

Clinical and Laboratory Manifestations of Systemic Lupus Erythematosus in Pakistani Lupus Patients

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Abstract.- Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies. These antibodies bind to circulating antigens to form immune complexes that get deposit on the tissue and bring about the symptoms of SLE. The main objectives of this study were to enlighten the clinical manifestations common in Pakistani SLE patients; to quantify the wide range of serum autoantibodies and their association with SLE. A total of 61 SLE patients fulfilling ACR criteria and 61 healthy blood donors were included in this study. Enzyme Linked Immunosorbent Assay technique was used for the detection and quantification of autoantibodies like ANA, dsDNA, anti-SSA, anti-SSB, anti-histone, and anti-Sm. Complications include mucocutaneous involvement, lupus nephritis, serositis, bull eye maculopathy, cardiovascular problem, Raynaud's phenomenon as well as various syndromes overlapped with SLE. Immunological parameters like ANA and anti-dsDNA showed a significant association ($p < 0.05$) with SLE. The mean of anti-SSA was found to be significantly higher in Pakistani SLE patients as compared to other autoantibodies. Furthermore, anti-SSA and anti-histone showed a significant difference ($p < 0.05$) with the known cut-off value. Thus one can conclude that SLE is a multifactorial disorder and the variety of autoantibodies are might be the primary cause of tissue damage in such patients.

Key words: Systemic lupus erythematosus, antinuclear antibodies, anti-double stranded deoxyribonucleic acid, anti-histone, anti-Smith, soluble substance A, soluble substance B.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is one of the examples of systemic autoimmune diseases in which there is production of wide spectrum of serum autoantibodies. These autoantibodies bind with circulating antigens to form immune complexes. Some of them cause tissue inflammation while others are responsible for direct cytotoxicity. In SLE, autoantibodies target nuclear and cytoplasmic macromolecules, lipid components, blood cells, plasma proteins and other antigens. The pathology of SLE remains unclear but autoantibodies like ANA, anti-dsDNA, anti-histone, anti-Sm, anti-SSA, anti-SSB are thought to play an important role in the development and progression of the disease. One hundred and sixteen autoantibodies are found to be associated with SLE. The production of these autoantibodies could be antigenic driven, the result of polyclonal B-cell activation, impaired apoptotic pathways or because

of the idiotypic network dysregulation (van Bruggen *et al.*, 2007). Diagnosis of SLE can be difficult as patients present with a broad range of symptoms so the American College of Rheumatology (ACR) has developed a diagnostic criteria to diagnose SLE. This ACR criterion includes serositis, oral ulcer, arthritis, photosensitivity, hematological disorders, renal disorder, positive antinuclear antibody test, immunological disorder, malar rash, discoid rash, and neurological disorder (Hochberg, 1997). In Pakistan, data on the characteristics of SLE is scarce. Thus the main objectives of this study were to enlighten the clinical manifestations common in Pakistani SLE patients, and to quantify the wide range of serum autoantibodies and their association with Pakistani SLE patients.

MATERIALS AND METHODS

A total of 61 SLE patients as well 61 healthy controls were enrolled in this study. The study was carried out after ethical approval obtained from the Board of Higher studies, University of the Punjab and patients were included after informed consent. Sixty-one SLE patients, clinically identified according to the ACR criteria were tested for

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different autoantibodies [ANA (Orgentec 538), dsDNA (Orgentec 604), SSA (Orgentec 508), SSB (Orgentec 509), anti-histone (Orgentec 507), Sm (Orgentec 510)] by an ELISA assay. Statistical analysis like univariate analysis, Mann-Whitney U test, Chi-square, Pearson correlation, one-sample t test, Analysis of Variance (ANOVA) were carried out by SPSS ver 13. Specificity, sensitivity, negative predictive value, positive predictive value were calculated by using online software "Diagnostic Test statistics" (Maceneaney and Malone, 2000).

Orgentec 538 antinuclear antibody (ANA) screen assay

Purified antigens are bound to microwells and if antibodies are present in the diluted serum, they will bind to the respective antigen. Washing of the microwells removes unspecific serum components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate-antibody-antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end product. The intensity of this yellow color was measured photometrically at 450nm (Migliorini *et al.*, 2005).

