**ABSTRACT**

**Introduction:** Systemic Lupus Erythematosus (SLE) is an inflammatory autoimmune disease where an interplay between acute phase proteins and cytokines are involved in disease activation. **Aim and Objectives:** This case control study was performed to investigate interrelationship between high sensitivity C-reactive proteins (hs-CRP), Interleukin-6 (IL-6) levels and disease activity among SLE patients. **Materials and Methods:** One hundred forty one clinically diagnosed SLE cases were included and disease activity was noted by SLE Disease Activity Index (SLEDAI). Serum IL-6 levels were measure by cytokine multiplex assay. Serum hs-CRP, C3 and C4 levels were measure by nephelometer. The Pearson correlation test was used for correlation between hs-CRP, IL-6 and SLEDAI. **Results:** Based on SLEDAI, 126 patients (89.4 %) had active disease and 15 patients (10.6%) had inactive disease. Mean hs-CRP levels in SLE patients were significantly higher (12.1 + 11.5 mg/L) than controls (2.41 + 1.37 mg/L) \( (P < 0.0001) \). Hs-CRP levels among active SLE were significantly higher (13.5 + 11.4 mg/L) as compared with inactive SLE (4.4 + 2.9 mg/L) \( (P=0.002) \). Similarly, IL-6 levels in SLE patients were significantly higher among active SLE (26.9 + 15.5 pg/ml) as compared with inactive SLE (13.9 + 10.2 pg/ml) \( (P=0.0001) \). An inverse correlation between IL-6 and hemoglobin levels between active and inactive SLE was noted \( (r=-0.46, P<0.0001) \). **Conclusion:** This study suggests a good correlation between hs-CRP, IL-6 and SLE disease activity indicating their direct involvement in inflammatory conditions associated with disease.

**KEY WORDS:** High sensitivity C-reactive protein levels, interleukin-6 levels, systemic lupus erythematosus, systemic lupus erythematosus disease activity index

**Introduction**

Systemic lupus erythematosus (SLE) is a multi-factorial chronic inflammatory autoimmune disease characterized by the production of a wide spectrum of autoantibodies and immune complex deposition leading to multiple organ damage. An inflammatory response is mediated through the influx of proinflammatory cytokines. These cytokines further induce production of various acute-phase reactant proteins such as high sensitivity C-reactive proteins (hs-CRP), lectin proteins, hepcidin, etc., and these proteins are established as inflammatory markers. CRP is one of the primary acute phase proteins, mainly synthesized by hepatocytes in response to proinflammatory cytokines such as interleukin-6 (IL-6),...
tumor necrosis factor-α (TNF-α) and IL-1 β (IL-1 β). Raised hs-CRP levels have been reported in response to a variety of cellular abnormalities such as tissue inflammation, infection, cardiovascular disease, and thromboembolism.\textsuperscript{[5,6]}

In SLE, hs-CRP concentration rises sharply during acute inflammatory conditions. Positive correlations have been reported between hs-CRP levels and SLE disease activity index (SLEDAI).\textsuperscript{[5,6]} Several studies have reported significance of various proinflammatory cytokines such as IL-6, TNF-α, and IL-1 β in SLE patients indicating their pathophysiological role mainly in active SLE patients.\textsuperscript{[7,8]} There is a paucity of data regarding the relationship among inflammatory cytokines like IL-6, raised CRP and various risk factors contributing to SLE disease severity. In this study, the possible correlation between CRP and serum IL-6 levels with disease activity among Indian SLE patients was evaluated.

