Original Article

Serum Vascular Endothelial Growth Factor (VEGF) as a Biomarker for Disease Activity in Lupus Nephritis

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Abstract

***Background*: Previous studies have shown that serum VEGF levels were elevated in patients with active systemic lupus erythematosus (SLE), especially in those with lupus nephritis (LN). In this case control study, we aimed to compare serum levels of VEGF in SLE patients between LN, non-LN and healthy participants to determine the association between serum VEGF levels and the activity and histological classes of lupus nephritis.**

***Methods*: Blood samples were obtained from 92 SLE patients (46 LN and 46 non-LN) and 26 controls. Data were collected from medical records. Serum VEGF assays were performed by specific, enzyme-linked immunosorbent assay kits (ELISA). Laboratory investigations included urinalysis, urine protein–creatinine ratio, serum creatinine, albumin and VEGF levels. Blood pressure, renal biopsy result and treatment were recorded. LN activity was evaluated using the renal subscale of the British Isles Lupus Assessment Group (rBILAG, 2004). The rBILAG measures blood pressure (diastolic and systolic), urine protein, serum creatinine, calculated glomerular filtration rate (GFR), presence of active urinary sediments and histological evidence of active nephritis.**

***Results*: Serum VEGF was elevated in SLE patients with LN compared with the non-LN group and healthy controls. The levels found were significantly higher in the sera of patients with active nephritis compared to those with quiescent nephritis (P = 0.024). The study did not find a statistically significant relationship between serum VEGF levels and histological classes of LN.**

***Conclusion*: There was no significant difference of serum VEGF level between LN and non-LN SLE groups and between the non-LN group and healthy controls. However, there were increased levels of serum VEGF in the LN group, especially in patients with active nephritis as compared to quiescent nephritis group. This reflects the role of VEGF in the pathogenesis of lupus nephritis, however the clinical potential of this biomarker needs further study.**

Keywords: *serum vascular endothelial growth factor (VEGF), biomarker, systemic lupus erythematosus (SLE), lupus nephritis (LN), renal British Isles Lupus Assessment Group (rBILAG)*

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder with a broad spectrum of clinical presentations (1). Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus (SLE) and is associated with substantial morbidity and mortality. Approximately 35% of adults with SLE have clinical evidence of nephritis at the time of diagnosis, with an estimated total of 50–60% developing nephritis during the first 10 years of disease (2, 3). Tests for proteinuria, urine protein–to–creatinine ratio, creatinine clearance, anti-dsDNA and complement levels currently used in the diagnosis of lupus nephritis are inadequate (4). They lack sensitivity and specificity for differentiating renal activity and damage in lupus nephritis (5). Significant renal damage can occur before the impairment of renal function and initial detection by laboratory parameters. Whether cytokine measurements can assist in distinguishing between flare and chronic damage, detecting renal remission in lupus nephritis, recognising early renal flare in known lupus nephritis, assessing the duration of immunosuppressant use and reducing the need for invasive renal biopsy is the focus of the current research.

Vascular endothelial growth factor (VEGF) is an endothelial-specific growth factor that promotes endothelial cell proliferation, differentiation and survival; mediates endothelium-dependent vasodilatation; induces micro-vascular hyper permeability and participates in interstitial matrix remodelling. In contrast to neoplastic diseases, the significance of angiogenesis and angiogenic factors in the pathogenesis of connective tissue diseases has not been very well investigated. Very few studies have investigated the role of VEGF in adult patients with SLE, and they gave inconsistent results. With previous studies showing abnormalities in production of VEGF and abnormal VEGF levels in SLE patients (6, 7), it would be beneficial to see the relationship between the levels of serum VEGF and involvement of particular organs in disease pathology. This study aimed to compare serum levels of VEGF in SLE patients between lupus nephritis, non-LN SLE and healthy participants and to determine the association between serum VEGF levels, lupus nephritis activity and histological classes of lupus nephritis