Sample buffer concentrate (5 X) was diluted with deionized water to a final volume of 100 ml prior to use. Similarly, buffered wash solution concentrate (50 X) was diluted with deionized water to a final volume of 1000 ml prior to use. These solutions remained stable for at least 30 days after preparation. 100 μ l of controls and prediluted patient samples (1:100) were poured into the wells of the microtitration plate; incubated for 30 minutes at room temperature. The contents of the microwells were then discarded and washed 3 times with 300 μ l of wash solution. 100 μ l of the enzyme conjugate was dispensed into each well and incubated for 15 minutes at room temperature. The contents of the microwells were again discarded and washed 3 times with 300 μ l of wash solution. TMB substrate solution (100 μ l) was dispensed in to each well and incubated for 15 minutes at room temperature. 100 μ l of the stop solution was added to each well of the

modules and left untouched for 5 minutes at room temperature. Optical densities were read at 450 nm and 630 nm as bi-chromatic measurement was preferable. The cut-off value and index value was calculated by using following formulas:

$$\text{OD Cut-off} = \text{OD Calibrator Control} \times \text{Specific Factor}$$

$$\text{Index Value} = \text{OD sample} / \text{OD cut-off}$$

Orgentec 604 double stranded DNA (dsDNA) screen

Human recombinant double-stranded DNA (dsDNA) is bound to microwells and antibodies to this antigen, if present in diluted serum or plasma, bind to the respective antigen. After washing, horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate-antibody-antigen complex. After another step of washing, an enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. Here 1M HCl was used as a stop solution. The intensity of the color was measured photometrically at 450 nm. The amount of color was directly proportional to the concentration of IgG antibodies present in the original sample. The standard curve of dsDNA was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on graph paper with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis (Enger, 2000).

Orgentec 508 soluble substance A (anti-SSA/Ro) assay

The autoantigen SSA is a ribonucleoprotein consisting of a Uridine rich RNA and associated proteins. For an Indirect ELISA, the microplate is coated with SSA and if antibodies for this antigen, present in the serum; they bind to the respective antigen. After incubation, washing of the microwells remove unspecific serum and plasma components. Enzyme conjugated with antihuman IgG, immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. After following another step of washing, an enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction by forming a

yellow end-product. The intensity of this yellow color was measured at a wavelength of 450 nm and the amount of color was directly proportional to the concentration of IgG antibodies present in the original sample. Using the mean absorbance value for each sample, the corresponding concentration in U/ml was determined from the standard curve (Langguth *et al.*, 2007).

Orgentec 509 soluble substance B (anti-SSB/La) assay

Anti-SSB is an indirect solid phase enzyme immunometric assay (ELISA), which is designed for the quantitative measurement of IgG class autoantibodies directed against the extractable nuclear antigens SSB (La). Highly purified SS-B is bound to microwells and this type of ELISA is divided in to three phases. Phase I is the binding of present autoantibodies and in the phase II, there is a formation of sandwich complexes. In the last phase, enzymatic color reaction takes place and the intensity of this color is directly proportional to the concentration of autoantibody in which we are interested. The SSB concentration of the samples was determined from the standard curve by matching their mean absorbance readings with the corresponding standard concentrations (Milic *et al.*, 2009).

Orgentec 510 anti-Smith (anti-Sm) assay

Antibodies in the diluted serum against highly purified Sm, bind to the antigen precoated in to the microwells. After washing, horseradish peroxidase conjugate solution recognized the autoantibodies bind to the immobilized antigens, forming a conjugate/antibody/antigen complex. After washing, an enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of 1M HCl stopped the reaction by forming a yellow end-product. Optical density was measured at 450. The Sm concentration of the samples was determined from the standard curve (Reichlin and Van Venrooj, 1991).

Orgentec 507 anti-histone assay

Highly purified histone proteins H1, H2A, H2B, H3 and H4 are bound to microwells of the microtitration plate. Antibodies against total

histones bind to the coated antigen. After incubation and washing, enzyme labeled antihuman globulin is reacted with the antibody that has attached to the antigen. The uncombined labeled enzyme is washed from the well and a substrate is added. The presence and concentration of the antibody that has reacted with the antigen is shown by the change in color of the substrate. More intense is the color, higher is the concentration of antibody in the serum. Using the mean absorbance value for each sample, the corresponding concentration in U/ml was determined from the standard curve (Hasan *et al.*, 2004).