Materials and Methods

Study population
One hundred and forty-one patients (131 females and 10 males; mean age at evaluation 27 ± 11 years, range 14–54 years) fulfilling the American College of Rheumatology classification criteria for SLE were recruited.\textsuperscript{[9]} This retrospective case-control study was conducted over a period of 4 years (2010–2013). Pregnant and postmenopausal women, smokers, patients with diabetes and with significant hyperlipemia were excluded. One hundred and fifty age and sex-matched healthy individuals were included as controls. The study was approved by the Institutional Ethics Committee and written consent was obtained from all patients and controls. Disease severity was evaluated by calculating the SLEDAI score.\textsuperscript{[10]} A score equal to or more than 10 was considered to be active.\textsuperscript{[11]}

Laboratory investigations
Venous blood samples from all patients and healthy controls were collected for investigating hemoglobin levels, serum CRPs, and IL-6 levels. Sera were stored in aliquots at −80°C until tested. Twenty-four-hour urine was collected for urinalysis. Serum hs-CRP levels, complement component (C3, C4) levels were measured using a Nephelometer (BN Prospek, Dade Behring, Germany). Serum cytokine levels were detected by bead-based MILLIPLEX MAP technology (Millipore Corporation, Billerica, MA, USA.) in all patients and healthy controls.

Statistical analysis
Statistical analyses were performed using GraphPad Prism version 5.05 (GraphPad Prism Software Inc., California, USA.) for Windows. Comparisons of serum levels of hs-CRP and IL-6 in SLE patients with or without active disease were performed using nonparametric Mann–Whitney U-test. Means between two groups were analyzed using two-tailed unpaired t-test. Fischer’s exact test was used to determine an association between laboratory investigations, clinical manifestations, and the presence of autoantibodies in SLE patients. The Pearson’s correlation test was used to analyze correlations between various laboratory measures and SLEDAI scores. The value of $P \leq 0.05$ was considered statistically significant.

Results

The mean age of SLE patients at the time of evaluation was 29.4 ± 9.8 years where mean disease duration was 2.7 ± 1.9 years. Mean SLEDAI score was found to be 16.9 ± 7.6. Of 141 SLE patients, 69 patients (48.9%) were lupus nephritis (LN) and 72 patients (51.1%) were without renal involvement (non-LN). Based on SLEDAI, 126 patients (116 females and 10 males) (89.4%) were categorized as active SLE (SLEDAI > 10) and 15 patients (all females) (10.6%) as an inactive SLE (SLEDAI < 10). Mean CRP levels, urine proteins and serum creatinine levels were significantly higher in active SLE than inactive SLE ($P < 0.01$), whereas mean hemoglobin level was significantly lower among active SLE patients ($P = 0.0029$) [Table 1]. Reduced levels of C3 alone were significantly higher in active SLE as compared with inactive SLE ($P = 0.0010$). Among active SLE, 57.1% showed arthritis and 52% had renal involvement. Statistically significant difference was noted for arthritis and renal manifestations when active and inactive SLE groups were compared ($P = 0.0502$, $P = 0.0266$, respectively). Similarly, hematological disorders like low Hb, leukopenia and thrombocytopenia were higher as in active SLE than inactive SLE (44.4% vs. 13.3%).

The mean hs-CRP levels among SLE patients were significantly higher (12.6 ± 20.9 mg/L) as compared to healthy individuals (3.41 ± 1.37 mg/L). Among SLE patients, mean hs-CRP levels were significantly higher in active SLE (13.6 ± 21.9 mg/L) as compared to inactive SLE (4.8 ± 3.8 mg/L) ($P = 0.0029$). When hs-CRP levels with SLEDAI were compared, a positive correlation ($r = 0.298$, $P = 0.0005$) was observed [Figure 1a]. Mean serum IL-6 levels among SLE patients were 68.1 ± 68.0 pg/mL, which was 4.8 times higher than healthy controls (14.16 ± 5.83 pg/mL) and this difference was statistically significant ($P < 0.0001$). Mean IL-6 levels were significantly higher among active SLE (74.9 ± 68.5 pg/mL) as compared to inactive SLE (19.0 ± 3.8 pg/mL, $P = 0.0056$). IL-6 levels were significantly elevated among LN patients (80.4 ± 61.0 pg/mL) as compared to non-LN patients (57.9 ± 72.7 pg/mL, $P = 0.0488$). When IL-6 levels were compared with SLEDAI positive correlation was observed ($r = 0.271$, $P = 0.001$) [Figure 1b].

A significant positive correlation was also noted between IL-6 levels and hs-CRP levels in SLE patients ($r = 0.411$, $P < 0.0001$).

