Estimation of S100 calcium binding protein A8/ calgranulin A (S100A8) level in Systemic Lupus Erythematosus patients (nephritis patients)

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Abstract. Systemic Lupus Erythematosus (SLE) is a multifactorial autoimmune disease, in which genetic and environmental factors interact to determine susceptibility and phenotype. The aim of the current study were to detect the analytic estimation of serum S100 calcium binding protein A8/ calgranulin A (S100A8) level in Systemic Lupus Erythematosus patients (nephritis patients). Methods: This investigation was conducted at Najaf public laboratories and Biological Therapy center in Marjan Teaching Hospital in Babylon, Iraq. During the period from October, 2022 till May, 2023. This investigation was included 120 subjects women divided into two groups 90 patients group (all patients 90 has ANA positive, 60 only from these women has positive anti-double stranded antibody suffering from SLE) that age ranges from 20-39 years and control group was composed of 30 female healthy persons. Parameters that estimate in this investigation include: urea concentration and S100 calcium binding protein A8/ calgranulin A (S100A8) estimate by using enzyme linked immune sorbent assay. As well as studying the relation between this biomarker level, Body Mass Index (BMI), age, and Hb, Leukocyte count and urea. Results: The results showed a significant increase (P-Value< 0.05) in urea concentration, and significant increase (P-Value < 0.05) in level of S100 calcium binding protein A8/ calgranulin A (S100A8) in systemic lupus erythematosus patients as comparison with healthy groups. Enzyme Linked Immune Sorbent Assay measure of serum S100 calcium binding protein A8/ calgranulin A (S100A8) indicated more significant levels for systemic lupus erythematosus patients than healthy controls. Conclusion: The current study concluded that a S100 calcium binding protein A8/ calgranulin A (S100A8) is a prognostic marker and early detection of systemic lupus erythematosus with renal disease associated with some complication.

1 Introduction

Systemic lupus erythematosus (SLE) is an inflammatory, multisystem, heterogeneous autoimmune disorder characterized by a multitude of autoantibody production and immune complex deposition, causing damage to multiple organs. Dysfunction of T and B cells are

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believed to be critical factors involved in the pathogenesis of disease [1]. Renal involvement occurs in 40–70% of all SLE patients and is a major cause of morbidity and hospital admissions [2]. Macrophages and dendritic cells may play a role in the initiation as well as the progression of LN [3]. S100 proteins can act intracellularly and extracellularly. Intracellular S100 isoforms control immune system functions, such as transcription regulation, trafficking activity, intracellular receptors, free radicals scavenger, and cytoskeleton rearrangement, to name a few examples [4]. S100 proteins, in particular, function as cytokines and bind with Receptor for Advanced Glycation Endproducts (RAGE) and Toll-Like Receptor 4 (TLR-4) to activate the pro-inflammatory signaling cascade, thus increasing immune cell recruitment for their proliferation and differentiation [5]. This protein also enhances the expression of MMPs (Matrix metalloproteases) and CAMs (Cell adhesion molecules) required for tissue remodelling and chemotaxis, respectively [6]. In vitro induction of NETs has recently demonstrated functional involvement of the S100 protein group [7]. Macrophages are key players in the role of an active immune system. Bacterial LPS (lipopolysaccharides), also known as lipoglycans and endotoxins, cause macrophage activation. This promotes S100A8 monomer expression by activating the TLR-4 receptor on macrophage surfaces [8].

2 Methods

2.1 Subject population

The study was directed in Najaf public laboratories and Biological Therapy center in Marjan Teaching Hospital in Babylon, Iraq. 120 subjects women were tested by the Antinuclear antibody and anti-double strand antibody test, 60 only from these women has positive anti-double stranded antibody suffering from SLE, the control group was composed of (30) healthy persons. The samples were gathered during the period from October, 2022 till May, 2023.The patients age and control rang (20 – 39) years. Patient and control group numerals were divided into groups by Hb concentration, age, and body mass index.

