Triggering Receptor Expressed on Myeloid Cells-1 and Clinical Disease Activity in Patient with SLE Nephritis 24 Months Follow-Up Study

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ABSTRACT

Objective: Is the TREM-1 can be used as a predictor of early disease activity or follow-up of a lupus patient. SLE pathogenesis is a multifactorial disease, the Traditional biomarker used in an everyday assessment of SLE patients such as ANA, Anti-DNA, C3, C4, creatinine clearance, and kidney biopsy. TREM-1 is a member of the immunoglobulin super family with an established role in innate and adaptive immune response and possible role in SLE nephritis.

Methods: thirty-five SLE patients classified according to SLICC Revised Criteria for Diagnosis of SLE 2012 and 20 healthy control group. All patients underwent full clinical and lab assessment and kidney biopsy were taken through the study according to clinical assessment of SLE activity with consent taken. TREM-1 was done for all patient group at an early study and after 24 months of SLE disease activity.

Results: this results showed increased level of TREM-1 early and marked elevation at the end of the study and highly significant difference within the patients and between patient and study group P<0.05. There is a positive correlation between TREM-1 and renal biopsy and anti-DNA, C3, C4 SELENA score

Conclusion: TREM-1 can be used as biomarkers for monitoring and/or predicting SLE disease activity but, needs to be further investigated with large-scale multicenter trials. A combination of novel markers with conventional clinical parameters to enhance the sensitivity and specificity for the early prediction of renal disease/flares and prognosis in patient with lupus nephritis

Key Ward: TREM-1 and early lupus activity.

Introduction

Systemic lupus erythematosus (SLE), is arguably the most serologically and clinically diverse autoimmune disease, with diverse clinical manifestation. SLE is still remaining one of the greater challenges to both physician and investigator. SLE patients have excess production of autoantibodies and immune complexes, excess complement activation, consumption, and insidious onset of inflammation in SLE patients.14

SLE pathogenesis is multifactorial and multistage disease, with various genetic, epigenetic, environmental, and immunoregulatory factors contributing to the susceptibility, onset, progress and prognosis of SLE disease activity. Undoubtedly, no single biomarker will be sufficient ultimately to diagnose, monitor and stratify all patients with SLE. Traditional biomarker used in an everyday assessment of SLE
patients such as ANA, Anti-DNA, C3, C4, creatinine clearance, and biopsy\(^1\).

SLE is a complex autoimmune disease characterized by the loss of tolerance to self-nuclear antigens. Accumulating evidence shows that Toll-like receptors (TLRs), previously proven to be critical for host defense, are implicated in the pathogenesis of autoimmune diseases by recognition of self-molecules. There is evidence for the involvement of TLRs, including TLR2/4, TLR5, TLR3 and TLR7/8/9, in SLE pathogenesis\(^18\).

TREM-1 is a member of the immunoglobulin superfamily with an established role in innate and adaptive immune response. TREM-1 is a recently identified molecule involved in monocyctic activation and inflammatory response. It belongs to a family related to Natural Killer cell receptors and is expressed on neutrophils, mature monocytes, and macrophages. The engagement of TREM-1 synergizes with several Toll-Like Receptors activation and amplifying the inflammatory response to acute or chronic inflammatory conditions\(^6\).

TREM-1 was first characterized in infections, was highly upregulated and has been implicated as an amplifier of inflammation functioning as an important coactivator of the TLR signaling pathways. Although patients with lupus exhibit increased serum levels of soluble TREM-1 (sTREM-1), the role of TREM-1 in SLE remains unknown\(^10\).

Activation of TREM-1 leads to the production of multiple pro-inflammatory cytokines and chemokines, which, can synergize with innate immune stimuli to amplify inflammatory responses. Activation of the TREM-1 pathway has been reported in sepsis, pneumonia, autoimmune disease like SLE, RA, Ulcerative colitis, chronic obstructive pulmonary disease, pancreatitis, gout, and peptic ulcer disease. Thus, implicating that sTREM-1 levels could serve as a biomarker for these diseases\(^3\).

