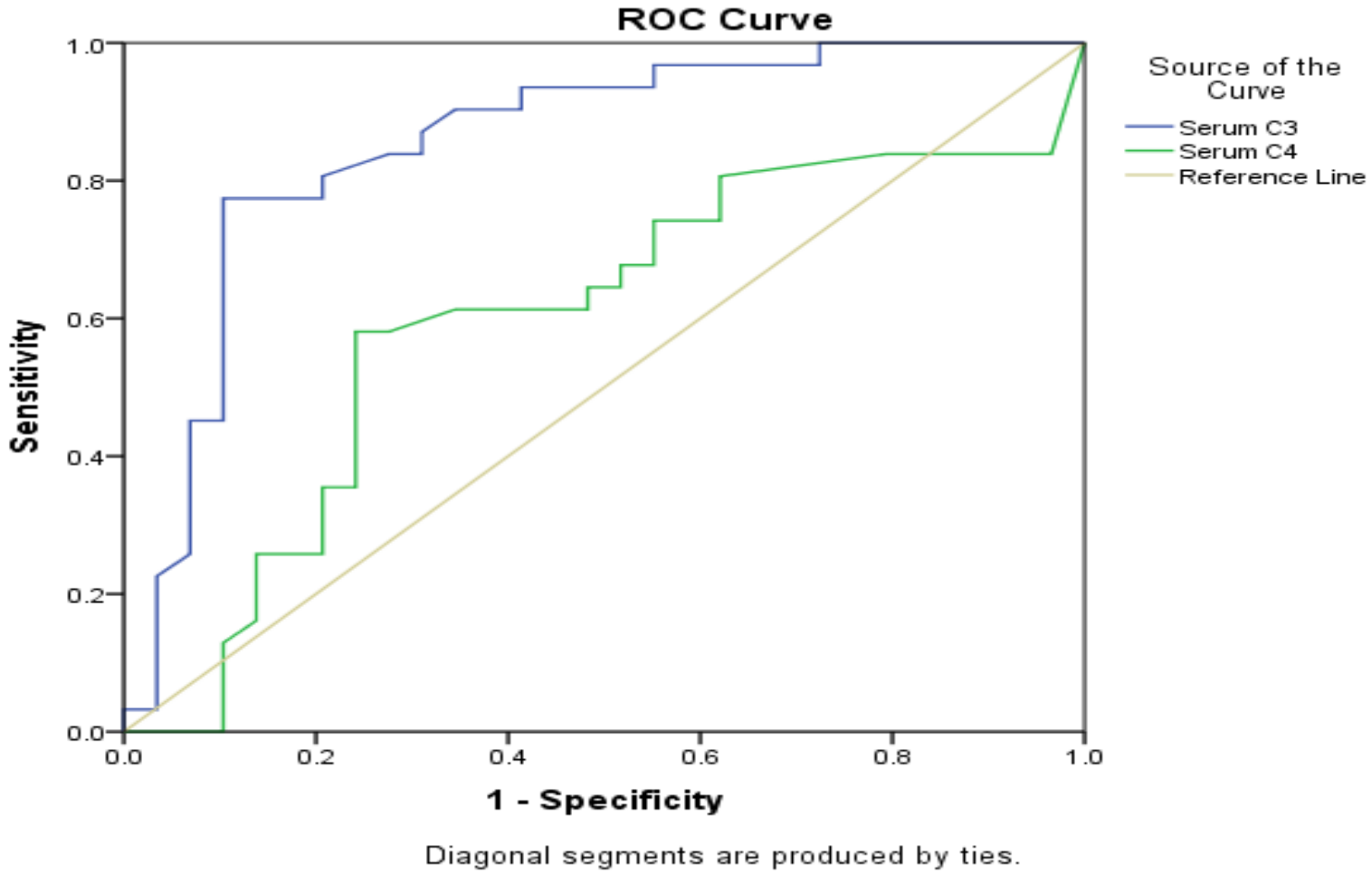

Figure 2: ROC curve of serum C_3 and serum C_4 in diagnosis of lupus nephritis activity. Area under curve (AUC) of serum C_4 =0.596 and serum C_3 =0.855

Table V: Sensitivity and specificity of uNAG at different cutoff value in diagnosis of lupus nephritis (n=60)

uNAG	Sensitivity	Specificity
61	0.966	0.903
62	0.966	0.935
65	0.931	0.935
68	0.897	0.935
70	0.897	0.968
71	0.862	0.968

Table VI: Distribution of lupus nephritis patients by uNAG (n=60)

uNAG	Lupus nephritis		Total
	Active	Inactive	
≥62	28 [TP]	2 [FP]	30
<62	1 [FN]	29 [TN]	30
Total	29	31	60

Table VI shows lupus nephritis patients by uNAG. Lupus nephritis activity was diagnosed by SLEDAI 2K (renal). Among 29 active lupus nephritis cases raised uNAG was found in 28 cases and among 31 inactive lupus nephritis cases raised uNAG was found in 2 cases.