2.2 Exclusion criteria

All other autoimmune diseases include (rheumatoid arthritis, multiple sclerosis, diabetes, pernicious anemia, graves' disease, hashimota thyroiditis), blood disease, smoking, male with SLE and also other disease that are related to patient were excluded from the study.

2.3 Collection of blood samples

The blood samples were drawn from vein by sterilized synergies with 5 milliliters. The sample put in the two labeled tubes, first group of tubes contains EDTA as anti-coagulants to prevent clotting of blood to be used for hematological studies. The second group of tubes was without anti-coagulant as gel tubes, for blood to be used for preparing serum for following biochemical and biomarkers. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 10 minutes, and then serum was separated and freezing at -80 ºC until time for performed the laboratory analysis for study.

2.4 Body Mass Index (BMI)

Electronic balance and height tool, were employed for account the weight and height, then applied the neutralization below:

BMI = Weight [kg]/Height [m2].

2.5. Measurements of serum urea concentration

The assessment of serum urea concentration were provided by using urea kit (Biolab. France) and measured manually by Spectrophotometer (Milton Roy Company,U.S.A)

2.6 Biomarker measurement

The assessment of serum S100 calcium binding protein A8/ calgranulin A(S100A8) concentration is provided by (sunlong- china) sandwich immunoassay technique (enzyme linked immunosorbent assay-automated microtiter plate), ELISA reader (Bio kit SL1549HU)

2.7 Statistical analysis

For statistical analysis, IBM-SPSS statistics 24 was used to test treatment responses versus controls. T-test and repeated measures ANOVA, and Standard Error were applied to test the variability and the statistical significance of this experiment. In addition,. Treatment effects are considered statistical significant as P value < 0.05. For creation the plots and tables, we used SigmaPlot 9.0 software, and Microsoft excel [9, 10].

3 Results

3.1 Urea concentration

Urea concentration as shown in figure (1) the result indicated the presence of significant increase (P-Value< 0.05) in urea level of systemic lupus erythematosus patients (55.39± 3.78)mg/dl, in comparison with that of control (28.30±1.36) mg/dl.
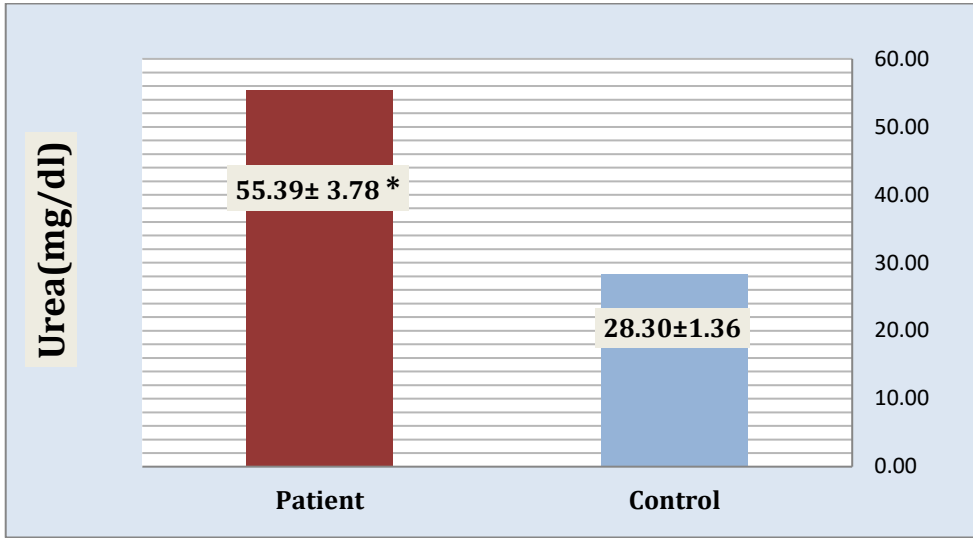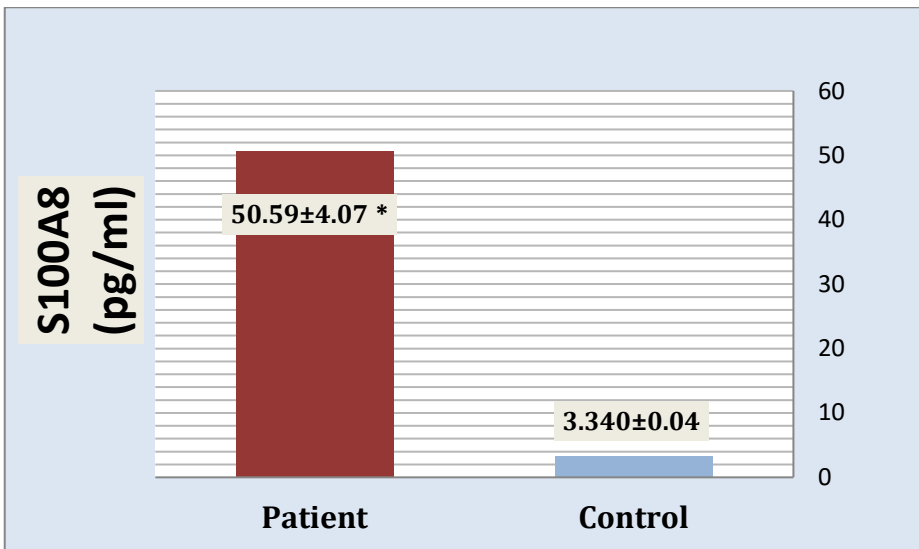
Fig. 1. Urea concentration (mg/dl) in control and systemic lupus erythematosus patients.