Materials and Methods

Patients and Data Collection

We recruited 92 SLE patients who attended rheumatology clinics or who were admitted to medical wards in University Sains Malaysia, Kelantan between January 2012 and October 2012. Patients were divided into those with lupus nephritis and those with non-LN SLE. Healthy controls, matched for age and gender, were recruited from among hospital staff and medical students. Participation in the study was voluntary. Patients were considered eligible if they were 18 to 55 years old and fulfilled four or more of the 1997 American Rheumatism Association (ARA) Revised Criteria for SLE diagnosis (8). Lupus nephritis patients were defined as those who fulfilled the ACR diagnostic criteria for LN (9). Patients with systemic sepsis, malignancy, diabetes mellitus, pregnancy, other autoimmune disease, SLE-like conditions such as drug induced lupus or skin lupus (who did not fulfill the 1997 ARA criteria), urinary tract infection or those who were menstruating while on haemodialysis were excluded. The following clinical and demographic data were retrieved from patient medical records: age, gender, race, duration of SLE, system involved and current immunosuppressive treatment (if the patient had been treated for at least 2 weeks). Lupus nephritis activity was evaluated based on the renal subscale of the British Isles Lupus Assessment Group (rBILAG, 2004). BILAG scoring for each lupus nephritis patient was determined manually from the collection of clinical and laboratory results, which consisted of blood pressure (diastolic and systolic), urine protein, serum creatinine, calculated glomerular filtration rate (GFR) and assessment of histological evidence of active nephritis. The study protocol was explained to all participants and their informed consent was obtained by signature. This study has been reviewed and approved by the Research Ethics Committee (human), University Sains Malaysia.

Lab Measurements

Spot urine (10 mL) was collected for urine protein–creatinine ratio (UPCR), protein dipstick test and full microscopy examination and these were processed in our chemical pathology laboratory. Calculated glomerular filtration rate (eGRF) for lupus nephritis patients was obtained using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

formula. Venous blood (6 mL) was taken from each patient and healthy control, and this was divided into two plain tubes: 2 mL was sent to the chemical pathology laboratory for serum albumin and renal function testing and 4 mL was sent to the immunology laboratory for serum VEGF assay. Blood for VEGF assays was allowed to clot at room temperature for 1 hour, and the tube was centrifuged at 1500 rpm for 15 minutes. The sera obtained were aliquoted into different vials and stored at -25 °C until assayed for VEGF. One allocated, trained laboratory technician randomly coded the serum and carried out the test. The technician was not aware of any laboratory data concerning participants, including their clinical status. Serum VEGF assays were performed using commercially available, specific, enzyme-linked immunosorbent assay kits (Quantikine; R&D systems Inc., Minneapolis, Minnesota, USA) according to the manufacturer's instructions. The most suitable recombinant human cytokine or receptor was used to produce the standard curve in each assay. Both standards and samples were assayed as duplicates and the range of inter-assay variation were given by the manufacturer (6.2–8.8%). These methods have been illustrated in detail elsewhere (6). The sensitivity of the assay was < 9.0 pg/mL. Concentrations of VEGF in samples were verified by discursion from the standard curve.

Statistical Analysis

All data analysis was carried out using Statistical Program for Social Sciences version 19. Age distribution was compared between the three groups using a one-way ANOVA test described by mean and standard deviation. Association among categorical variables (gender and ethnic) was reported as frequency and percentage and measured by Fisher's exact test using the STATA program. Duration of SLE diagnosis was not normally distributed, thus it was analysed using the Mann-Whitney U test and presented in median value and interquartile range.

The level of all studied serum VEGF deviated significantly from the normal distribution according to the Kolmogorov-Smirnov normality test, median value and interquartile ranges were calculated as measures of central tendency. The Mann-Whitney U test was used to compare between two groups while the Kruskall-Wallis test was used to compare three or more groups. This was followed by post hoc comparison using multiple Mann-Whitney U tests with Bonferroni's correction (between groups). Correlations between serum VEGF and numerical variables were performed using Spearman's rank correlation coefficient. Statistical significance was denoted by two-tailed p values lower than 0.05.