Table VII: Validity test of uNAG in diagnosis of lupus nephritis activity (n=60)

Statistics	Value	Low 95% CI	High 95% CI
Kappa	0.900		
Accuracy	0.950	0.841	0.982
Sensitivity	0.966	0.853	0.988
Specificity	0.935	0.830	0.966
Positive Predictive Value (PPV)	0.933	0.825	0.965
Negative Predictive Value (NPV)	0.967	0.858	0.998

Table VII shows validity parameters of uNAG in diagnosis of lupus nephritis activity. uNAG showed very good agreement in diagnosis of lupus nephritis activity according to Kappa statistics. uNAG in diagnosis of lupus nephritis activity showed accuracy, sensitivity, specificity, PPV and NPV were 0.950, 0.966, 0.935, 0.933 and 0.967 respectively.

Table VIII: Correlation of uNAG with SLEDAI-2K (renal) and proteinuria of the patients (n=60)

	r value	p value
SLEDAI-2K (renal)	0.656	<0.001
Proteinuria	0.240	0.065
Serum C3	-0.556	<0.001
Anti ds DNA Ab titre	0.248	0.056

Above table shows uNAG had positive correlation with SLEDAI-2K (significantly) and proteinuria. uNAG had negative correlation with serum C_3.

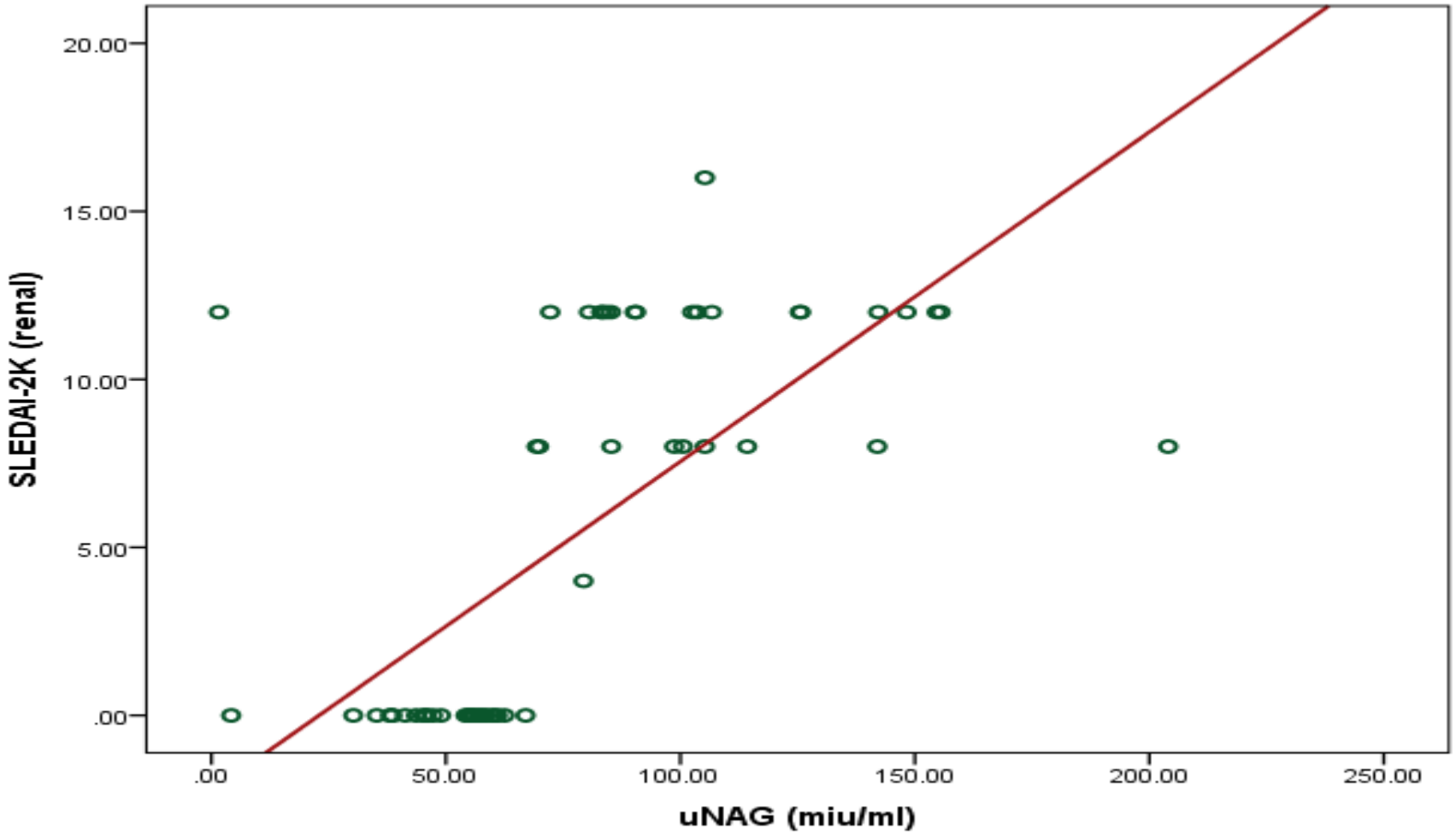

Figure 5: Correlation of uNAG with SLEDAI-2K (renal) in the study subjects

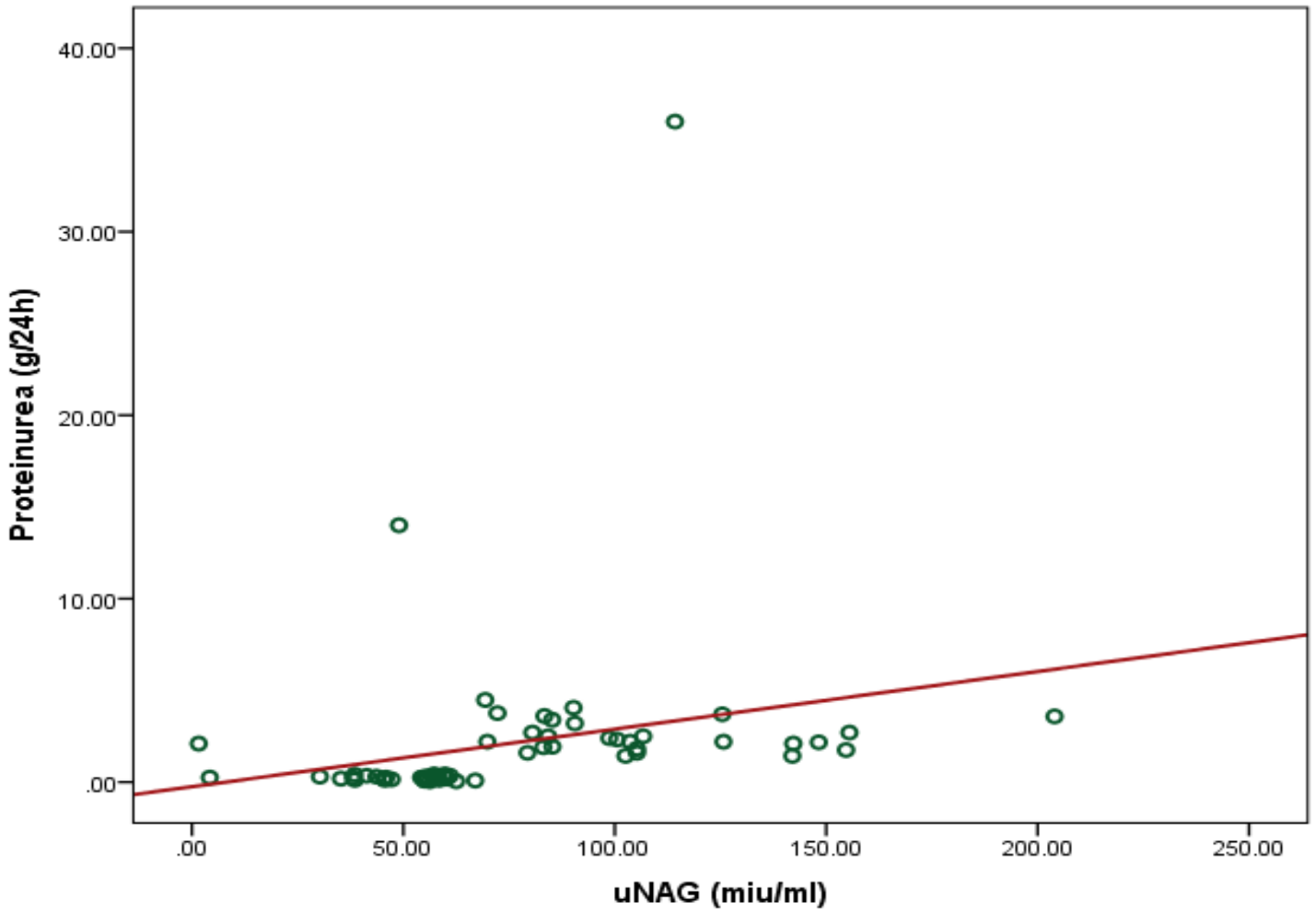

Figure 6: Correlation of uNAG with proteinuria in the study subjects

8. Discussion

Adult 60 biopsy proven lupus nephritis were enrolled in this study. The present study findings were discussed and compared with previously published relevant studies.