RESULTS

During a period of 16 months, a total of 1,650 patients came to Rheumatology and Nephrology department of different hospitals of Lahore that covered major population of Lahore. Of these, 61 were the SLE patients who fulfilled ACR criteria. Serological tests were carried out at the Pathology Department of the Services Institute of Medical Sciences, Lahore. Of the 61 SLE patients, fifty-five (90.16%) were females and six (9.83%) were males. The female versus male ratio was 9.16:1. Of the 55 females, 36 were married and 19 were unmarried. Mean age at diagnosis was 30.35 ± 1.687 (12-68 years). SLE patients were diagnosed according to ACR criteria, most of the SLE patients (36.06%) fulfilled 4, and some of them (8.2%) also fulfilled 10 of the 11 ACR criteria. Mucocutaneous involvement was found in SLE patients such as malar rash in 9.84%, discoid rash in 18.03%, and photosensitivity in 18.03% of the SLE patients. Various syndromes were overlapping SLE and the most common one was Rheumatoid arthritis 31.14 % but cases of Sjogren's syndrome 16.39 %, Scleroderma 3.27%, Secondary Antiphospholipid syndrome 3.27%, and of Budd-Chiari Syndrome 1.63% were also found along with SLE. Antiphospholipid syndrome patients were proved clinically either by thrombosis along with positive anticardiolipin antibodies or by lupus anticoagulants. Renal involvement was found in 32.78% patients characterized by proteinuria and red cell cast. Serositis mainly pericardial effusion 6.55% and oral ulcer 3.27% were found in the SLE

patients but there was no report of seizures. Three SLE patients (4.91%) had eye problem, one had Bull eye maculopathy and the other two had optic atrophy. One (1.63%) SLE patient had cardiovascular problem and 29.5% were suspected of having Raynaud's phenomenon.

Immunological parameters used for the diagnosis of SLE are summarized in Table I. When a systemic autoimmune disease is suspected, a common practice is the performance of a general screening test for a panel of antinuclear antibodies. The wells of the ANA screen test (Orgentec 538 Diagnostika GmbH, Germany) are coated with a mixture of 8 different antigens like RNP-70, RNP/Sm, Sm, SSA, SSB, Scl-70, Centromere B and Jo-1. This assay permits a perfect screening for the corresponding antinuclear antibodies in patient serum. Mean value of ANA absorbance in SLE patients was 1.288 ± 0.117 and that of index value was 3.59 ± 0.327 . SLE is also characterized by the presence of autoantibodies against native double stranded DNA. These antibodies are useful to monitor the therapy and to predict disease progression but in this study, we could not study the association of therapeutics and autoantibodies levels. In the anti-dsDNA kit (Orgentec 604, Diagnostika GmbH, Germany), human recombinant DNA is used as antigen so this dsDNA kit permits the detection of lupus-specific autoantibodies. Mean value of dsDNA concentration in SLE patients was 17.51 ± 1.335 while in controls it was 3.505 ± 0.481 . Here ANA Index value was 7 times increased in cases over controls while dsDNA concentration was more than 3 folds increased in SLE cases with their respective controls. Chi-square test of association was applied to evaluate the association of ANA, dsDNA with SLE and level of significance was at $p \leq 0.05$ (Table II).

Mann-Whitney U test is a non-parametric significance test; the data deviated from normal distribution so Mann-Whitney test was carried out by using SPSS ver 13 (Table III). In this study, it was applied for assessing whether two observations are from the same distribution. Table IV represents the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of ANA and anti-dsDNA. Here 95% confidence interval (CI) calculated with Binomial expansion. In this study,

the positive predictive value (PPV) of ANA and of anti-dsDNA was 100%.

Table I.- Immunological markers for the diagnosis of SLE.

Immunological Markers	No. of positive patients	% of positive patients
ANA	46	75.40
dsDNA	44	72.13
Anti-Sm	18	29.5
SSA	29	47.5
SSB	10	16.4
Anti-histone	2	3.3

ANA, Antinuclear antibodies; dsDNA, Anti-double stranded deoxyribonucleic acid; Anti-Sm, Anti-Smith; Anti-SSA, Anti-Soluble substance A; Anti-SSB, Anti-Soluble substance B

Table II.- ANA and dsDNA frequencies in SLE patients (n=61) and control group.