Results

Clinical Data among Groups

The characteristics of SLE patients with and without lupus nephritis are shown in Table 1. Participants recruited into the study comprised 92 patients with SLE (46 with LN and 46 non-LN) and 26 healthy controls. No significant differences in age, gender, ethnicity and duration of disease were seen. The mean age of SLE patients with LN was 28.48 ± 9.93 years old, while mean age for the non- LN group was 32.39 ± 11.46 years old. The mean age for healthy controls was 33.19 ± 10.30 years old. In the SLE with LN group, 44 (96%) were females and two (4%) were males, while in the non-LN group, all 46 (100%) patients were females. In the healthy control group, all 26 (100%) were females. Median disease duration for the LN group was 33 months and 44 months for the non-LN group.

There were more SLE patients with LN who were on high-dose steroids (prednisolone > 10 mg/day) compared to those with non-LN SLE (Table 2). Among the LN group, 27 (59%) patients were on high-dose steroid medication, 17 (37%) patients were on low-dose steroid medication (prednisolone ≤ 10 mg/day) and two (4%) patients were not on steroid medication. While in the non-LN group, 11 (24%) patients were on high-dose steroids, 23 (50%) were on low-dose steroids and another 12 (26%) patients were not on steroid therapy. In the SLE with LN group, 13 (28%) patients were on azathioprine and 33 (72%) patients were not on azathioprine. While in the non-LN group, nine (20%) patients were on azathioprine and 37 (80%) patients were not on azathioprine. There were more non-LN patients on hydroxychloroquine compared to patients with LN. In the LN group, 26 (57%) patients were on hydroxychloroquine and 20 (43%) patients were not. In the non-LN group, 38 (83%) patients were on hydroxychloroquine. In the LN group, two (4%) out of 46 patients were on mycophenolate mofetil in while no patients in the non-LN group were on this medication. In the LN group, eight (17%) out of 46 patients received pulse cyclophosphamide therapy due to having active disease while

participating in the study. Among these, one patient was indicated for pneumonitis (low-dose therapy) and seven patients were indicated for nephritis (National Institute of Health (NIH) regime or high-dose therapy). Only one (2%) of the non-LN group received pulse cyclophosphamide therapy for mesenteric vasculitis (low-dose therapy) during the study.

Among the 46 patients with lupus nephritis, 20 (44%) patients had severely active nephritis (rBILAG category A), 14 (30%) patients had moderately active nephritis (category B), five (11%) patients had mildly active nephritis (category C) and seven (15%) patients had inactive disease (category D) as shown in Figure 1. Category D represents stable lupus patients.

Among the 46 patients with lupus nephritis, 16 (35%) had normal renal function (chronic kidney disease (CKD) stage 1), 17 (37%) had mild renal impairment (CKD stage 2), seven (15%) had moderate renal impairment (CKD stage 3), one (2%) had severe renal impairment (CKD stage 4) and five (11%) had established renal failure (CKD stage 5) (Figure 2).

Out of the 46 lupus nephritis patients, 38 had a renal biopsy. Results showed that two patients (5%) were in class I (minimal change glomerunephritis (GN)), four (10%) patients were in class II (mesangial GN), six (16%) patients were in class III (focal segmental GN), 18 (47%) patients were in class IV (diffuse proliferative GN), four (11%) patients were in class V (membranous GN) and four (11%) patients were in class VI (advanced sclerosing GN). The WHO classification for renal biopsies in active LN patients is shown in Figure 3.

Table 1. Demographic characteristics of study population

Variables	SLE with LN (n = 46)	SLE without LN (n = 46)	Healthy control (n = 26)	P-value
Age (mean ± SD)	28.48 (9.93)	32.39 (11.46)	33.19 (10.30)	0.111 [a]
Gender n(%)				
F	44 (96%)	46 (100%)	26 (100%)	0.347[b]
M	2 (4%)	0	0	
Ethnic n(%)				
Malay	43 (93%)	42 (91%)	24 (92%)	0.794[b]
Non-Malay	3 (7%)	4 (9%)	2 (8%)	
SLE duration in month (median/ IqR)	33 (60)	44 (63)		0.255[c]

[a]One-Way ANOVA
[b]Fisher's Exact test
[c]Mann-Whitney test

Table 2. Immunosuppressive treatment received by the SLE patients in study population