This study was done to determine plasma concentrations of TREM-1 in patients with early and after 24 months of SLE presentation of their disease, and their association with clinical, disease activity, and laboratory parameters of the disease. Is the TREM-1 is one of the biomarkers of lupus activity?

**Patient and Methods**

This study was carried out on 35 SLE patients who were attending the Rheumatology outpatient clinic, from July 2014-2016. All SLE Patients classified according to SLICC Revised Criteria for Diagnosis 13. Clinical and laboratory evidence of renal disease defined as varying combinations of the following: urine protein >0.5 gm/24h, creatinine clearance < 60 ml/min, diastolic blood pressure >90 mmHg and serum creatinine >1.5 mg/dl.

All patients were subjected to thorough history taking and full clinical examination, the clinical disease activity was recorded using the (SELENA SLEDAI disease activity INTRANET scoring assessment) records two times, first time of presentation of the disease diagnosis as early disease activity, a second time after 24 months of disease course as follow-up assessment. Clinical active disease considered when SELENA score more than six (>6)\(^2\).

Ultrasonography (U/S) assessment for both kidneys was done by the aid of a state of art, (GE Ultrasound machine, Logic 9 model), and by using a deep probe (3-5 Mhz). Images were taken while the patients were at supine as well as at lateral positions.

Follow-up of the patient with renal lupus activity underwent renal biopsy for the patient who has proteinuria, biopsy was performed during the course of assessment and management of the disease. Renal biopsy was done by percutaneous puncture ultrasound guided and all renal biopsies were assessed by a pathologist and classified according to International Society of Nephrology (ISN) and Royal Pathology Society (RPS) classification of lupus nephritis 2003 as follow: normal Minimal mesangial LGN (type I), mesangial proliferative LGN (type II), focal proliferative glomerulonephritis (type III), diffuse glomerulonephritis DLGN (type IV), diffuse,
membranous glomerulonephritis (type V), and advanced sclerosing glomerulonephritis (type VI). Laboratory investigations (all laboratory assessment done in same lab area): CBC, ESR Westergren method, ANA and anti-ds-DNA antibodies using the indirect immunofluorescent antibody test, TREM-1, C3 and C4 levels, uric acid, creatinine clearance, estimation of GFR, MDRD, CK-Epi, Cockcroft scale, protein 24 hours in urine, calcium, phosphorus and albumin and total serum protein, as well as routine urine analysis for urinary casts, hematuria and pyuria were measured in early lupus and 24 month after activity.

TREM-1 assay: Quantitative determination of human Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) concentrations in plasma by collecting 3 ml blood thrown into a plain tube (red cover) for measurement of sTREM-1 levels. Serum was collected after centrifugation of the sample for 15 minutes at 1000 x g. The plasma was collected in sterile microtubes and frozen at 

Measurement of GFR

1. Cockcroft–Gault and Modification of Diet in Renal Disease Formulas GFR is accepted as the best overall measure of kidney function. Normal values, which are related to age, sex, and body size, are approximately 130 ml per minute per 1.73 m2 in young men and 120 ml per minute per 1.73 m2 in young women. Mean values decline as person’s age.

2. Estimated GFR by Modification of Diet in Renal Disease (MDRD) Study equation and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)

However, it is important to pay attention to potential inaccuracies due to the non-steady state of serum creatinine, co-morbidities that cause, and the use of medications that interfere with the measurement of serum creatinine. 

Stages of Chronic Kidney Disease

Stage 1 Kidney Disease ( Healthy kidneys or kidney damage with normal or high GFR >90 mL/min; Stage 2 Kidney Disease Kidney damage and mild decrease in GFR LEVEL: 60 to 89 mL/min; Stage 3A & 3B Kidney Disease with Moderate decrease in GFR between 30 to 59 mL/min; Stage 4 Kidney Disease, Severe decrease in GFR between 15 to 29 mL/min and Stage 5 Kidney Disease GFR level: Less than 15 mL/min or on dialysis(kidney failure).

Exclusion Criteria

- Malnutrition patient or patient under age of 16 years or more than 45 years
- All patient has Creatinine >150mmol.
- Pregnancy or hyperthyroidism and any patient with concurrent infection.
- Uncontrolled hypertension or drug-induced hypertension are excluded.
Remission or disease activity less than 6 BILAG scores.
Ethical committee approval of studies done and Informed consent Taken from each patient before a start of the study.