Parameters	Status	n	Mean	χ^2
ANA absorbance	Cases	61	1.288 ± 0.117	$p < 0.0001$ $\chi^2 = 73.842$
	Controls	61	0.199 ± 0.0134	
ANA index value (U/ml)	Cases	61	3.592 ± 0.327	
	Controls	61	0.444 ± 0.0249	
dsDNA conc. (U/ml)	Cases	61	17.513 ± 1.335	$p < 0.0001$ $\chi^2 = 68.821$
	Controls	61	3.505 ± 0.481	

Table III.- Mann-Whitney U test for ANA in SLE patients (n=61)

Parameters	ANA patient value (U/ml)	ANA index value
Mann-Whitney U Test	354	218
p-value	0.00	0.00

Table IV.- Sensitivity, specificity, positive and negative predictive value of ANA and dsDNA for the clinical diagnosis of SLE (n=61).

Antibodies	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	PPV (%)	NPV (%)
ANA Screen	95.4 (62-85)	100 (94-100)	100 (92-100)	80.26 (69-88)
dsDNA Screen	72.13 (59-82)	100 (94-100)	100 (91-100)	78.2 (67-86)

PPV, positive predictive value; NPV, negative predictive value

Mean value of anti-Sm antibody concentration in SLE patients was 12.59 ± 1.970 while the minimum concentration was 1 U/ml and the maximum concentration was 70 U/ml. Mean value of anti-SSA antibody concentration in SLE patients was 63.54 ± 9.86 , the minimum range was 2 and the maximum one was 200. The mean value of anti-SSB antibody concentration in SLE patients was 23.961 ± 6.719 ; the minimum range was 1 and the maximum one was 200. Mean value of anti-histone antibody concentration in SLE patients was 12.25 ± 4.49 (Table V). Table VI represented that there was no significant association of anti-Sm and anti-SSB autoantibodies with SLE but a significant association of anti-SSA and of anti-histone with SLE was found as the p-value is < 0.05 . Anti-SSA autoantibodies were present in the sera of SLE patients with serositis, photosensitivity, and lupus nephritis. Anti-SSA autoantibodies were also found in patients with Sjogren's syndrome secondary to SLE but anti-SSB autoantibodies were found in the sera of only those SLE patients who have Sjogren's syndrome or co-existed with SSA antibodies in lupus patients.

Table. V.- Anti-Sm, SSA, SSB, histone autoantibodies frequencies in SLE patients (n=61).

Parameter*s	Mean (U/ml)	Range	Minimum (U/ml)	Maximum (U/ml)
Anti-Sm	12.59 ± 1.9709	69	1	70
Anti-SSA	63.541 ± 9.863	199	1	200
Anti-SSB	23.961 ± 6.719	199	1	200
Anti-histone	12.25 ± 4.495	198	2	200

*For Abbreviations see Table I.

Table. VI.- One sample t-Test for anti-Sm, anti-SSA, anti-SSB, anti-histone autoantibodies in SLE patients (n=61).

Autoantibodies	Cut-off value=15 U/ml		
	t	p	Results
Anti-Sm	1.223	0.226	> 0.05
Anti-SSA	4.921	0.00	< 0.05
Anti-SSB	1.334	0.187	> 0.05
Anti-histone	12.835	0.006	< 0.05

*For Abbreviations see Table I.

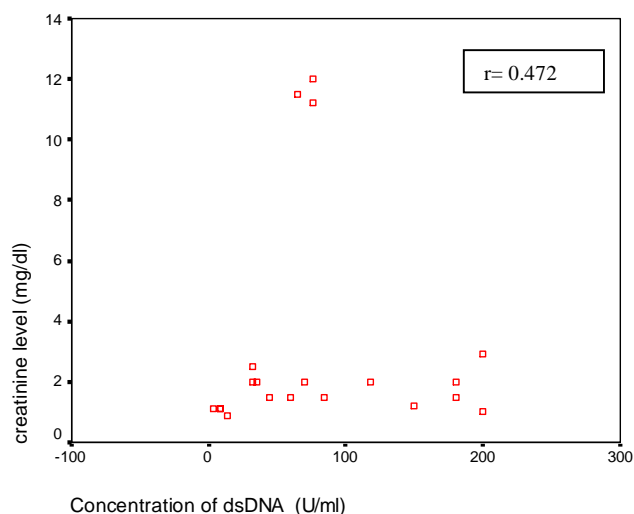


Fig. 1. Relationship of dsDNA with creatinine level in SLE patients (n=61).