Treatment	SLE with LN *n* (%)	Non-renal SLE *n* (%)	*P*-value
Steroid			
No	2 (4%)	12 (26%)	0.001[a]
Low dose	17 (37%)	23 (50%)	
High dose	27 (59)	11 (24%)	
Azathioprine			
No	33 (72%)	37 (80%)	0.328[a]
Yes	13 (28%)	9 (20%)	
Hydroxychloroquine			
No	20 (43%)	8 (17%)	0.007[a]
Yes	26 (57%)	38 (83%)	
Mycophenolate mofetil			
No	44 (96%)	46 (100%)	0.274[b]
Yes	2 (4%)	0	
Cyclophosphamide			
No	38 (83%)	45 (98%)	0.015[b]
Yes	8 (17%)	1 (2%)	

[a]Pearson chi square test
[b]Fisher's exact test

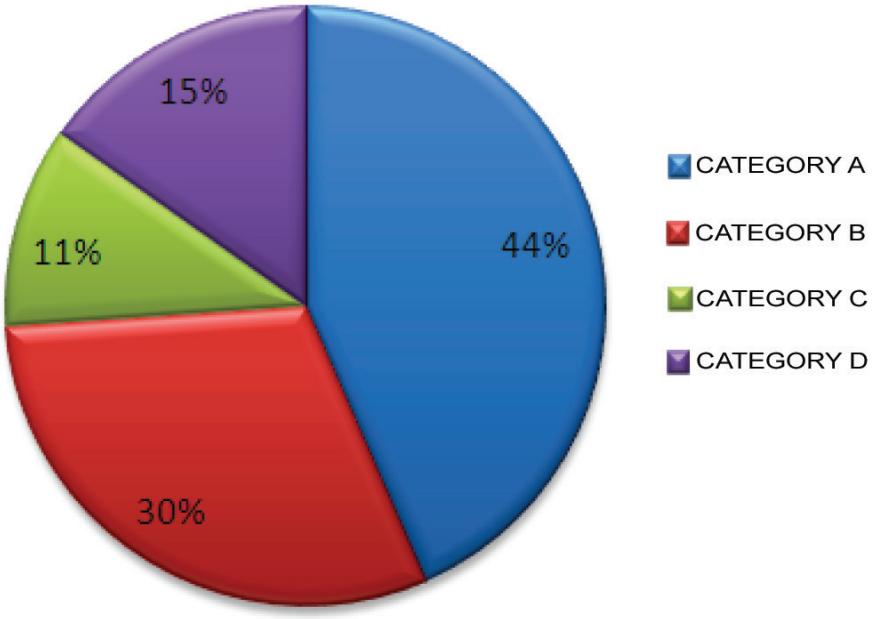

Figure 1. The severity of LN activity among studied LN patients

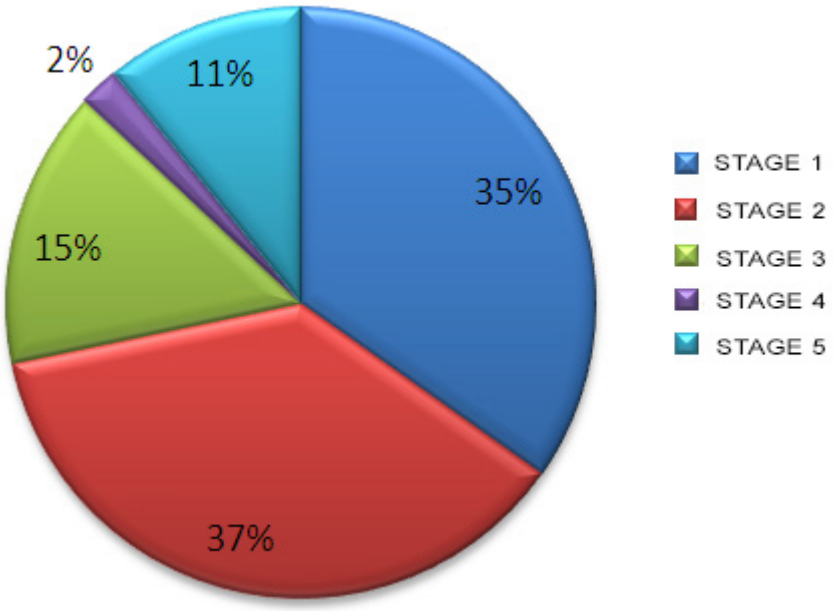

Figure 2. The CKD staging of studied LN patients

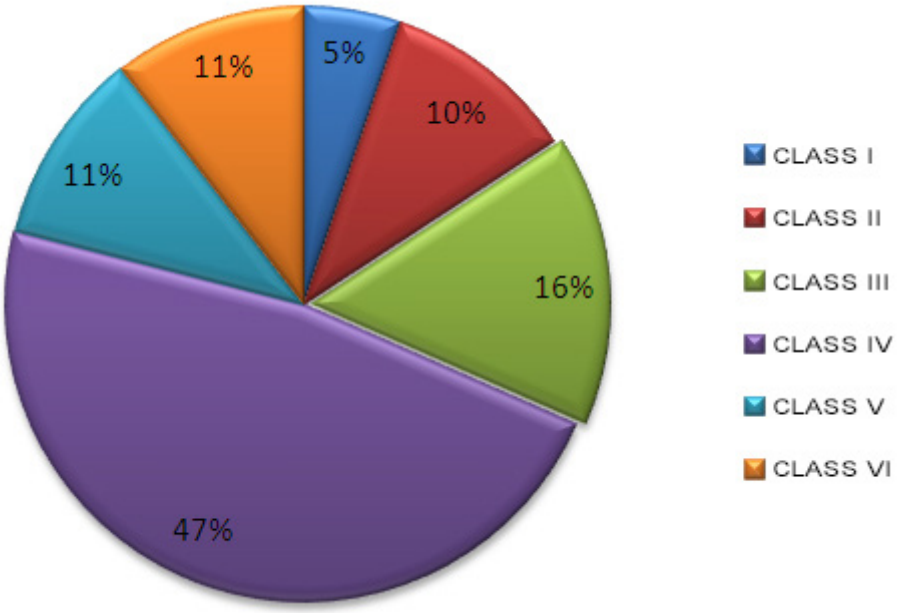

Figure 3. The renal histology (WHO Classification 1995) of 38 LN patients with renal biopsy

Comparison of VEGF levels between SLE patients with LN, without LN and healthy controls

The serum VEGF level for each group is shown in Table 3. Serum VEGF was detected in all groups, and the LN group had the highest median level. Median values were 533.65 pg/mL in the LN group, 436.14 pg/mL in the non-LN group and 343.00 pg/mL in healthy controls. Levels were compared using the Kruskal-Wallis test for non-parametric data, and median VEGF levels of the three groups were significantly different (P = 0.003).

Comparison of VEGF levels among groups according to severity of lupus nephritis (active/quiescent) based on renal BILAG score

This comparison was done using the Kruskal-Wallis test for non-parametric data. The result showed the highest median VEGF level in severe nephritis (category A), which was 658.91 pg/mL. This was followed by moderate nephritis (category B) at 603.20 pg/mL, mild nephritis (category C) at 574.63 pg/mL and inactive renal disease (category D) at 273.68 pg/mL as shown in Table 4. The levels of all four groups were significantly different (P = 0.018).

Comparison between active (categories A and B) and quiescent (categories C and D) nephritis showed a significantly higher concentration of serum VEGF in the sera of patients with active nephritis (P = 0.024) (Table 5a). However, no significant difference was noted between active nephritis and the non-LN group (Table 5b).

Comparison of VEGF levels among groups according to histological type of LN

The comparison of serum VEGF levels between classes of renal histology is shown in Table 6a. The levels were compared using the Kruskal-Wallis test for non-parametric data. The highest median level was seen in class II LN, which was 877.75 pg/mL and the lowest was in class III LN at 273.75 pg/mL. There was no significant difference between groups. The comparison of median serum VEGF levels between proliferative GN (classes III and IV) and non-proliferative GN (all other classes) also demonstrated no significant difference, as shown in Table 6b.