Limitation of the study: Several limitations of this study should be noted. This study was performed on a relatively small sample size with wide variability in clinical and biopsy finding (should be done on the similar histopathologic picture). Also, most of the patient take an antibiotic before start of the study (relation between TREM-1 and infection cannot be confirmed or ruled out in this study). The third point is that the sample of the patient is not same body weight so, creatinine clearance and GFR estimation is not precisely predictable for renal function as the standardized measurement.

Statistical Methods: Statistical analysis was done by using SPSS statistical package for social science ver.21. The qualitative data presented in the form of number and percentage Chi-square test was used to compare between qualitative data. The qualitative data presented in the form of mean, standard deviation and range. Student’s t-test was used to compare two quantitative data groups. Pearson correlation coefficient was done to study the relation between variables. Values of p<0.05 were considered significant p<0.01=highly significant value.

Results
Thirty-five SLE patients were included in this study, with and 20 healthy persons as a control group, demographic data and clinical characteristic in the study group of early and after 24 months follow-up (table 1).
Six patients out of 35 (17.1%) associated with history of bronchial asthma with 4 cases (11.42%) have history of DM with pregnancy and 22.9% was developed postprandial hyperglycemia after the 24 month follow-up, all patient was normal blood pressure at the start of the study, 10 out of 35 (31.42%) of the patient developed hypertension controlled on medication; (most patients was treated with captopril to control proteinuria); follow-up after 24 months showed significant increase in protein in urine.
The patient of this study showed positive ANA in 23 patients 65.70% vs 12 patients 34.30% negative value, Anti-DNA was positive in 100% of cases with titer 255.94±197.60 at the start of assessment and decreased to 125.11±109.44 after 24 months of study.
The patient in this study showed staging of kidney disease 14 cases (35) (40%) stage 1 progressive decrease to 8 (22.9%); stage 2 were (51.4 %) 18 cases (35); grade 3A was (8.6%) in 3 cases (35) deteriorate to 9 cases (25.8%) of cases.
Estimation of GFR (mL/min/1.73m²)in this study was showed 25 cases (71.4%) showed normal GFR at start of study (>130) with GFR progressive decrease in 6 out of 35 (17.2%) between 60-90 and 4 out of 35 cases (11.4%) showed marked decreased on GFR <60 and only one case showed rapid decreased < 30 at the end of the study (2.9%) of the patient (most patients was treated with captopril to control proteinuria); MDRD was showed same results of GFR. There is a significant difference between early and post assessment of GFR estimation 2.67 P<0.01. Also, a highly significant difference between patient and control group P <0.01.
Because the CKD-EPI equation performed better than the MDRD equation, especially at higher GFR, with less bias and greater accuracy; This study showed normal estimation of CKD-Epi in 26 of (35) cases 74.3% progressive decrease to 15 (42.9%) of the case; 14.30% showed mild CKD-Epi estimation 5 of (35) of cases increased to 17 of (35) 48.6% of cases; and 4 of (35) 11.40% moderately deterioration of to two cases 5.7% of the case and only one case (2.9%) showed rapidly deteriorate of CKD-Epi estimation.
Figure 1: showed normality distribution of the study pre-post TREM-1 assessment (A,B)

At the start of the study, creatinine clearance showed normal value in 60% of SLE patient 21 cases (35), progressively decreased to 42.9% 15 cases (35); also, creatinine clearance was low in 40% of SLE patients 14 (35); increased to 57.1% in 20 (35) after 24 months of disease activity. Proteinuria was mild at early study assessment in 21 (35) cases 60% of SLE patients decreased to 12 (35) cases 34.3%; with moderate proteinuria 14(35) 40% increased to 23 (35) 65.7% of SLE patients.

