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HAEMATOLOGICAL AND AUTOANTIBODY PROFILE IN SLE AND ITS CORRELATION WITH CLINICAL MANIFESTATIONS AND SEVERITY

General Medicine

Dr. Nirjhrini Dhal Associate Professor, Department of General Medicine, FMMCH, Balasore.

Dr. Sushant Bhuyan Associate Professor, Department of General Medicine, PGIMER Capital Hospital, Bhubaneshwar.

Dr. Naresh Chandra Pattanaik Professor, Department of Clinical Haematology, IMS and SUM Hospital, Bhubaneshwar.

Dr. Rohan Pattanaik* Postgraduate Student, Department of Biochemistry, S.C.B. Medical College and Hospital, Cuttack. *Corresponding Author

ABSTRACT

Aim of the study: There is high morbidity and mortality in Indian SLE patients, keeping in mind that scarcity of studies in SLE regarding Autoantibody and hematological profile in India. Present study was conducted to correlate the same with clinical manifestations and severity. **Methods and materials:** 80 SLE patients fulfilling revised ARA criteria 1997, admitted to department of Medicine were included in the study. Statistical analysis was done by Chi-square, p-value and Annova. **Results:** 80 consecutive SLE patients and 50 healthy controls were enrolled. Female patients in the age group of 15 - 35 years were commonly affected (54 out of 80). The commonest clinical manifestations were musculoskeletal like arthritis, oral ulcer, malar rash and nephritis was the major organ involvement. Disease severity was associated with SLEDAI scores. Patients with SLEDAI score < 10 had more musculoskeletal involvement, > 20 had vasculitis and 10 - 20 had renal involvement. SLE patients had significantly higher ESR ($P < 0.0001$), serum ferritin ($P < 0.0001$) and significantly lower values of hemoglobin ($P < 0.0001$), RBC ($P < 0.0001$) and TPC ($P = 0.03$) compared to control group. There was a significant positive correlation between serum ferritin and SLEDAI ($r = 0.34$), $P < 0.001$, indicating its value as a biomarker of disease activity. ESR was significantly high in patients with vasculitis ($P = 0.05$). Anti dsDNA was significantly higher in patients with renal involvement compared to vasculitis ($P=0.02$) and minor organ involvement ($P = 0.004$). Anti-histone antibody was also significantly high in serositis compared to renal ($P=0.03$) and minor organ involvement ($P=0.01$). **Conclusion:** SLE is a disease of women between 15 - 36 years. Common clinical manifestation are musculoskeletal which includes arthritis (70%), skin rash (70%), oral ulcer (70%). Nephritis (37.5%) is the most important major organ manifestation. Anemia (75%) is the commonest hematological manifestation in lupus.

KEYWORDS

SLE, Autoantibody, Arthritis, Nephritis, Vasculitis, Malar rash

INTRODUCTION:

Systematic lupus erythematosus (SLE) is a multi-factorial chronic autoimmune disorder characterized by dysfunction of T and B lymphocytes and affects various vital organ systems; 70 to 90 % of SLE patients are female.[1] The etiology of the disease is still unclear, although environmental, host genetic and hormonal factors have been proposed to play major roles in pathogenesis of SLE.[2]There are limited studies on Indian SLE patients. Although the prevalence of SLE patients in India is rare (3/100,000), the survival rates of these patients (70% at 5 years, 50% at 10 years) are very low compared to Western cohorts.[3]

Systemic lupus erythematosus (SLE) at its onset may involve one or more organs systems and over a time additional manifestations may appear after a variable period. The systems involved in SLE are musculoskeletal, cutaneous, renal, nervous system, hematological, vascular, pulmonary, gastrointestinal and ocular. Hematological manifestations (abnormalities of the formed elements of the blood, of the clotting and fibrinolytic factors and related systems) of SLE are diverse and often they are presenting manifestations of the disease.[4,5,6]The major hematological manifestations of SLE are anemia, leukopenia, thrombocytopenia, the antiphospholipid antibody syndrome (APLAS).