In this study, mean age of the lupus nephritis patients in active and inactive groups was 25.40 ± 8.07 years and 30.13 ± 10.81 years respectively.

There was no significant difference in age between active and inactive lupus nephritis patients. Similar finding was observed in the study of Mohsen et al. (2012).

Mean age of the lupus nephritis patients was 27.67 ± 9.75 years and female to male ratio was 14:1. Vozmediano et al. (2012) in their study found that mean age of the LN patients was 34.3 ± 13.6 years and female to male ratio was 9:1.

In this study it was observed that there was a high prevalence of anaemia among the active LN patients, about 86.2% of all active patients had anaemia Michalis et al (2000) found 132 cases of anaemia (122 women, 10 men) from a total of 345 screened SLE patients and the identified cases of anaemia were ACD (37%), IDA (35.6%), AHA (14.4%) and other causes (12.9%).

Edema was observed significantly higher in active than that of inactive lupus nephritis patients in this study. In agreement with this study, Nezhad and Sepaskhah (2008) found most frequent presenting feature are edema (61.1%), arthalgia and hypertension. Loss of protein, salt and water retention is the most common causes.

Systolic and diastolic blood pressure was significantly higher in active lupus nephritis than that of inactive lupus nephritis patients in this study. Naiker et al. (1997) found 38.0% hypertensive cases at the onset of clinical lupus nephritis.

In this study Hb%, RBC, WBC, platelet count were lower in active LN than that of inactive LN but no significant difference was observed between two groups. Lopes (2017) found cytopenia (83.3%), anaemia (56.1%), leukopenia (28.9%) and lymphopenia (76%).

In this study, serum C_3 was significantly low in active LN but C_4 was almost similar in both active and inactive lupus nephritis. Mohsen et al. (2012) found serum C3 less in active LN. Levo and Pick (1974) found that C3 level was more sensitive index of disease activity than those of C_4. Ricker et al. (1991) describes serum C3 level are diagnostically more sensitive and specific for systemic lupus erythromatosus activity than serum C_4 level.

Serum creatinine was higher in active lupus nephritis than that of inactive lupus nephritis in this study. Mohsen et al. (2012) found serum creatinine more in active lupus.

In this study proeinuria was significantly higher in active LN but in inactive LN it was significantly lower (<500 mg/24 hours). Satirapoj et al. (2015) found class IV or V had massive proteinuria with lower Serum albumin level than those of patients with class II.

Mean anti ds DNA ab titre in this study was 103.00±66.64 in active LN and 54.23±78.16 inactive LN which mean that high titre of anti ds DNA ab is present in active LN than that of inactive LN. Though showed higher titre but not statistically significant. Similar findings were observed by Mohsen et al. (2012) and Narayan et al. (2000).

Increased activity of urinary N acetyl beta-D glucosaminadase (NAG) can be used as an early indicator of damage tubular epithelium. In this study uNAG were significantly higher in active cases 104.558±32.76 than inactive cases 47.25±14.43. American Society for cell biology (ASCB) demonstrated the normal value of uNAG was 5-40 mIU/ml.

Marks et al. (2005) found elevated urinary NAG (p=0.001) in lupus nephritis patients. The level of urinary NAG was found significantly higher in lupus nephritis group than that of control group (Shan et al., 2013).These results suggest that the determination of urinary NAG activity may be a useful supplement to the routine biochemical analysis performed on the urine in cases of SLE.

In this study, uNAG had positive significant correlation with SLEDAI-2K and proteinuria. Mohsen et al. (2012) found positive correlation of uNAG with proteinuria but they did not find any correlation with SLEDAI.

There was a strong positive correlation between proteinuria and urinary NAG activity ($p<0.001$, $r=0.759$) (Erdener et al., 2005). uNAG had negative correlation with serum C_3 in this study. Mohsen et al. (2012) did not find any correlation with serum C_3.

Area under curve (AUC) of uNAG was 0.958, serum C_3 was 0.855, Anti ds DNA Ab titre was 0.847 and serum C_4 was 0.596 in diagnosis of lupus nephritis activity. According to this study result, uNAG is better than serum C_3, Anti ds DNA Ab titre and serum C_4 in diagnosis of lupus nephritis activity. The area under the curve (AUC) was 0.74 for the C_3, and 0.65 for C_4 (Birmingham et al., 2012).

uNAG showed very good agreement in diagnosis of lupus nephritis activity according to Kappa statistics. uNAG in diagnosis of lupus nephritis activity showed accuracy, sensitivity, specificity, PPV and NPV were 0.950, 0.966, 0.935, 0.933 and 0.967 respectively.

10. Limitations

There are some limitations in this study. Some are mentioned below:

1. It was a single centred study.
2. Sample size was not reflecting the whole country scenario.

11. Recommendations

1. Further large scale study should be carried out.