Association of Serum Ferritin Levels with Hematological Manifestations in Systemic Lupus Erythematosus Patients from Western India

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Abstract
Objectives: To identify the hematological manifestations and its association with serum ferritin levels in SLE patients from Western India.

Methods: Ninety clinically diagnosed SLE patients fulfilling ACR criteria were included. Disease activity was assessed at the time of evaluation using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Sera were tested for serum ferritin levels by ELISA (Calbiotech, USA). Autoantibodies such as ANA, anti-dsDNA by indirect immunofluorescence test (IFA- Bio-Rad, USA) and anti-cardiolipin antibodies (ACA) to IgG and IgM isotypes and Anti-β2 GP antibodies to IgG and IgM isotypes were detected by ELISA using commercially available kits (Euroimmun, Lubeck, Germany).

Results: Out of 90 SLE patients studied, 41 patients (45.6%) showed hematological abnormalities, where anemia (82.9%), leucopenia (26.8%), autoimmune hemolytic anemia (AIHA) (14.6%) and idiopathic thrombocytopenic purpura (ITP) were noted in (34.1%) patients. Mean±SD serum ferritin levels among SLE patients were 270.2± 266.0 ng/ml as compared to 29.0± 15.8 ng/ml healthy normal controls (p<0.0001). A positive correlation between serum ferritin levels and SLEDAI scores (r=0.2640, p=0.0124) and anti-dsDNA positivity was noted (r=0.32, p<0.0001). Serum ferritin levels were negatively correlated with hemoglobin levels (r=0.5964, p=0.0001), WBC count (r=0.1705, p=0.2316), platelet count ((r)=0.1701, P=0.2375), C3 levels (r=-0.4417, p=0.0034) and C4 levels (r=-0.0363, p=0.8215)

Conclusion: Serum ferritin is an excellent marker of SLE which can be used for an evaluation of disease activity particularly in active stage of the disease mainly in patients having hematological and renal manifestations.

Editorial Viewpoint
• Patients with autoimmune inflammatory diseases like SLE have an elevated serum ferritin level.
• Ferritin levels are found to correlate with inflammatory state and anemia of chronic disease.
• This study has found serum ferritin level as excellent marker of SLE disease activity especially in patients having hematological manifestations.

Introduction
Systemic lupus erythematosus (SLE) is a prototype autoimmune disease characterized by a wide variety of clinical manifestations and presence of numerous autoantibodies resulting in organ and tissue damage. Hematological abnormalities are common in SLE. Worldwide studies have shown varied incidence of hematological...
manifestations in SLE patients. The major hematological manifestations of SLE are anemia, leucopenia, thrombocytopenia, and antiphospholipid syndrome (APS). Hematological abnormalities in SLE patients require early diagnosis, careful monitoring and prompt therapeutic intervention.  

Ferritin is an iron-binding molecule that stores iron in a biologically available form for vital cellular processes while protecting proteins, lipids and DNA from the potential toxicity of this metal element. Ferritin plays a role in a large number of other conditions, including inflammatory, neurodegenerative and malignant diseases. A markedly elevated serum ferritin level has been found to be associated with inflammatory conditions such as adult-onset Still's disease, systemic juvenile idiopathic arthritis, and hemophagocytic lymphohistiocytosis / macrophage activation syndrome. Hyperferritinemia is also reported in chronic inflammatory conditions such as flares of SLE and flares of granulomatosis with polyangiitis, as well as active rheumatoid arthritis, flare of inflammatory bowel disease, and active graft-versus-host disease. 

Ferritin serves as the primary iron reservoir from which iron can be mobilized and used in the production of hemoglobin. In SLE, it is estimated that 30-60% of patients are anemic. One of the most frequent causes of anemia in SLE patients is iron deficiency anemia (IDA). Anemia of chronic disease (ACD) which does not usually respond to iron supplementation is another major cause of anemia in patients with SLE. Patients with autoimmune inflammatory diseases, such as SLE and rheumatoid arthritis commonly have an elevated serum ferritin which more likely reflects disease activity, especially in the case of SLE, than iron status. Elevated ferritin and transferrin levels were found to correlate well with the inflammatory state and anemia of chronic disease suggesting that hyperferritinemia could potentially play a role in regulating immunity where ferritin can be a potential biomarker for disease activity in SLE. This study was designed to identify the hematological manifestations and understand its association with serum ferritin levels in SLE patients from Western India.