* refer to significant differences (P-Value < 0.05)
Patients n=60, control n=30

3.2 S100 calcium binding protein A8/ calgranulin A(S100A8) level in systemic lupus erythematosus patients in comparison with control (healthy)

Figure (2) show the result of S100A8 level in systemic lupus erythematosus patients and control groups. These result indicated a significant increase (P-Value< 0.05) in mean level of S100A8 in patients with systemic lupus erythematosus (50.59±4.07) pg/ml, in comparison with that mean of control group (3.340±0.04) pg/ml.
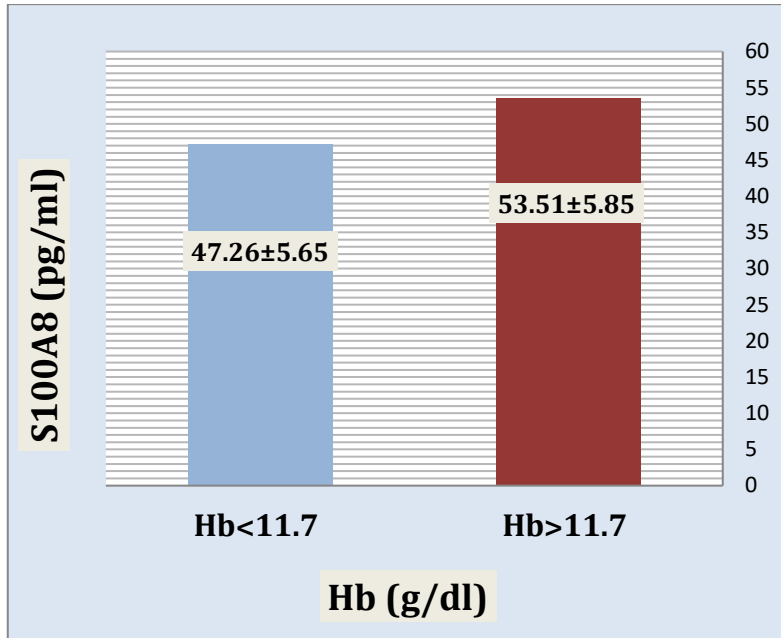

Fig. 2. S100A8 (pg/ml) level in patients with systemic lupus erythematosus and control groups.