A biopsy was taken from SLE patient showed 8 case type I of (35) 22.90%, 18 cases 51.40% type II minimal changed GN, 14.30% type III focal GN 5 cases out of (35), 4 out of (35) cases 11.40% showed biopsy diffuse proliferative GN. All cases are in active form no sclerosis, atrophic changes in a biopsy. No cases recorded as membranous type GN. TREM-1 has a significant relation between Renal biopsy P<0.05. Also, there was positive relation between biopsy in the study group of the lupus patient showed a significant relation between biopsy and CG-GFR, protein 24hours, creatinine clearance and creatinine in urine Z score was 6.5, 5.2, 5.6, 6.1 respectively and P<0.01. Also, there is a highly significant relation between ANA and Anti DNAs antibodies and biopsy where Z score 5.78, 6.56, P<0.01. But no significant correlation between proteinuria and biopsy as contradictory results P value>0.05.

SELENA score in this study showed 11(35) cases was in remission 31.40% progressive decreased to
2.9% at the start of the study; SELENA disease activity score was 24 (35) cases 68.6% with progressive increased to 97.1% after 24 months of assessment.

Assay of TREM-1 in this study, showed that overall patient in the study group TREM-1 has a Lower Limit of Detection LLD was in 68.57% > 70 pg/ml; it has a significant increased than control group p<0.05. Early assay of the TREM-1 level was 82.22±24.12 in 88.6% of cases vs to 34.3% after 24 months of disease activity. While late assay was 133.31±13.70 in 65.7% has a higher value (more than130pg/ml).

TREM-1 is a highly significantly higher in the patient than control group p<0.01 and a significant within-patient group P<0.05. TREM-1 is a significant correlation with renal pathology, SELENA score, Anti-DNA, complement level; that mean it can be used as an indicator of renal disease activity. But, no correlation between TREM-1 and disease activity index in SLE patient (ESR, WBCs, HB and GN staging in this study) where P>0.05.

TREM-1 is significantly correlated with C3, C4, and Anti-DNA P<0.05, and x2 also, showed a significant relation with C3, C4 and Anti-DNA P<0.05.

In comparison between study group (patient vs control group) and between early late assessment in the patient group (table1) as follow: the results showed no significant difference between patient and control group regarding age, weight, height P>0.05. Albumin was showed no significant difference between patient group but significant between patient and control group P<0.05. While total protein showed no significant difference in study group p>0.05. Proteinuria and protein 24/urine showed a significant difference between study group P<0.01. GFR, CKD-Epi, MDRD showed a significant difference (higher in early than late) within patient groups P<0.05 and patient and study group P<0.01.

### Table (1) clinical and demographic data of the study group

| Variable          | SLE N=35 |             | Control group N=20 |             |
|-------------------|----------|-------------|---------------------|-------------|
|                   | Early 0 Month | After 24 months | P value | P value |
| Age/years         | 40.91±8.58 | 36.22±9.24 | 1.97** |          |
| Height/cm         | 154.94±4.67 | 158.51±9.78 | -0.34** |          |
| Weight/kg         | 65.29±14.85 | 58.86±16.57 | 1.34** | -2.39** |
| Albumin / mmol    | 24.73±7.57 | 24.96±7.66 |          | -0.23 NS |
| Total Protein/mmol| 52.62±11.14 | 52.11±11.38 | 1.26 NS |          |
| Protein 24/urine mg/l | 0.52±0.32 | 1.24±0.98 | 4.15 NS | 0.06±0.32 |
| GFR               | 101.46±4.54 | 135.56±3.04 | 4.76 NS |          |
| CKEPI             | 102.06±4.65 | 141.69±4.78 | 6.34 NS |          |
| MDRD              | 102.86±6.90 | 133.64±23.32 | 3.22 NS |          |
| CREAT mg/dl       | 0.76±0.30 | 0.81±0.32 | 1.97** | 0.91±0.15 |
| Creat/Clear       | 75.86±0.17 | 75.88±17.13 | 2.62 NS | 112.90±18.71 |
| WBCS              | 5.99±1.73 | 6.07±3.49 | 0.13 NS | 7.09±2.92 |
| RBCS              | 4.32±0.58 | 4.00±0.91 | 1.77 NS | 4.76±0.52 |
| HB gm/dl          | 11.79±1.93 | 11.07±2.49 | 1.12 NS | 12.66±1.28 |
| UA/ mmol          | 291.06±120.18 | 340.60±48.96 | 2.67 ** |          |
| TG/ mmol          | 1.41±0.59 | 1.40±0.58 | 0.11 NS | 1.45±0.59 |
| Cholesterol/ mmol | 5.58±0.89 | 5.60±0.86 | 0.57 NS | 4.57±0.86 |
| Calcium/ mmol     | 2.17±0.23 | 2.16±0.22 | -0.51 NS | 2.27±0.22 |
| Phosphorous mmol  | 1.21±0.16 | 1.20±0.15 | -0.55 NS | 1.22±0.16 |
| Alk. Phosp.U/L    | 150.89±157.57 | 163.00±167.1 | 0.45 NS | 112.90±18.71 |
| ANTI DNA IU/ml    | 255.94±197.60 | 125.11±109.4 | -0.67 NS | Not done |
| C3mg/dl           | 0.67±0.30 | 0.71±0.29 | -0.13 NS | Not done |
| C4 mg/dl          | 0.61±0.32 | 0.59±0.35 | -0.24 NS | Not done |
| TREM-1/ pg/ml     | 82.22±24.12 | 133.31±13.70 | 4.34 NS | 27.31±5.88 |
| SLENA-SLEDAI      | 9.66±4.80 | 21.34±8.92 | 6.38 NS | Not done |