Table 3. Comparison of serum VEGF levels between SLE patients with LN, without LN and healthy control

Study group	*n*	Serum VEGF Median (IqR)	χ^2 stat[a] (df)	*P*-value
Healthy control	26	343.00 (198.21)		
SLE with LN	46	553.65 (398.64)	11.347 (2)	0.003
SLE without LN	46	436.14 (554.10)		

[a] Kruskal-Wallis test

Table 4. Comparison of serum VEGF levels between the severity of lupus nephritis based on renal BILAG score

Renal BILAG	*n*	Serum VEGF Median (IqR)	χ^2 stat[a] (df)	*P*-value
Category A	20	658.91(575.81)		
Category B	14	603.20(528.29)	10.025(3)	0.018
Category C	5	574.63(327.58)		
Category D	7	273.68(118.47)		

[a] Kruskal-Wallis test

Table 5a. Comparison of serum VEGF levels between active and quiescent LN

Median (IqR)				
Nephritis activity	Active (Category A+B) (n = 34)	Quiescent (Category C+D) (n = 12)	Z statistic	P-value
VEGF (pg/mL)	621.08 (534.72)	423.04 (368.25)	-2.251	0.024

Mann-Whitney test

Table 5b. Comparison of serum VEGF levels between active lupus nephritis and non-LN SLE

Median (IqR)				
Variable	Non-LN SLE (Category E) (n = 46)	Active LN (Category A+B) (n = 34)	Z statistic	P-value
VEGF (pg/mL)	436.14 (554.12)	621.08 (534.72)	-1.703	0.089

Mann-Whitney test

Table 6a. Comparison of serum VEGF levels between classes of renal histology based on WHO lupus nephritis classification.

Renal histology	n	Serum VEGF Median (IqR)	χ^2 stat[a] (df)	P-value
Class I	2	428.84 (-)		
Class II	4	877.75 (893.18)		
Class III	6	273.75 (752.08)		
Class IV	18	426.84 (397.74)	4.098 (5)	0.535
Class V	4	641.03 (431.61)		
Class VI	4	641.25 (639.13)		

[a] *Kruskal-Wallis test*

Table 6b. Comparison of serum VEGF levels between proliferative and non-proliferative GN

Median (IqR)				
Renal histology	Proliferative GN (Class III and IV) (n = 24)	Non-proliferative GN (other classes) (n = 14)	Z statistic	P-value
VEGF (pg/mL)	405.15 (409.98)	606.03 (510.24)	-1.271	0.204

Mann-Whitney test

Discussion

This is the first serum VEGF study in the SLE population done in Malaysia so far. In this study, we hypothesised that serum VEGF would be higher in SLE patients with active LN. In our study, a high median level of serum VEGF was observed in the lupus nephritis group and this was significantly higher than that in healthy controls. Moreover, serum VEGF level was highest in patients with active nephritis, although there was no significant difference between the LN and non-LN groups. There was also no significant difference found on this measure between the non-LN group and healthy controls. Two previous studies compared serum VEGF level between LN and non-LN SLE with controls from a normal population (10, 11) and showed a significant difference in serum VEGF between these three groups. Other investigators reported that patients with renal failure had significantly elevated plasma levels and over-expression of VEGF in renal tissue compared with SLE patients with normal renal function (12). It is postulated that this factor might be involved in glomerular endothelial repair (12). Another study observed significantly higher VEGF serum levels in SLE patients with severe and moderate changes in nail fold capillaroscopy compared to those with mild changes and healthy controls (13). This supports the idea that serum VEGF level may be a useful marker of disease activity and internal organ involvement in disease pathology. A more recent study observed that serum VEGF negatively correlates with plasma albumin, which suggests that disease activity may result in deterioration of nutritional conditions leading to an increase in serum VEGF levels (14). VEGF is also required for glomerular hypertrophy and proliferation in response to nephron reduction. Loss of VEGF is associated with glomerulosclerosis and tubulointerstitial fibrosis in the remnant kidney (14). These findings suggest that serum VEGF maybe a useful diagnostic marker of active lupus nephritis.