* refer to significant differences (P-Value < 0.05)
Patients n=60, control n=30

3.3 Comparison of biomarker in systemic lupus erythematosus patients according to Hemoglobin concentration

Figure (3) shows the results of serum S100A8 levels in Hb group patients of systemic lupus erythematosus, which revealed no significant difference (P-value >0.05) in S100A8 level between group of Hb less than 11.7(53.51±5.85) pg/ml and group of Hb more than 11.7 (47.26±5.65) pg/ml of systemic lupus erythematosus patients .


Fig. 3. S100A8 level in Hb groups less than11.7 and more than 11.7 of patients with systemic lupus erythematosus.

No significant differences (P-Value > 0.05).
Hb<11.7 n=28, Hb>11.7 n=32

3.4 Comparison of biomarker in systemic lupus erythematosus according to ages

Figure (4) explains the results of serum S100A8 level in all age groups of sickle cell anemia, these results mentioned significant increase (p< 0.05) in S100A8 level of age group (20-29) year about (70. 46±4.48) pg/ml comparison with that age group (30-39) (22.78±1.62) pg/ml.
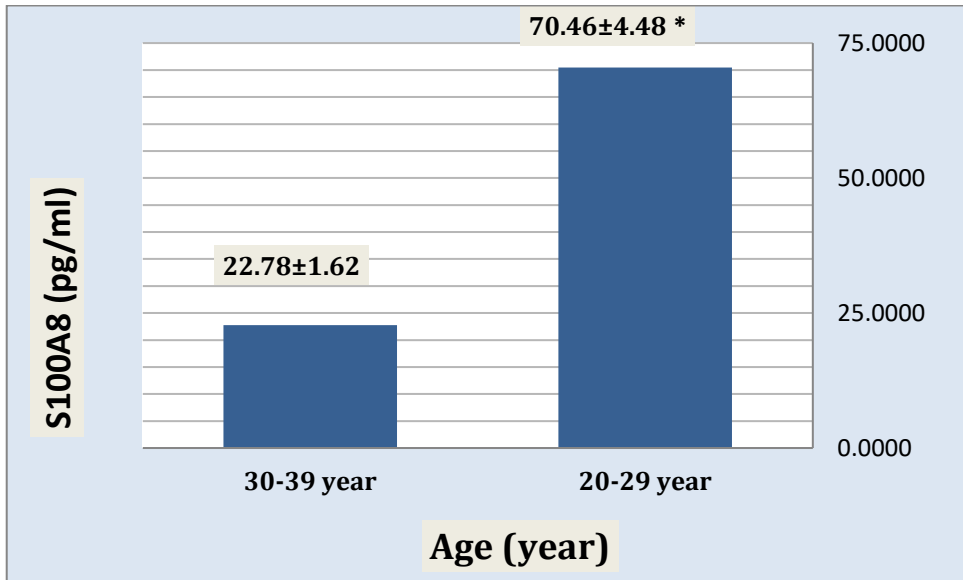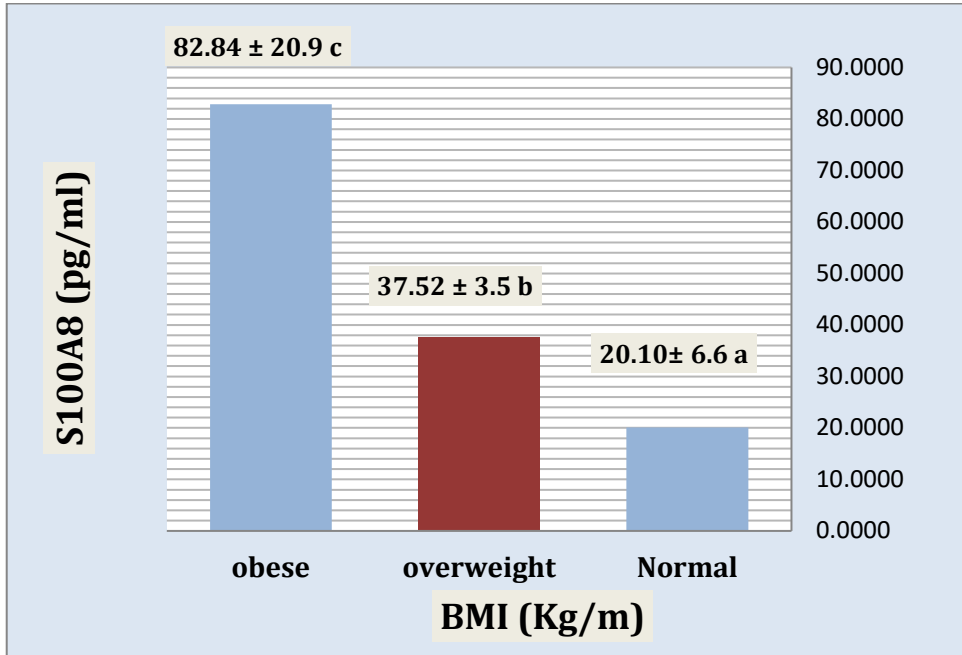
Fig. 4. S100A8 level (pg/ml) in both age groups of patients with systemic lupus erythematosus.

