

Sex Hormones and Peripheral White Blood Cell Subsets in Systemic Lupus Erythematosus Patients

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ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by antibodies to nuclear antigens, particularly anti-dsDNA. Imbalance between production and destruction of immune cells causes cytopenia. Sex hormones have immunomodulatory effects; estrogen increases the production of autoantibodies in SLE prone NZB/NZW mice. **Objective:** To investigate the relationship between sex hormones, anti-dsDNA, and lymphocyte subsets in Iranian patients with SLE. **Methods:** 38 SLE patients (28 females and 10 males) meeting 4 of 11 ACR revised criteria for SLE classification, and 20 age and sex matched healthy individuals (10 females and 10 males) participated in this study. Lymphocyte subsets were analyzed using flow cytometric analysis. Serum anti-dsDNA levels and sex hormones concentrations were determined using commercial ELISA and RIA kits, respectively. **Results:** The absolute count of white blood cells, lymphocytes, T lymphocytes (CD3⁺), T helper cells (CD3⁺CD4⁺), B cells (CD19⁺) and Nk cells (CD3⁻ CD16⁺CD56⁺) in SLE patients diminished significantly in comparison to control group (p<0.05). IgG anti-dsDNA antibody levels were significantly higher in patients compared to controls as expected (p<0.05). Prolactin increased significantly, while DHEAS showed a significant decrease in SLE patients compared with the controls (p<0.05), however the level of estrogen did not have any significant difference in SLE patients in comparison to controls. **Conclusion:** Increased concentration of prolactin together with a simultaneous decrease in serum DHEAS in SLE patients are associated with anti-dsDNA elevation and a decrease in almost all lymphocyte subsets.

Keywords: SLE, Anti-dsDNA, Estrogen, Progesterone, Prolactin, DHEAS

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by antibodies to nuclear antigens, especially anti-dsDNA and is accompanied by abnormal immunoregulation. The cause of SLE is unknown, but genetic, infectious and environmental factors are important and can activate the disease (1, 2). The disease affects women in child bearing ages more than men (3).

SLE is characterized by recurrent flares and for the laboratory follow-up, parameters such as anti-dsDNA antibodies, complements titer and leukocytopenia have been described (4).

Imbalance between the production and destruction of various types of immune cells can cause cytopenia. The main reason for cytopenia in SLE is unknown, but it appears that an increase of anti-lymphocyte antibodies is involved in cell destruction (5), and most patients develop anti-lymphocyte autoantibodies during their illness, and there is a positive association between disease flare and lymphopenia (6).

Sex hormones have profound immunomodulatory effects and are the strongest risk factor for developing SLE is female gender (7); female sex hormones enhance the immune responses, and accelerate the development of SLE and may be involved in the pathogenesis of the disease (8). In contrast androgens are immune suppressors and have protective effects in NZB/NZW lupus models in mice and inhibit anti-DNA antibodies production.

It has been noted that an important effect of female sex hormones is the ability to increase production of autoantibodies in SLE patients and in SLE prone NZB/NZW mice (9). Most SLE patients develop anti-lymphocyte autoantibodies and their titer increase with disease activity. These patients also have diminished subsets of lymphocyte populations.

In this study we have investigated the relationship between the amounts of sex hormones and lymphocyte subsets in patients with Systemic Lupus Erythematosus.

MATERIALS AND METHODS

Study Participants. The study group was comprised of 38 SLE patients (28 females and 10 males) with mean age of 27 years (16-47 years). All patients met 4 of 11 ACR (American College of Rheumatology) revised criteria for the SLE classification. Patients received no treatment or less than 10 mg/day prednisolone and/or 200 mg/day hydroxychloroquine, and none of them received any cytotoxic drugs. Twenty sex and age matched healthy individuals (10 females and 10 males) with a mean age of 28 years (18-42 years) participated as controls. All the female patients and controls were in their mid menstrual cycle (days 5-14), and none of them were taking birth control drugs.

This study was approved by Mashhad University of Medical Sciences ethics committee. Written informed consent was obtained from all participating patients and controls.

Detection of Lymphocyte Subsets. Three color antibodies CD4-FITC/CD8-PE/CD3-PreCP, CD3-FITC/CD19-PE/CD45-PreCP and two color antibody CD3-FITC/CD16+56-PE were purchased from IQ Products, The Netherlands. For staining cell surface markers, 20µl of fluorescent antibodies were added to 100µl of whole blood and incubated for 20 minutes at room temperature, then the RBCs were lysed using IQ Lyse solution (IQ Products, The Netherlands), and stained cells were washed twice

using PBS to exclude excess antibodies. Flow cytometry experiments were performed using FACSCalibur (Becton Dickinson, USA). The results were analyzed using Cellquest software (Becton Dickinson, USA).

Determination of Sex Hormones and Anti-dsDNA Concentrations. Sera from patients were separated and stored at -20°C until all were tested at the same time. For quantitative measurement of IgG anti-dsDNA antibodies, we used a commercial ELISA kit (Euroimmun AG, Germany). For determining sex hormones commercial RIA kits were purchased from Orion Diagnostica, Finland. The tests were performed according to the manufacturer's procedure.

Statistical Analyses. Data were presented as mean \pm standard error of mean (S.E.M.) and analyzed using SPSS version 11.5 software. The normal distribution of the investigated subsets was explored by the descriptive statistics test. Significance of differences between means was calculated using the Student's *t*-test. Mann-Whitney non-parametric tests were used for comparison of the variables with abnormal distribution. Statistically significant differences were accepted at a $p < 0.05$.