**Material and Methods**

This retrospective study was conducted in 90 clinically diagnosed SLE patients that were referred to our center over three years (2012-2014). All these SLE patients were diagnosed according to the American College of Rheumatology (ACR) criteria. The requisite ethical committee approval and a written consent was obtained from these patients. Clinical manifestations were noted in the proforma. Disease activity was assessed at the time of evaluation using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). The disease activity in all SLE patients were classified as mild, moderate and severe groups based on their SLEDAI scores (inactive <11, active ≥11). One hundred healthy age and sex matched normal healthy controls were also included. Hematological manifestations were assessed only at presentation. History of obstetric outcomes and thrombotic events were also evaluated in these patients. Renal biopsies of all lupus nephritis (LN) cases were examined by light microscopy using hematoxylin, eosin, Periodic Schiff (PAS) staining. Immunofluorescence microscopy was done using anti-IgG, anti-IgM, anti-IgA, anti-C3, anti-C4 and anti-fibrinogen fluorescein isothiocyanate conjugate (FITC). In LN patients the renal histology was classified according to revised WHO criteria. After blood collection, blood collected in EDTA was used for haemoglobin estimation and complete blood count (CBC) using automated blood counter, Sysmex KX-21, Japan. Serum ferritin levels were tested by ELISA (Calbiotech, USA). Sera were tested for autoantibodies such as ANA and anti-dsDNA by indirect immunofluorescence test (IFA- Bio-Rad, USA). Anti-cardiolipin antibodies (ACA) to IgG and IgM isotypes and anti-β2GP antibodies to IgG and IgM isotypes were detected by ELISA using commercially available kits (Euroimmun, Lubeck, Germany). APLA (ACA and anti-β2GP) tests were repeated at 3 monthly interval for confirmation. Serum complement C3 and C4 levels were tested using a nephelometer (BN ProSpec, Dade Behring, Germany).

**Statistical Analysis**

Mean ± standard deviation (SD) value was calculated for continuous variables and proportions for categorical variables. Means between two groups were analyzed by using Student’s unpaired t-test. Mann-Whitney U-test was used for comparisons between groups. Spearman correlation test was used to determine the relationship between ferritin and disease activity parameters. Pearson correlation test was used to analyze the correlations between various laboratory measures and SLEDAI scores. To compare the ratios between groups, chi-square test was used. A ‘p’value <0.05 was considered statistically significant.

**Results**

Out of 90 SLE patients studied, 85 patients (94.4%) were females and 5 patients (5.6%) were males. Age of the patients ranged from 12-55 years (mean 25.3 years). Clinical severity revealed that 51 patients (56.7%) were in an active stage at the time of evaluation (SLEDAI >11) and remaining 39 patients (43.3%) were in an inactive stage (SLEDAI < 11). Renal involvement in the form of lupus nephritis (LN) was
observed in 29 patients (32.2%). It was observed that 41 patients (45.6%) showed hematological abnormalities. Among patients having hematological abnormalities, female to male ratio was 19.5:1. Anemia (Hb <10 gm/dl) was detected in 34 patients (82.9%) with mean±SD hemoglobin level in SLE 9.9±1.9 gm/dl v/s 14.5±1.32 gm/dl among normals, leucopenia (WBC <4000/ml) was noted among 11 patients (26.8%) with mean±SD WBC count 7100±2500 /ml in SLE v/s 6400±1600 /ml among normals. Autoimmune hemolytic anemia (AIHA) was noted in six patients (14.6%). Idiopathic Thrombocytopenic purpura (ITP) platelets <150 X10^9/l) was noted in 14 patients (34.1%) with mean±SD platelet count 221.6±104.8 X10^9/l in SLE v/s 231.4±60.3X10^9/l among normals. Evan’s syndrome (immune thrombocytopenia (ITP) and autoimmune Haemolytic Anaemia (AIHA) with a positive direct antiglobulin test (DAT) was found in one patient (2.4%). Other clinical manifestations as per ACR criteria revealed that rash (malar/discoid) was seen in 39 patients (43.3%), photosensitivity in 33 patients (36.7%), arthritis in 48 patients (53.3%), serositis in 10 patients (11.1%) and CNS involvement in four patients (4.4%).