* refer to significant differences (P-Value < 0.05)
age 20-29 n=35, age 30-39 n=25

3.5 Comparison of biomarker in systemic lupus erythematosus patients according to BMI

Figure (5) the statistical analysis revealed the presence of a significant decrease (p< 0.05) in serum S100A8 level of normal weight group (20.10± 6.6) pg/ml, in comparison with яverweight and obese groups (37.52 ± 3.5) pg/ml and (82.84 ± 2 pg/ml respectively.

Fig. 5. S100A8 level (pg/ml) in normal weight, overweight and obese groups of patients with systemic lupus erythematosus.

Different letter refer to significant differences (P-Value < 0.05).
Normal weight n=20 overweight n=15 obese n=25

3.6 Comparison of biomarker in systemic lupus erythematosus patients according to leukocyte count

Figure (6) the statistical analysis revealed to significant increase ($p<0.05$) in serum S100A8 levels of leukocytosis group (59.2925) pg/ml as compared to the normal WBCs count group (34.2100) pg/ml in systemic lupus erythematosus patients.
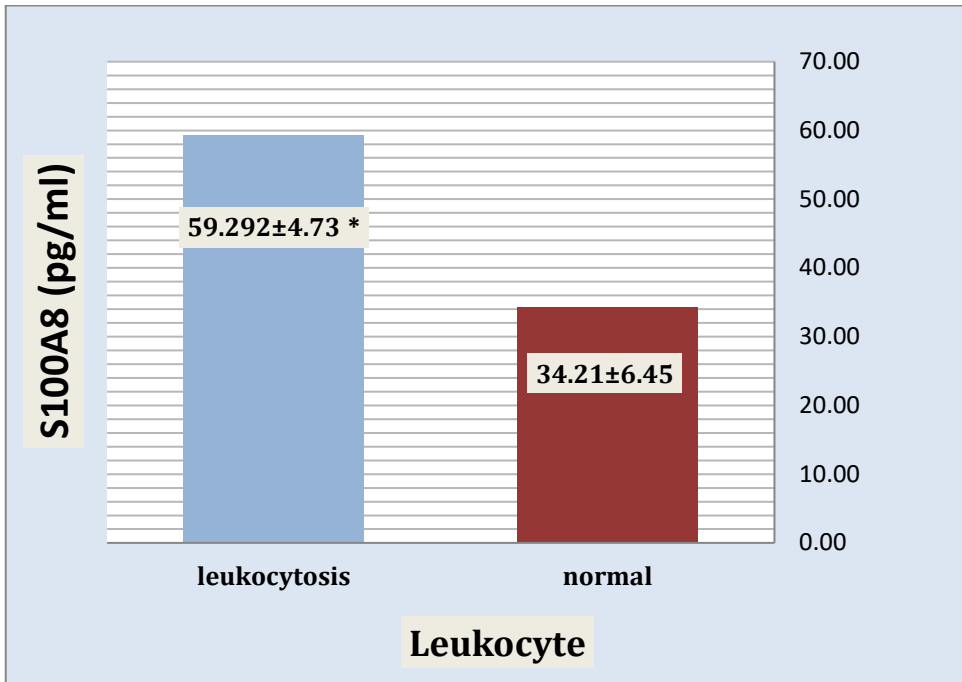
Fig. 6. S100A8 (pg/ml) level in leukocytosis group and normal WBCs count group of systemic lupus erythematosus patients.
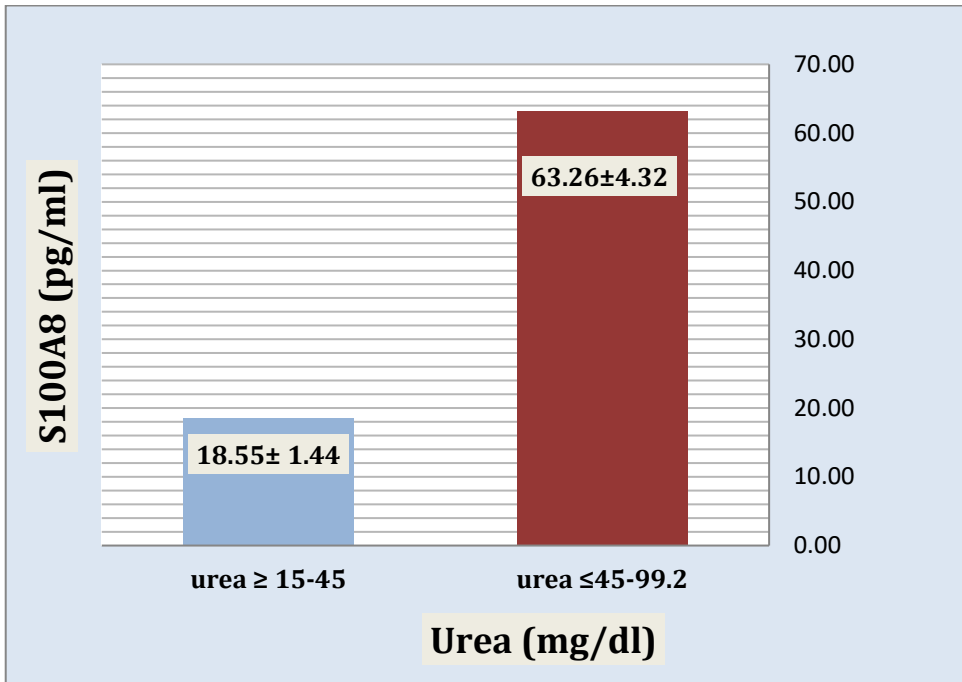
* refer to significant differences (P-Value < 0.05).
Normal WBC s n=20, leukocytosis n=40

3.7 Comparison between biomarkers in systemic lupus erythematosus patients according to urea

Figure (7) the statistical analysis revealed to significant increase(p<0.05) in serum S100A8 levels of urea ≤ 45-99.2 group (63.26±4.32) pg/ml as compared to the the urea ≥ 15-45 group (18.55± 1.44) pg/ml of systemic lupus erythematosus patients.

Fig. 7. S100A8 (pg/ml) level in urea $\leq$ 15-45 and urea $\geq$45-99.2 groups of systemic lupus erythematosus patients.