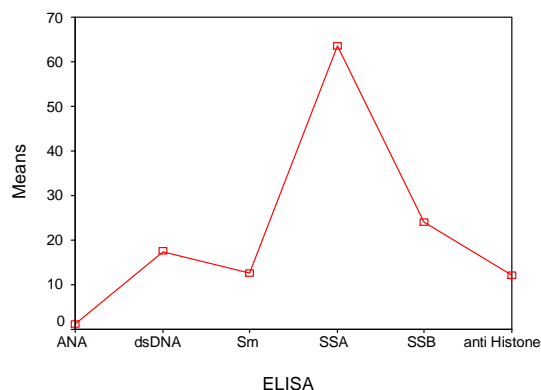


Fig. 2. Mean plot of different autoantibodies detected by ELISA. For Abbreviations see Table I.

Figure 1 shows a positive correlation between anti-dsDNA and creatinine. High levels of creatinine and proteinuria are the indicators of lupus nephritis. Cut-off value for anti-dsDNA was 20 U/ml and the normal range of serum creatinine was 0.5-0.7 mg/dl. The diagram represented showed the positive correlation between anti-dsDNA and lupus nephritis. Analysis of Variance (ANOVA) was carried out by SPSS ver 13 in order to check whether or not the means of several groups are all equal. Through ANOVA using F-test, the Figure 2 represented that the mean of SSA was significantly higher, and the p-value was 0.000. Thus, the differences in the clinical presentation or in the

autoantibody profile of SLE patients were most probably due to genetic differences. There is a need for more work at the molecular level in this aspect of the Pakistani population.

DISCUSSION

The region variation in the presentation of SLE is related to different genetic and environmental factors. Hassan *et al.* (2004) demonstrated that lupus disease gets activate during sunny season. Lahore is the second largest city of Pakistan, the total estimated population of which is approximately 10 million; this makes Lahore the fifth largest city in South Asia and the 23rd largest city in the world. Lahore is bounded on the north and west by the Sheikhpura District, on the east by Wagah, and on the south by Kasur District. Lahore is in the Punjab province of Pakistan and covers a total area of 404 km². The weather of Lahore is extreme during the months of May, June, and July, when the temperature reaches to 50^oC. In August, the monsoon season starts while December, January and February are the coldest months when the temperature drop to -1^oC (Punjab portal, 2007).

In Pakistan, data on the characteristics of SLE is scarce, but it is not uncommon in the South East Asian region. Different characteristics were viewed in Asian Lupus population like lupus is more common in females than males (Ratio: 8-28 females to 1 male). 45-98% of the SLE patients showed cutaneous and mucoskeletal involvement and 6-100% had lupus nephritis. The survival rate was up to 5-years in 65-98% of the SLE patients. In Asian lupus population main causes of mortality are infection and active disease while the main organs involved are kidney and CNS. In the present study, female versus male ratio was approximately 9:1; here more female lupus patients were affected than the males, so hormonal factor might be one of the reasons involved in the pathogenesis of SLE. Due to long course of the disease and inadequate follow-up of patients, mortality rate could not be calculated.

Tench *et al* (2009) calculated fatigue score and found that fatigue in SLE patients is significantly associated with hypothyroidism, anemia, poor sleep quality, anxiety, depression, and also with higher disease activity (Tench *et al.*,

2009). The subject population presented with fever, fatigue, arthritis, lupus nephritis in combination with mucocutaneous lesions. Fatigue was claimed by 100% of the lupus patients but it was found to be difficult to evaluate because of the involvement of multiple factors like anemia, pain, poor sleep quality, depression, and lack of social support. Ultraviolet radiation (UV) is one of the environmental factors that cause the disease to flare up. Mechanisms like modulation of autoantibody location, apoptosis induction, up-regulation of adhesion molecules and cytokines, induction of nitric oxide synthase, UV generated antigenic DNA are involved in photosensitivity (Ahmed *et al.*, 2002). In this study, 18.03% of the lupus patients met one of the ACR criteria that were photosensitivity and one of the mechanisms mentioned above might be responsible for photosensitivity.