The hematological changes, though very commonly seen, are not properly evaluated or estimated and are not given enough representation in the American College of Rheumatology (ACR) criteria for diagnosis of SLE. It is only natural to expect hematological manifestations more often than others, since blood and blood vessels together contain diverse number of antigens than any other organ in the body and in SLE auto antibodies is known to develop against any antigen or tissue. Ferritin is an acute-phase reactant and is thus elevated in inflammation, autoimmune disorders, chronic infections, and liver disease. High concentrations of serum ferritin levels have been reported with patients with active SLE as compared to inactive SLE.[7,8,9] Elevated levels of urinary ferritin have also been reported in lupus nephritis (LN) patients. Beyond its role as an acute phase protein, ferritin plays an important role in iron storage and recycling. Ferritin stores iron in a non-toxic and soluble form and releases it in a controlled fashion. Ferritin plays an important role in host immune response as it is evident from its increased concentration during infection in order to counter infective agents that attempt to bind iron from the host tissue.[10] An increased immune response augments the migration of the ferritin from the plasma to within the cells, so that iron is not available to the infective agent. Iron, an important molecule regulates ferritin expressions.[11]

SLE is an autoimmune disease characterized by B cell hyperactivity resulting in overproduction of autoantibodies against cytoplasmic, nuclear, and surface antigens and immune complex formation[12,13]. The majority autoantibodies found in SLE are targeted at intracellular nucleoprotein particles. 98% of the patients have antinuclear antibodies and anti-double-stranded DNA antibodies are found in 50-80% of patients[14].These autoantibodies are frequently targeted against intracellular antigens of the cell nucleus(double and single stranded DNA(dsDNA and ssDNA, respectively), histones, and extractable nuclear antigens (ENAs).Most of the autoantibodies are not specific for SLE and might be produced non-specifically as a result of polyclonal B cell activation[15,16].

Autoimmune disease detection protocol starts with determination of ANA (antinuclear antibody). Positive ANA test leads to further investigation of extractable nuclear antigens (ENA)[17]. the prevalence of anti-dsDNA autoantibody 70% in SLE, giving a higher diagnostic sensitivity than the similarly disease-specific anti-sm auto-antibodies (30%). In SLE, the level of circulating immune complexes (CIC) increases resulting in deposition in tissues and activation of classical complement pathway[7].However, there is limited knowledge on autoantibody profile in Indian SLE patients. The present study is an attempt to observe comprehensibly autoantibody profile and hematological parameters in a cohort of SLE patients enrolled prospectively.

AIM OF THE STUDY:

This study was carried out to correlate auto antibodies and hematological parameter with different clinical manifestations and

severity of disease based on SLEDAI score.

MATERIALS AND METHODS:

Eighty (80) consecutive adult SLE patients (diagnosed by revised ARA criteria 1997) from the OPD and indoors of department of medicine S.C.B M.C.H, were included in the study. Exclusion criteria were Pediatric SLE, Drug induced lupus and overlap syndrome patients. Age and sex matched individuals were taken as controls.

CLASSIFICATION CRITERIA FOR SLE (1997):

1) Malar Rash: Fixed erythema, flat or raised over the malar eminences
2) Discoid rash: Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur
3) Photosensitivity: Exposure to ultraviolet light causes rash
4) Oral Ulcers: Includes oral and nasopharyngeal ulcers observed by physician.
5) Arthritis: Non erosive involving two or more peripheral joints characterized by tenderness, swelling or effusion.
6) Serositis: Pleuritis or pericarditis documented by ECG, rub, or evidence of pericardial effusion.
7) Renal disorder: Proteinuria > 0.5 g/dl or 3+ or cellular caste.
8) Neurological: Seizures or psychosis without other causes.
9) Hematological: Haemolyticanemia, Leucopenia (<4000/ul) or Lymphopenia(<1500/ul) or Thrombocytopenia(<100,000/ul) in the absence of offending drugs
10) Immunolgical: Anti-dsDNA, anti-sm and/or anti-phospholipid antibodies.
11) Antinuclear: An abnormal titer of ANAs by immunofluorescence or an equivalent antibodies assay at any point in time in the absence of drugs known to induce ANAs.

Four out of eleven criteria were satisfied for diagnosis.

Minor organ involvement was defined as presence of arthritis skin involvement oral ulcer.

Hematological test like CBC, ESR, detection of ANA and anti dsDNA by indirect immunofluorescence using commercial kit EuroimmunMedizinchelabordignostica, AGD23560 Lubek (Deuchland, Seekamb).