GFR, MDRD, CKD-e = mL/min/1.73 m2, p value* = significant p<0.05 **= highly significant P<0.01. NS=Non-significant P>0.05. SELENA-SLEDAI (systemic lupus disease activity index score)
Creatinine and creatinine clearance showed a non-significant difference with patient group P>0.05 and significant between patient vs control group P<0.05. Hematological manifestation and bone panel showed a non-significant difference within patient group P>0.05; while there was a significant difference between patient and control group except for WBCS and alkalinephosphatase where P<0.05. Complement C3, C4 and Anti-DNA showed a non significant difference within patient group P>0.05. SELENA score higher in late than early in study group P<0.05.

Discussion

SLE is multifactorial disease and multiple immune dysregulations. Early detection of nephritis in SLE patient is compulsory for start of medication especially immunosuppressive agent or biological therapy. Also, detection of renal activity through the course of the disease may adjust patients dose of steroid or increase or decrease immunosuppressive agent to reduce renal damage.

Renal biopsy is still one of the best choice (gold standard), sometimes it’s mandatory of diagnosis and assessment of nephritis in SLE patient, and plan of medication can be changed after biopsy proven stages of renal disease, also it helpful in the assessment of the patient in activity or in remission.

Renal function tests such as creatinine or urine protein or estimation of GFR may not be sufficient to estimate SLE nephritis. Renal tissue damage can occur before changes in this parameter. Also, overestimation or underestimation usually depend on another factor such as age, body weight, nutritional state, and can change late in the course of the disease; so it’s not sensitive enough or specific to detect early or late disease course in SLE nephritis.

So, promising biomarkers needed to help in early detection or predict flare of nephritis, but most of this biomarker is too much expensive to evaluated in longitudinal studies of lupus nephritis; but certainly helpful in most cases.

This study uses TREM-1 to detect early renal involvement in SLE nephritis, or predict progression of the disease; overall the disease activity course of patient SLE have nearly normal or slightly elevated creatinine and mild-moderate deteriorate in GFR in more than 50% of the patient in the study group. But, assay of the TREM-1 is significantly elevated in the early than the control group and significantly elevated than an early level of this study and highly significant between the patients and control group (all control group below minimum detection level). The investigators try to evolved or discovery a valid biomarkers ‘panels’ as a novel ‘bio-signatures’ of SLE to detect early lupus activity and or nephritis rather than clinical assessment for a patient with SLE. In addition, TREM-1 is readily detected in biological fluids of patients suffering from a variety of disease.

Mok et al, (2005), explained our results in his study; which described TREM-1 as a costimulatory receptor on myeloid cells, potently amplified the function of TLR and Nod-like receptors which resulted in enhanced cytokine production; TREM-1 could serve as a biomarker for acute inflammation during infection and noninfectious inflammatory responses.