Table 1: Laboratory investigations done in systemic lupus erythematosus patients ($n=172$)

| Laboratory parameters | Active ($n=149$) | Inactive ($n=23$) | $P$ |
|-----------------------|-----------------|------------------|-----|
| 24 h urine proteins (g) | 1.9±1.4         | 1.1±0.9          | 0.028 |
| Serum creatinine (mg/dl) | 2.0±1.4        | 1.3±0.2          | 0.0152 |
| Hemoglobin (g/dl) | 8.7±2.4        | 10.3±2.1         | 0.0030 |
| Complement C3 (<90 mg/dl) | 69.4±39.4    | 90.9±36.5        | 0.017 |
| Complement C (<10 mg/dl) | 10.5±7.4     | 15.5±9.0         | 0.014 |
| hs-CRP (mg/L) | 13.3±11.5      | 4.9±4.5          | <0.0001 |

The data represent mean±SD. Statistical $P$ value calculated by Mann–Whitney U-test. hs-CRP: High-sensitivity C-reactive protein, SD: Standard deviation.
The association of cytokines with disease activity and organ damage to the musculoskeletal system in active SLE patients as compared to inactive as well as healthy controls (n = 141). Hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6; SLEDAI: SLE Disease Activity Index; r, Pearson’s correlation coefficient

P < 0.0001. An inverse correlation between serum IL-6 and hemoglobin levels was seen among SLE patients (r = −0.214, P = 0.0106). Mean IL-6 levels were higher (62.2 ± 49.1 pg/mL) in patients with anemia (Hb ≤ 12.0 g/dL) than those without anemia (24.6 ± 23.1 pg/mL). This difference was found to be statistically significant (P = 0.0006).

Discussion

CRP is an inflammatory marker produced in response to IL-6 stimulation by hepatocytes and adipocytes.\[12\] Hs-CRP levels have been found elevated in SLE but its correlation with SLE disease activity was reported to be controversial.\[13,14\] Bertoli at al. had reported that SLE patients from Hispanic, African American, and Caucasian populations show a significant association of hs-CRP levels with the disease activity. It was reported that elevated hs-CRP levels are associated with multiple organ damage, including renal, cardiovascular, and musculoskeletal manifestations in SLE.\[6\] Lee et al. also had reported an association of hs-CRP levels with the disease activity and organ damage to the musculoskeletal system in their multiethnic cohort study including Asian SLE patients.\[15\]

In this study, significantly higher levels of hs-CRP were noted in active SLE patients as compared to inactive as well as healthy controls (P < 0.01). Pathogenic role and statistically significant association between IL-6 levels and disease activity was also reported in SLE patients by Sabry et al. in Egyptian SLE patients and Chun et al. in Korean population where higher serum levels of IL-6 in active SLE and its association with disease activity had been reported.\[15,16\] In our study, a significantly higher level of both hs-CRP and IL-6 in clinically active SLE (P < 0.05) with serositis was noted which was similar to study reported by Sproink et al.\[17\] Enoecon et al. also had reported increased CRP levels among SLE patients compared to controls, without any association with IL-6 levels and SLEDAI.\[18\] There are a few reports suggesting cardiovascular risk in active SLE patients with higher hs-CRP levels and an increased cardiovascular morbidity.\[13,19,20\] The limitations of our study were that in this study the impact of measurement of immune parameters such as hs-CRP and IL-6 during remission or relapses could not be evaluated which needs to be evaluated in details in patients with varied disease activity.

The relationship between anemia and inflammatory conditions in autoimmune disorders has been well documented.\[20\] Ripley et al. had reported low hemoglobin levels with the increased serum IL-6 which was similar to our study where an inverse correlation between IL-6 and hemoglobin levels was found.\[21\] This study supports the finding that a triangular measurement of hs-CRP, IL-6 and disease activity will be helpful in assessing the disease severity among active SLE patients.

Conclusion

This study suggests a good correlation between hs-CRP, IL-6 and SLE disease activity indicating their direct involvement in inflammatory conditions associated with disease.

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Conflicts of interest

There are no conflicts of interest.

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