ANA-negative lupus covers approximately 5% of the SLE population; these patients appear similar to those with subacute cutaneous lupus erythematosus clinically and serologically. The age of onset as well as sex predominance is similar to the ANA positive SLE patients. It is likely that there is an overlap between ANA-negative lupus patients and those having subacute cutaneous lupus erythematosus. Systemic involvement in ANA-negative lupus patient is not severe while lupus nephritis is generally mild (Conrad *et al.*, 2002). In this study, 75.4% of the SLE patients were ANA positive while 24.6% of the patients were negative. The cases that were ANA negative fulfilled > 5 of the revised ACR criteria but these patients were negative probably because of the prolong medication. In order to confirm ANA negative patients as SLE patients, dsDNA was performed by ELISA as a preferable screening marker. Berden and his colleagues (1999) proposed that 40-80% of the SLE patients have detectable antibodies to dsDNA; these pathogenic autoantibodies bind to nucleosomes and entered into the bloodstream to get settled into the renal glomerular basement membrane in order to initiate glomerulonephritis. This series of events was later demonstrated in animal models (Crispin *et al.*, 2008). The present study showed a significant association of ANA and of anti-dsDNA with SLE.

Koshak and Mughales (2000), found a highly positive correlation between ANA and dsDNA. In Thailand, positive ANA was reported in 91% of the lupus patients; while the positive predictive value for the diagnosis of SLE in Thai population was 57% with the negative predictive value of 97% (Koshak and Mughales, 2000). In this study, the positive predictive value (PPV) of anti nuclear antibody and of double stranded DNA was 100%. In 1991, Smeenk *et al* found that the specificity of ELISA for dsDNA is 100% and sensitivity is 88%; similarly in this study, the specificity of dsDNA was 100% while the sensitivity was 72.13%. Clinical association of anti-SSA with SLE has been found in the Japanese population (Crispin and Tsokos, 2010). Goeb *et al* (2007) studied that anti-SSA antibodies are found in 30-60% of Primary Sjogren's syndrome patients and are not considered specific to the disease because they are also seen in 30% of the SLE patients while anti-SSB are found to be specific for Sjogren's syndrome and can co-exist with SSA antibodies in more than 50% of the patients (Goeb *et al.*, 2007). There is a high prevalence of anti-Sm antibodies in Asians lupus patients than their western counterparts. The frequency of anti-Sm antibodies was found to be highest in Indian (25.3%), Singaporean (26%), and Vietnamese (36%) lupus patients (Izmirly *et al.*, 2010). In this study, one sample t- test was applied to compare the mean score of a sample for different autoantibodies to a known cut-off value.

There are two ways in which certain drugs may interact with lupus: they will either induce lupus or flare-up the disease activity (Olsen, 2004). Antibodies to histones H1 and H3 are markers with high specificity of 93.6-96.4% for drug induced lupus (Leber and Lakdawala, 2009). In this study, two (3.3%) SLE patients manifested autoantibodies; such patients were the cases of drug-induced lupus. In the present study, the most affected organs in SLE patients were kidney, joints and skin. In all of the cases, there is inflammation; and deposition of antibodies and complement. Mannik *et al* (2003) detected IgG that bound to a number of non-DNA antigens, including ribonucleoprotein complex, RNA binding protein, a subunit of the C1 complement component and nuclear particles consisting of several different polypeptides (Mannik

et al., 2003). In Malaysian lupus patients, a clinical association was found between dsDNA and lupus nephritis (Rahman and Isenberg, 2008). In this study, the incidence of lupus nephritis in lupus patients was comparable to the other studies. There are 100 types of arthritis and two of them are rheumatoid arthritis and lupus. There are specific as well as overlapping symptoms associated with lupus and rheumatoid arthritis for example the bone mineral density of matched SLE and RA patients was reduced to a similar extent (Bang *et al.*, 2007). The incidence of arthritis was lower than that of the oral ulcers in Saudi lupus population (Hussain *et al.*, 2005). In the present study, rheumatoid arthritis factor was found to be positive 31.14% of the SLE patients while oral ulcer was found in 3.27% of the SLE patients so unlike Hussain *et al* study; the cases of rheumatoid arthritis were more than the cases of oral ulcers in Pakistani lupus population.