* refer to significant differences (P-Value < 0.05).
urea $\leq$15-45 n=17 urea $\geq$ 45-99 n=43

4 Discussion

Fig. 1. showed a significant increase in urea concentrations of systemic lupus erythematosus patients in comparison with control group. The current result in accordance with study of Rani *et al*., (2021) who reported that Lupus nephritis (LN) develops as a result of immunological abnormalities [11]. The pathogenesis of LN is a complex process, involving the deposition of autoantibodies in the glomerulus. The glomerular filtration rate (GFR) is widely accepted as the best overall measure of kidney function, enabling a statement of the complex functions of the kidney in a single numeric expression [12]. The endogenous marker of GFR commonly employed is creatinine, but it does not complete the requirements of an ideal marker because apart from being subjected to tubular secretion it is also influenced by the muscle mass and gender of the patient Generally, 74% of lupus patients develop clinically relevant nephritis at some time during the course of their illness [13]. The results of figure Fig. 2. revealed significant significant increase in level of S100A8 in patients with systemic lupus erythematosus in comparison with that of control group. The current study agree with recent study by Kim *et al*., (2022) has been documented that the expression of S100A8 in serum, urine, and saliva is significantly higher in patients with SLE than in HCs and is associated with disease activity markers [14]. Platelets contain S100A8/A9 in membrane-enclosed vesicles, enabling rapid cell surface deposition upon activation. Furthermore, platelet S100A8/A9 protein levels were increased in SLE patients, particularly in those with CVD, and may be a future therapeutic target. [15]. The results of Fig. 3 revealed no significant difference in S100A8 level between group of Hb less than 11.7 and group of Hb more than 11.7 of systemic lupus

erythematosus patients. A study of Schneider *et al.*, (2016) also has been shown that S100a8—whose expression was increased in mutant erythroblasts, monocytes and macrophages—is functionally involved in the erythroid defect caused by the *Rps14* deletion, as addition of recombinant S100a8 was sufficient to induce a differentiation defect in wild-type erythroid cells, and genetic inactivation of *S100a8* expression rescued the erythroid differentiation defect of *Rps14*-haploinsufficient HSCs [16]. S100A8 may be involved in the block of erythroid differentiation that can occur in MDS, as inactivation of S100A8 alone rescued erythroid cells to normal hematopoiesis [17]. The result of Fig. 4 referred to significant increase in S100A8level of age group (20-29) year comparison with that age group (30-39) of systemic lupus erythematosus patients. increase in S100A8 in age (20-29)year may be discuss as a level of its marker appear in low degree of inflammation at earlier stage all biomarkers activate cellular immunity –T-lymphocytes and release of different cytokines also infilteration and migration anumber of leukocyte such as monocytes, macrophage and neutrophils and these associated with disease activity at ages(20-29)years [18] . The results of Fig. 5 indicate the presence of significant decrease in serum S100A8 level of normal weight groups in comparison with overweight and obese group. Activated neutrophils and monocytes are the main sources of extracellular S100A8/A9 and diabetes, dyslipidemia, obesity, and smoking are associated with elevated circulating protein levels. S100A8/A9 seems to be involved in atherogenesis, plaque vulnerability, and post-ischemic myocardial damage [19]. The result of Fig. 6 revealed to significant increase in serum S100A8 levels of leukocytosis group as compared to the normal WBCs count group in systemic lupus erythematosus patients. A study of Gebhardt *et al.*, (2008) also has been shown that by interacting with RAGE, S100 proteins activate NF-κB, inducing the production of pro-inflammatory cytokines leading to the migration of neutrophils, monocytes, and macrophages [20]. The result of Fig. 7 showed significant increase in serum S100A8 levels of urea $\leq$ 45-99.2 group as compared to the the urea $\geq$ 15-45 group of systemic lupus erythematosus patients. Another study on S100A8/9 in glomerulonephritis suggest that increased S100A8/9 expression actually reflects a change in the immune cell phenotype versus a simple increase in the number of infiltrating immune cells within the kidney [21]. Another study has been show that elevated levels of pro-inflammatory S100A12 and heterodimeric S100A8/A9 in serum and urine samples from patients with juvenile-onset SLE and in serum of adult-onset patients with SLE were reported to correlate with renal disease [22, 23, 24].

5 Conclusion

The current study concluded that S100 calcium binding protein A8/ calgranulin A(S100A8) was a prognostic marker for detection of systemic lupus erythematosus with renal disease (LN) associated with some complication.

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