Antibody to different antigen by immune-blot strips coated with 14 different antigens: nRNP, Sm, SSA, SSB, RO-52, SCL-70, PM-SCL, JO-1, CENPB, PCNA, dsDNA, nucleosome histone, robo-P-protein, AMA-M2.

C3, C4 estimation done by Turbidometry method.

PT, APTT estimated by automated coagulometer (SYSMEX-XT500)

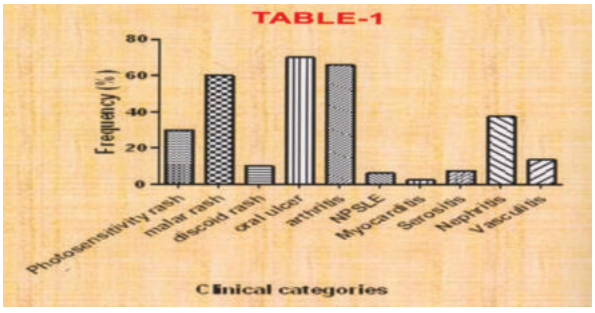
Frequency of auto antibody, clinical manifestations were statistically analyzed by Chi-square, p values and Annova and inference was drawn.

SLEDAI SCORE

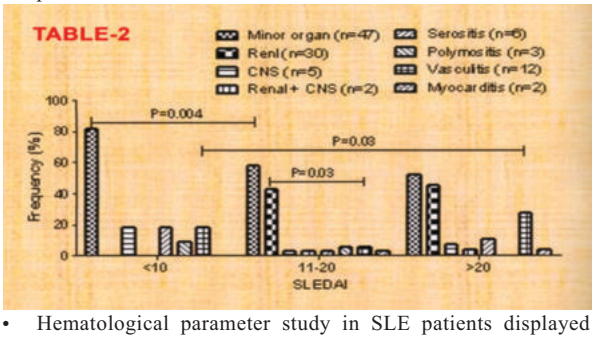
DESCRIPTOR	WEIGHTED SCORE
Seizure	8
Psychosis	8
Organic Brain Syndrome	8
Visual	8
Cranial nerve	8
Lupus headache	8
Cerebrovascular Accident	8
Vasculitis	8
Arthritis	4
Myositis	4
Casts	4
Hematuria	4
Proteinuria	4
Pyuria	4
New malar rash	4
Alopecia	4
Mucous Membrane	4
Pleurisy	4
Pericarditis	4
Low complement	2
Increased DNA binding	2
Fever	1
Thrombocytopenia	1
Leukopenia	1
Total Score	115

RESULTS:

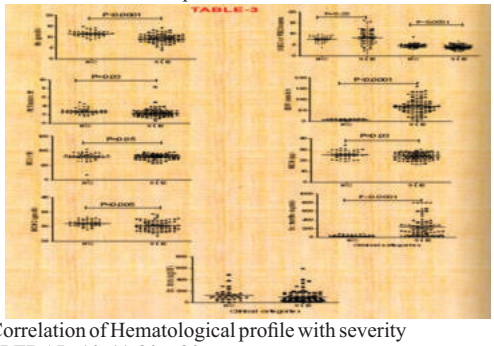
- Eighty (80) patients (Revised ACR 1997 criteria) and fifty (50) control [Female-77(96%), Male -3(4%)].
- 51.25% patients were 26.-35year age group.



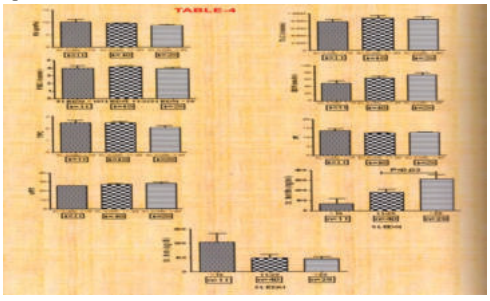
- Arthritis (70%), oral Ulcer (70%), Malar Rash (60%), Nephritis (37.5%), Photo sensitivity (30%), vasculitis (13.75%), Discoid Rash (10%), Serositis (7.5%), NPSLE (6.2%), Myocarditis (2.5%) as presented in Table-1.
- Involvement of age is not associated with involvement of organ.
- Minor organ involvement was significant in <10 SLEDAI score patients compared with 11-20 score (P=0.004).
- Vasculitis more frequent in >20 SLEDAI Score (P=0.03).
- Most patients in 11-20 score had renal involvement (P=0.03) as presented in Table-2.