The mean±SD serum ferritin levels among SLE patients were 270.2±266.0 ng/ml as compared to healthy normal controls 29.0±15.8 ng/ml (p<0.0001). There was no statistically significant difference noted among females and males for ferritin levels (p > 0.05). A positive association between serum ferritin levels and disease activity among SLE patients measured by SLEDAI scores was noted (r = 0.2640, p=0.0124) (Figure 1). Among different clinical categories of SLE, patients in active group had higher ferritin levels (299.7±269.8 ng/ml) as compared to inactive group (232.3±259.4) and LN patients had higher levels of ferritin (327.4±289.3 ng/ml) as compared to non-LN. A statistically significant difference was noted in serum ferritin levels in SLE patients with hematological manifestations such as anemia, leucopenia and thrombocytopenia (382.9±264.8 ng/ml) as compared with patients without hematological abnormalities (173.9±282.7) (p<0.0001) (Figure 2). Serum ferritin levels were negatively associated with hemoglobin levels among SLE patients (r=-0.5964, p=0.0001), WBC count (r=-0.1705, p=0.2316) and platelet count (r=-0.1701,P=0.2375) (Figure 3). There was no statistically significant difference for serum ferritin levels for other clinical manifestations such as rash (malar and/discoid), photosensitivity, arthritis, serositis (pleuritis and/pericarditis) and CNS involvement when active and inactive SLE groups were compared (p>0.05) (Figure 4). Among total SLE patients studied, 80 patients (88.9%) had reduced C3 levels (<90 mg/dl) and 77 patients (85.6%) had reduced C4 levels (<15 mg/dl). Figures 5 and 6 shows correlation between serum ferritin levels and C3 and C4 levels respectively in all SLE patients studied. A statistically significant difference was noted between ferritin levels and C3 levels (p=0.0034) where as there was no statistically significant difference noted when ferritin levels were compared with C4 levels (p=0.8215). Serum ferritin levels were higher among patients with reduced C3 levels (359.3±269.0 ng/ml), reduced C4 (338.6±277.4 ng/ml) and patients having reduced levels both for C3 and C4 (342.2±277.6 ng/ml) as compared to total SLE patients. Anti-nuclear antibodies (ANA) were present in all patients (100%) patients, anti-dsDNA antibodies were present in 80 patients (88.9%). Anti-cardiolipin antibodies with IgG isotype (ACA-IgG) were detected in 13 patients (14.4%) and IgM isotype (ACA-IgM) were detected in 11 patients (12.2%). Anti-β2 GP antibodies (anti-β2GP-IgG) were detected in 25 patients (27.8%), whereas anti-β2GP IgM in 22 patients (24.4%). A statistically significant difference was noted.
between serum ferritin levels and anti-dsDNA positivity. \((r=0.32, p<0.0001)\).

**Discussion**

Hematological manifestations such as haemolytic anaemia, leukopenia, lymphopenia, and thrombocytopenia are the most commonly seen manifestations among patients with SLE at the time of disease onset. Most SLE patients exhibit anaemia at some point during their disease course. The causes of anaemia in SLE are mainly due to immune or nonimmune pathogenic mechanisms. Hematological disorders are also included in the revised 1997 American College of Rheumatology classification criteria for systemic lupus erythematosus. Worldwide studies have shown varied incidence of hematological manifestations in SLE patients. Hematological abnormalities reported from different parts of India show regional variations. Study from Southern India had reported hematological manifestations in 82% SLE patients at the time of presentation which was the most common initial presentation. A positive correlation with platelet count and a negative correlation with hematocrit levels were reported by Seyhan et al. This finding was not similar to our finding. Our SLE patients showed a negative correlation of serum ferritin levels with platelet count \((r=-0.1701, p=0.2375)\) and with hematocrit levels \((r=-0.6429, p=0.0002)\).

A positive correlation with serum ferritin levels and disease activity evaluated by SLEDAI scores. Limitation of most of these studies was a small sample size. Seyhan, 2014 had observed that serum ferritin levels in SLE was higher than in the control group where a significant positive correlation with ANA, anti-dsDNA titer, and SLEDAI score was reported. Nishiya et al had reported higher ferritin levels in SLE in controls and had a positive correlation with anti-dsDNA and a negative correlation with complement levels. A positive clinical correlation of ferritin levels in SLE patients with hematologic manifestations and serositis was reported. Recently Tripathi et al had observed high levels of serum ferritin in SLE patients from Eastern India and a significant positive correlation between serum ferritin levels and SLEDAI and anti-dsDNA autoantibody positivity was reported, whereas a negative correlation of serum ferritin levels was reported with C3 and C4 levels. It was also reported that patients with renal involvement had higher ferritin levels than SLE patients without renal involvement.

A positive correlation with serological anti-phospholipid syndrome and interferon \(\gamma\) (IFN \(\gamma\)), may be involved in the pathogenesis of ACD among SLE as these cytokines inhibit proliferation of erythrocyte progenitors modulate iron metabolism. This needs to be studied in SLE patients with hyperferritinemia. Erythrocyte derived microparticles have also been detected in patients with high ferritin levels. These circulating microparticles derived as a result of cell damage may further lead to apoptotic debris. This suggested a hypothesis that the released cellular components such as phospholipids and DNA due to defect in apoptotic clearance mechanism in autoimmune inflammatory conditions may generate autoantibodies to these cell constituents. Raised ferritin levels have also been found to be associated with inflammatory diseases in which antibodies are produced to these molecules. There is always a need for biomarker or combination of biomarkers for identification and evaluation of disease activity and prognosis in SLE. It is important especially in countries where inflammatory diseases are more prevalent with load of infectious disease burden, serum ferritin levels may in general be a better marker of inflammation. Serum ferritin is possibly such an excellent marker of SLE which can be used for an evaluation of disease activity particularly in active stage of disease mainly in patients having hematological and renal manifestations.

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**References**