In the eye, immune complex deposits in the vascular endothelium of the conjunctiva, sclera, in the basement membrane of the ciliary body, cornea, along the peripheral nerves of the ciliary body, cornea and along the peripheral nerves of the ciliary body and conjunctiva. This will alter the tissue structure as well as the function and such SLE patients with retinopathy have a high morbidity rate. Optic nerve disease occurs in about 1% of the SLE patients while retinal disease is common in about 10% of the SLE patients (Sivaraj *et al.*, 2008). In 2003, Shahnaz *et al* found Cytomegalovirus retinitis in an AIDS patient with SLE and end-stage renal disease, who was receiving azathioprine and low-dose corticosteroids (Shahnaz *et al.*, 2003). In this study, 3 (4.91%) SLE patients had eye problem; all of them were receiving low-dose of corticosteroids. In an SLE patient of Bull's eye maculopathy, there was an increase pigmentation of macula retina which was further accompanied by degeneration.

CONCLUSIONS

The present study showed that SLE is a highly heterogeneous, complex disease; presenting differently from patient to patient with no single clinical or immunological feature required to make a confirm diagnosis. SLE was a rare disease among Pakistani population. Circulating antinuclear

antibodies were commonly seen in SLE patients and their presence form part of the diagnosis. Pathogenic autoantibodies were found to be the main cause of tissue damage in lupus patients. The deposition of autoantibodies in organs, such as the kidney, joints or the skin, resulted in chronic inflammation. The differences in the clinical presentation of lupus patients or in the autoantibody profile of SLE patients were most probably due to genetic differences. There is a need for more work at the molecular level in this aspect of the Pakistani population.