- Hematological parameter study in SLE patients displayed significantly Higher ESR (P< 0.0001) and serum Ferritin (P< 0.0001) compared to healthy controls. In contrast HB (P< 0.0001), RBC (P< 0.0001) and TPC (P=0.03) were significantly lower in patients than control as presented in Table-3.



- Correlation of Hematological profile with severity
- SLEDAI <10, 11-20, >20.
- Serum Ferritin significantly elevated in >20 than 11-20. Other hematological parameter were comparable among various score group, failed to demonstrate any association with SLEDAI score as presented in Table-4.

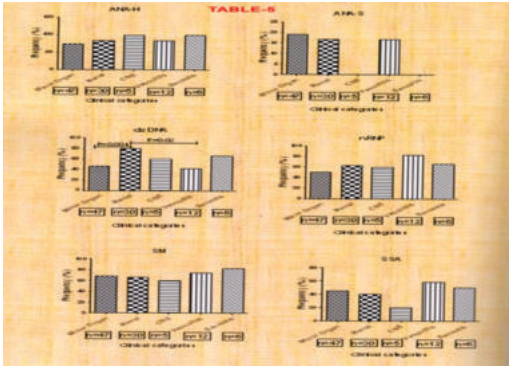


Serum ferritin significantly decreased in patients with longer duration of disease (>36 months and 25-36 months) than <12 months.

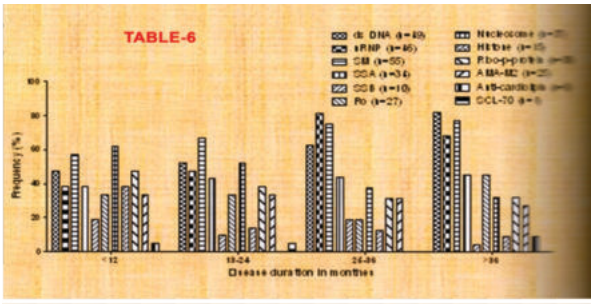
Vasculitis is associated with high ESR value as compared to vasculitis with minor organ damage (p<0.05). No association was found with other hematological profiles in SLE.

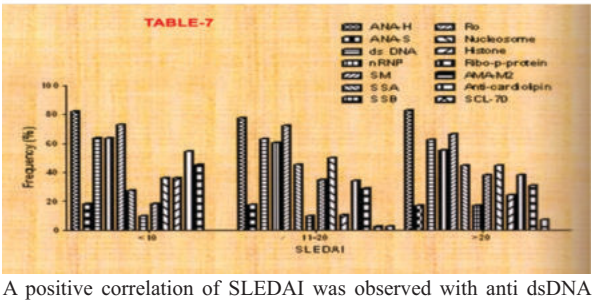
Auto antibodies profile in SLE: Smith antigen positivity significantly decreased in >45years patients compared to 15-25 years (P=0.04)

Other two antibodies had no association with age. Prevalence of Anti-DS-DNA was significantly higher in renal involvement (P=0.004). Anti-histone antibody increased in serositis than renal (P=0.03) and minor organ involvement (P=0.01) as presented in Table no-5.



No association between duration of disease and auto antibodies profile in SLE patients and SLEDAI score as presented in Table 6, 7.





A positive correlation of SLEDAI was observed with anti dsDNA (P=0.006, r=0.33) and a negative correlation with C3(P=0.003, r= -0.25).

DISCUSSION:

The mean age of patients at presentation was 27.17 +/- 8.4 years. Most patients were in the age group 26 - 35 years. The gender composition was skewed towards female (96.04%) compared to males (3.96%) in SLE group. In comparative study from different regions of India, the female predominance as well as the affection of younger age group was evident.[3, 18]The median duration of disease was 3 years before presentation to a tertiary care hospital indicating lack of awareness of the problem leading to late referral.

On comparative analysis of the clinical profile from different parts of country, it was observed that musculoskeletal symptoms were early presentation of lupus. [25]

Previous studies had shown that organ involvement tends to be higher with increasing age of patients and duration of disease.[8] In the present study, no significant association of age and disease duration was observed with organ involvement. However, renal involvement increases with age of the patients and duration of disease. Long standing SLE can therefore be a cause for nephritis in Indian population, besides, genetic susceptibility and improper control of disease activity.