However, Zhihong et al 2013 revealed TREM-1 induces anti-apoptotic protein Bcl-2 and increased macrophage survival which abundant in any type of infection or inflammation; TREM-1 activation can propagate inflammation by modulating the survival of inflammatory cells and increased inflammatory cytokine production.

Serum sTREM-1 level correlated with elevated erythrocyte sedimentation rate (p = 0.002), but neither with SLE Disease Activity Index nor with clinical or laboratory features of SLE.

TREM-1 up-regulation in SLE might play a role in innate immune response and autoimmunity.

Yair et al, 2013 stated that serum sTREM-1 level was significantly elevated in 78 unselected patients with SLE compared to controls, also determined serum sTREM-1 level of 20 SLE patients who presented with a concurrent infectious disease not statistically significant.
compared with the above 78 patients without an infection. Serum sTREM-1 level correlated with elevated erythrocyte sedimentation rate (p = 0.002), but neither with SLE Disease Activity Index nor with clinical or laboratory features of SLE.

Also, Yong et al., 2016 in his study on nephritis-prone 129/SvJ strain demonstrate the elevated TREM-1 expression of renal tissue indicate that TREM-1 plays a critical role in the pathogenesis of inflammatory nephritis. Moreover, his studies demonstrated, for the first time, that TREM-1 is amenable to therapeutic targeting for nephritis. TREM-1 blockade markedly reduced inflammation in immune-mediated nephritis.

This study showed highly significant correlation between renal pathology, SELENA score, Anti-DNA, complement level and TREM-1 levels; this relation more significantly with early value of TREM-1 serum level that mean it can be used as indicator of renal disease activity this result agree with Yong et al (2016), but he did in animal model he found that the TREM-1 level play role in lupus nephritis by increased local production of TREM-1 in the inflamed kidney might contribute to disease pathogenesis. TREM-1 can be expressed by the inflammatory infiltrate and renal epithelial cells in chronic disease.

Our study agree with results of Laurie et al 2013 Elevated sTREM-1 levels were detected in serum of SLE patients with nephritis and TREM-1 was detected in renal biopsies from SLE patients but not controls and might act as a prognostic indicator of disease, can be as amenable to therapeutic targeting for nephritis more than renal disease was measured by proteinuria and renal pathology.

Iman et al, 2016 agree with our results in which TREM-1 in plasma a significantly higher levels in SLE patients than the healthy control group and especially patients with the total high disease activity. TREM-1 levels were significantly correlated with parameters of disease activity, i.e. SLEDAI score, IL-6, hypoalbuminemia.

On the other hand, we did not find significant differences in sTREM-1 levels in relation to age, disease duration, medications, ESR. Our preliminary data indicated that sTREM-1 levels may be an additional useful marker of disease activity in SLE; demonstrate that sTREM-1 has a significant relation with biopsy and SELENA assessment and C3, C4 in early disease activity and carry a possibility of TREM-1 abnormalities play role in the immune response appear to be an indispensable role.

Stock and Putterman (2013), revealed sTREM-1 is higher in SLE patients than healthy subjects. However, they have revealed contradictory results regarding the ability of sTREM-1 levels to differentiate between infection and activity in febrile SLE patients.

Also, Serum sTREM-1 level was significantly elevated in patients with SLE compared to that of the controls P < 0.01 and no statistically significant of TREM-1 compared with that of the patients with or without infection. Serum sTREM-1 level did not correlate with SLE disease activity. So, elevated serum sTREM-1 level suggests an increased shedding of TREM-1 in SLE and a possible novel pathway of innate immune response in autoimmunity.

Kim et al (2009), in his study, agree with our results and he demonstrates that the TREM-1 is more specific a prognostic indicator of disease, it can be targeted as new therapeutic value for nephritis that might greatly enhance the quality of SLE nephritis management.

The conclusion is TREM-1 can be used as biomarkers for monitoring and/or predicting SLE disease activity but, needs to be further investigated with large-scale multicenter trials.

Elevated TREM-1 levels in SLE serum and correlated with hematological manifestations; and renal manifestations. Disease activity, ESR, GFR, anti-DNA, and low complement level. A combination of novel markers with conventional clinical parameters to enhance the sensitivity and specificity for the early prediction of renal disease/flare and prognosis in patient with lupus nephritis.
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