Our study found a significant increase of median serum VEGF level as severity of nephritis increased, and the highest median VEGF level was seen in the severe nephritis group (rBILAG category A). However, post hoc comparison revealed significant difference only when the severe (rBILAG category A) and moderate (rBILAG category B) nephritis groups were compared with inactive disease. Comparison between active (categories A and B) and quiescent (categories C and D) nephritis showed a significantly higher concentration of serum VEGF in the sera of patients with active nephritis (P = 0.024). These results showed that although serum VEGF is not as sensitive as rBILAG scoring, it can differentiate between active and quiescent nephritis. This finding supports the idea that circulating serum VEGF level correlates with disease activity in LN. It suggests that the higher the nephritis activity, the higher the serum VEGF produced. This is likely to be because VEGF is involved in promoting endothelial cell proliferation, differentiation and survival; mediating endothelium-dependent vasodilatation; inducing microvascular hyperpermeability and participating in interstitial matrix remodelling (15). Previous studies (16) using urinary VEGF reported that urinary VEGF mRNA levels are higher in active Class IV nephritis as compared to Class II nephritis and decrease in response to treatment. However, in our study, which used serum VEGF, we did not find significant differences between WHO stages of LN, especially between proliferative (classes III and IV) and non-proliferative classes. However, VEGF level was low in proliferative GN as compared to non-proliferative GN. Low renal VEGF correlated with peritubular capillary rarefaction, fibrosis and proteinuria. Reduction in renal VEGF could cause endothelial cell injury, which may lead to tubular atrophy, interstitial fibrosis and proteinuria (17). Although this result was not statically significant, this discovery may act as a predictive factor for short-term loss of kidney function. It may be also be an important tool to enable early intervention and treatment before kidney function deteriorates severely. It is possible that serum levels of VEGF increase before the renal content of VEGF decreases, so this may allow precise intervention timing.

Our findings imply that serum VEGF may have potential pathogenic effects in all types of lupus nephritis including mesangial and membranous nephritis, therefore serum VEGF measurement cannot replace kidney biopsy in the diagnostic process to determine the histological type of LN. This finding remains to be confirmed in larger numbers of patients displaying each of these histological subtypes or using other VEGF parameters, such as urinary VEGF, which is a direct product or consequence of kidney inflammation or injury.

When levels of serum VEGF were compared among different activities of lupus nephritis, there was elevation as disease activity increased.

This showed significant results when active nephritis (severe and moderate) was compared with quiescent (mild and inactive) nephritis. However, there was no significant difference in serum VEGF levels when severe, moderate and mild disease activity were compared individually. This result supports the idea that serum VEGF may play a role in lupus nephritis, especially in active nephritis, but that it is not sensitive to variation in nephritis activity as measured by the conventionally used rBILAG index for evaluation of disease activity.

In our study, we did not find a difference in VEGF level between WHO histological classes of LN. This finding showed that serum VEGF could not differentiate between proliferative and non-proliferative types of LN that might influence the specific treatment.

Conclusions

Our study demonstrated that the level of serum VEGF was elevated in SLE patients with LN as compared to those with non-LN SLE and healthy controls. Post-hoc comparison showed a significant elevation of serum VEGF level in LN compared with healthy controls. This study also demonstrated that serum VEGF level was increased in active nephritis group as compared to quiescent nephritis group. However, we failed to find a significant elevation in serum VEGF level between each rBILAG category of activity of LN. It also could not differentiate between proliferative and non-proliferative classes of lupus nephritis. Therefore, it is unlikely at this juncture that serum VEGF level alone can replace conventional clinical parameters, such as the rBILAG score and renal biopsy result used to determine the activity of LN. This reflects the complex immunopathogenesis of LN.

Other VEGF parameters such as urine and renal tissue VEGF should be compared with serum VEGF in future studies as these parameters appear to be more encouraging than a serum biomarker, possibly because they are direct products or consequences of kidney inflammation or injury. Future directions in SLE biomarker research should focus on combination of novel biomarkers with conventional clinical parameters to enhance sensitivity of tests used to predict renal flares and prognosis in LN.

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Conflict of Interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

Authors' Contributions

Conception and design: RI
Analysis and interpretation of the data: RI
Drafting of the article: RI
Critical revision of the article for important intellectual content: WSWG
Final approval of the article: WSWG, NSMA
Provision of study materials: WSWG, NSMA
Statistical expertise: NSMA
Obtaining of funding: WSWG
Administrative, technical, or logistic support: WSWG, NSMA
Collection and assembly of data: RI

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