We observed a significant association of minor organ manifestation (arthritis, skin rash, mucosal ulcer) with lower SLEDAI (<10) (P=0.004) while high scores, that is more than 20 SLEDAI score, correlated with vasculitis (P=0.03). Further, most patients with SLEDAI scores between 11-20 displayed significant renal involvement (P=0.03). Higher SLEDAI scores is an indication of significant disease activity.

In a study on Chinese SLE patients by Hu et al, hematological abnormality was detected in 86% of patients while in a study by Mathew et al from South India, 92.2% had hematological changes.[26, 27] The commonest abnormality observed was anemia out of 80 (75%) cases. In the present study, anemia (<7 gm/dl) was observed in 8 cases, the lowest being 4 gm/dl. In a previous study, anemia was found to be significantly associated with nephritis and poor survival while in another observation; anemia was associated with various organ damage such as renal, cardiovascular and pulmonary manifestations [20]. However, no significant association was observed in our study.

Ferritin is an acute phase reactant and has been observed to be elevated in systemic lupus erythematosus.[7,9]It is used as a biomarker of disease activity. In the current investigation, plasma ferritin levels were significantly high in SLE compared to healthy control. Beyan et al observed rise in serum ferritin levels in Turkish SLE patients, a significant variation in ferritin levels before and after treatment, and a positive correlation between serum ferritin and SLEDAI.[7] A study on Korean SLE patients corroborates the observation that serum ferritin is a good marker of disease activity in SLE.[8]

Higher prevalence of thrombocytopenia has been reported earlier in Indian patients: North India (11%), South India (12%), Western India (33.3%).[3,18,28]In present study, thrombocytopenia was found in 1.2% cases.
Leucopenia was found in 10 out of 80 cases (12%), similar to previous observation reported from North India (16%), South India (14.7%) and Western India (43.3%).[3,18,28] A prevalence of leukocytosis in 27 out of 80(33.5%) cases was observed in our cohort. Lymphopenia was observed in 28 out of 80 (35%) cases.

In present study we observed a significant association of ESR with SLE. SLE patients had higher ESR compared to healthy controls. Patients with vasculitis displayed significantly higher ESR values compared to minor organ involvement. No significant difference was observed in serum levels of other hematological parameters such as MCV, MCH, MCHC, PT, aPTT between patients and controls.

Anti dsDNA and Sm antibodies are more specific in SLE [21,23]. ANA is a good screening test and was positive in 98% of cases. A homogeneous pattern indicates activity to dsDNA and histone protein. A speckled pattern indicates antibody directed to nRNP, Sm, SSA, Ro-52, SSB. In the current investigation, we found positivity in 97.5% cases to ANA (homogeneous: 80%; speckled: 17.5%), 61.25% to anti dsDNA, 68.75% to anti Sm, 57.5% anti nRNP, 37.5% anti Rib-p-Protein, 18.5% anti nucleosome, 18.75% anti histone, 42.5% anti SSA, 12.5% anti SSB, 31.25% anti AMA-m2, 33.75% anti Ro, 3.75% anticardiolipin, 1.25% anti SCL-70.

SLE is a heterogenous disease marked by overproduction of autoantibodies which are produced against multiple antigens. Although anti dsDNA and anti Sm antibodies are specific for SLE, the relevance of other autoantibodies needs to be studied by long term follow up.

CONCLUSION:

Observation from our study revealed SLE occurs predominantly in females. Prevalence is more between 15-36 years age group and less common in >45 years age group. Predominant clinical features were arthritis, skin rash, oral ulcer and vasculitis. NPSLE and myocarditis are uncommon. Mean duration of presentation to a tertiary care hospital is three years. Anemia is the commonest hematological abnormality and iron deficiency is important component of anemia in our study. Thrombocytopenia is extremely rare. Leucopenia occurs in modest number of cases. ESR is high in SLE and there is significant

association with Vasculitis. Serum ferritin has got significant correlation with SLEDAI and serves as a biomarker.

Auto antibodies revealed ANA (97.5%), anti dsDNA (61.25%), anti-smith(68.75%). Anti-cardiolipin antibody is extremely rare. Anti-dsDNA correlates with Nephritis and SLEDAI Score. Other antibodies (anti-nRNP, anti-RO-52, anti-SS-A, anti-SS-B, anti-SCL-70) although increased are not associated with duration of disease, severity of disease and organ involvement.

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