REFERENCES

- AHMED, T.A., IKRAM, N., HUSSAIN, T., FAROOQUI, A., HALEEM, A., BASHIR, M. AND MOIN, S., 2002. Clinical and laboratory features of SLE in Pakistani patients. *J. Pak. med. Assoc.*, **52**: 12-5.
- BANG, H., EGERER, K., GAULIARD, A., LÜTHKE, K., RUDOLPH, P.E., AND FREDENHAGEN, G., 2007. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arth. Rheum.*, **56**: 2503-11.
- CONRAD, K., SCHÖBLER, W. AND HIEPE, F., 2002. Autoantibodies in Systemic Autoimmune Diseases. A Diagnostic Reference. Autoantigens, autoantibodies, *Autoimmunity*. **1**: 2. Pabst Science Publishers.
- CRISPÍN, J.C. AND TSOKOS, G.C., 2010. IL-17 in Systemic Lupus Erythematosus. *J. Biomed. Biotech.*, **10**: 1155.
- CRISPIN, J.C., OUKKA, M., BAYLISS, G., COHEN, R.A., VAN BEEK, C.A., STILLMAN, I.E., KYATTARIS, V.C., JAUNG, Y.T. AND TSOKOS, G.C., 2008. Expanded double negative T cells in patients with SLE produce IL-17 and infiltrate the kidneys. *J. Immunol.*, **181**: 8761-8766.
- ENGER, W., 2000. The use of laboratory tests in the diagnosis of SLE. *J. clin. Pathol.*, **53**: 424-432.
- GOEB, V., SALLE, V., DUHAUT, P., JOUEN, F., SAMIL, A., DOCROIX, J.P., TRON, F., LELOET, X. AND VITTECOQ, O., 2007. Clinical significance of autoantibodies recognizing Sjogren's syndrome A (SSA), SSB, Calpastatin and alpha-Fodrin in Primary Sjogren's syndrome. *Clin. exp. Immunol.*, **148**: 281-287.
- HASAN, T., PERTOVAARA, M., YLI-KERTTULA, U., LUUKKAALA, T. AND KORPELA, M., 2004. Seasonal variation of disease activity of systemic lupus erythematosus in Finland: a 1 year follow up study. *Ann. Rheum. Dis.*, **63**:1498-500.
- HOCHBERG, M.C., 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis. Rheum.*, **40**: 1725.
- HUSSAIN, W.M., KARIMA, T.M., FATANI, M.I., BUKHARI, S.Z. AND SHAHZAD, M.T., 2005. Systemic lupus erythematosus in Makkah, Saudia Arabia. *Kuwait med J.*, **37**: 267-270.
- IZMIRLY, P.M., LLANOS, C., LEE, L.A., ASKANASE, A., KIM, M.Y. AND BUYON, J.P., 2010. Cutaneous manifestations of neonatal lupus and risk of subsequent congenital heart block. *Arth. Rheum.*, **62**: 1153 – 1157.
- KOSHAK, E.A. AND MUGHALES, J., 2000. How the serological correlation between ANA and dsDNA can enhance cost effectiveness? *Annls Saudi Med.*, **20**: 5-6.
- LANGGUTH, D.M., MORRIS, S., CLIFFORD, L., WILSON, R.J., NEIL, J., HOGAN, P.G. AND WONG, C.W., 2007. Specific testing for isolated anti-52KDa SSA/Ro antibodies during standard anti-extractable nuclear antigen testing is of limited clinical value. *J. clin. Pathol.*, **60**: 670-673.
- LEBER, M.J. AND LAKDAWALA, V.S., 2009. Systemic lupus erythematosus. *Rheumatology*, **27**: 1-23.
- MACENEANCY, P.M. AND MALONE, D.E., 2000. The meaning of diagnostic test results: a spread sheet for swift data analysis. *Clin. Radiol.*, **55**: 227-235.
- MANSON, J.J. AND ISENBERG, A., 2005. *Systemic Lupus Erythematosus*. Orphanet Encyclopedia.
- MIGLIORINI, P., BALDINI, C., ROCCHI, V. AND BOMABARDIERI, S., 2005. Anti-Sm and anti-RNP antibodies. *Autoimmunity*, **38**: 47-54.
- MILIC, V.D., PETROVIC, R.R., BORICIC, I.V., MARINKOVIC-ERIC, J., RADUNOVIC, G.L., JERICIC, J.D., PEINOVIC, N.N. AND DAMIANOV, N.S., 2009 Diagnostic value of salivary gland ultrasonographic scoring system in Sjogren's syndrome: A comparison with scintigraphy and biopsy. *J. Rheumatol.*, **36**: 1495-1500.
- OLSEN, N.J., 2004. Drug induced lupus autoimmunity. *Best. Prac. Res. Clin. Rheum.*, **18**: 677.
- PUNJAB PORTAL. Government of the Punjab. Retrieved on 2007-12-11.
- RAHMAN, A. AND ISENBERG, D., 2008. Systemic Lupus Erythematosus. *N. Engl. J. Med.*, **358**: 929-939.
- REICHLIN, M. AND VAN VENROOJ, W.J., 1991. Autoantibodies to the URNP particles: Relationship to clinical diagnosis and nephritis. *Clin. exp. Immunol.*, **83**: 286-290.
- SHAHNAZ, S., CHOKSI, M.T. AND TAN, I.J., 2003. Bilateral Cytomegalovirus retinitis in a patient with SLE and End-stage renal disease. *Mayo Clin. Proc.*, **78**: 1412-1415.
- SIVARAJ, R.R., DURRANI, O.M., DENNISTON, A.K., MURRAY, P.I. AND GORDON, C., 2008. Ocular manifestations of SLE. *Rheumatology*, **46**: 1757-1762.

TENCH, C.M., MCCURDIE, I., WHITE, P.D. AND D'CRUZ, D.P., 2009. The prevalence and associations of fatigue in systemic lupus erythematosus. *Rheumatology*, **39**: 1249-1254.

VAN BRUGGEN, B., WALGREEN, T.P., RIJKE, W., TAMBOER, K., KRAMERS, R.J., SMEENK, M., MONESTIER, G.J. AND FOURNIE, J.H., 2007.

Antigen specificity of anti-nuclear antibodies complexed to nucleosomes determines glomerular basement membrane binding *in vivo*. *Eur. J. Immunol.*, **27**: 1564–1569.

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