RESULTS

38 SLE patients (28 females and 10 males) with a mean age of 27 years (16-47 years) and 20 age and sex matched controls with a mean age of 28 years (18-42 years) participated in this study. In all patients we quantified the following lymphocyte subpopulations: T cells (CD3^+), T helper cells ($\text{CD3}^+\text{CD4}^+$), T cytotoxic cells ($\text{CD3}^+\text{CD8}^+$), B cells (CD19^+) and NK cells ($\text{CD3}^-\text{CD16}^+\text{56}^+$) (Table 1). The absolute counts of white blood cells, lymphocytes, CD3^+ T cells, $\text{CD3}^+\text{CD4}^+$ T helper cells as well as CD19^+ B cells, and $\text{CD3}^-\text{CD16}^+\text{56}^+$ NK cells were significantly lower in patients compared to controls. Although the absolute count of $\text{CD3}^+\text{CD8}^+$ T cytotoxic cells had a slight increase in the patients, but the difference was not significant compared to controls. The mean level of IgG anti-DNA antibodies in SLE patients was considerably higher as expected. The increase of estrogen and progesterone levels in patients was not statistically significant, however prolactin concentration was significantly higher and DHEAS level was significantly lower in patients in comparison to controls.

We then analysed the results separately in female and male patients and controls. Table 2 shows that differences between female patients and controls are similar to differences between all the patients and controls except for a decrease in CD19^+ B cells in SLE patients which was not statistically significant compared to female controls. When the progesterone level in the male patients and controls was analysed separately as shown in Table 3, a significant decrease in progesterone level was spotted in the male patients.

Table 1. Lymphocyte subsets, and anti-dsDNA and sex hormone levels in SLE patients and controls

Sam- ples	WBC	lymph	CD3	CD4	CD8	CD19	NK	CD4/ CD8	Anti- DNA	Estr	Prog	Prola	DHEA
SLE patients	5733.8 ±339.8	1423.6 ±112.2	1096.5 ±93.9	609.8 ± 59.6	432.3 ± 44.3	158.9 ± 18.8	128.6 ± 16.9	1.5 ± 0.15	265.7 ±43.0 IU/ml	94.4 ± 19.5 pmol/l	1.7 ±0.7 nmol/l	24.4 ± 3.1 mIU/l	576.8 ±117.6 umol/l
Normal controls	7919.0 ±445.7	2091.0 ±170.3	1510.5 ±126.1	979.2 ±85.7	413.8 ± 36.5	237.1 ± 31.4	247.4 ± 20.6	2.5 ± 0.19	52.0 ± 8.7 IU/ml	60.8 ± 14.2 pmol/l	0.57 ± 0.10 nmol/l	11.2 ± 1.2 mIU/l	1725.3 ±231.1 umol/l
	P<0.05	P<0.05	P<0.05	P<0.05		P<0.05	P<0.05	P<0.05	P<0.05			P<0.05	P<0.05

Table 2. Lymphocyte subsets, and anti-dsDNA and sex hormone levels in female SLE patients and controls

Sam- ples	WBC	lymph	CD3	CD4	CD8	CD19	NK	CD4/ CD8	Anti- DNA	Estr	Prog	Prola	DHE A
Female SLE patients	5497.8 ±448.8	1417.8 ±149.3	1090.7 ±124.1	624.0 ± 78.2	420.0 ±58.2	178.6 ±21.8	101.2 ±19.2	1.7 ± 0.21	300.6 ±57.04 IU/ml	134.6 ± 27.8 pmol/l	2.5 ±1.1 nmol/l	25.08 ± 4.3 mIU/l	613.6 ±160.6 umol/l
Female controls	7736.3 ±599.1	2051.9 ±208.3	1503.1 ±159.0	988.3 ±111.9	399.1 ±44.4	243.5 ±38.7	220.7 ±27.2	2.5 ± 0.20	52.0 ± 6.3 IU/ml	92.9 ± 23.6 pmol/l	0.67 ±0.18 nmol/l	13.7 ± 2.03 mIU/l	1646.8 ±340.6 umol/l
	P<0.05	P<0.05	P<0.05	P<0.05			P<0.05	P<0.05	P<0.05			P<0.05	P<0.05

Table 3. Lymphocyte subsets, and anti-dsDNA and sex hormone levels in male SLE patients and controls

Sam- ples	WBC	lymph	CD3	CD4	CD8	CD19	NK	CD4/ CD8	Anti- DNA	Estr	Prog	Prola	DHEA
Male SLE patients	6227.2 ±463.7	1321.7 ±125.0	1018.6 ±115.6	580.0 ± 89.4	457.9 ± 65.8	119.4 ± 34.4	183.4 ± 27.4	1.3 ± 0.21	203.9 ± 62.7 IU/ml	26.4 ± 4.9 pmol/l	0.2 ± 0.02 nmol/l	23.0 ± 4.2 mIU/l	508.8 ±165.9 umol/l
Male controls	8120.0 ±692.7	2134.1 ±286.2	1709.6 ±207.9	969.2 ±137.7	429.9 ± 61.3	230.2 ± 52.6	276.9 ± 29.8	2.5 ± 0.36	39.0 ± 8.0 IU/ml	25.6 ±1.9 pmol/l	0.46 ± 0.04 nmol/l	8.6 ± 0.74 mIU/l	1803.8 ±328.8 umol/l
	P<0.05	P<0.05	P<0.05	P<0.05		P<0.05	P<0.05	P<0.05	P<0.05			P<0.05	P<0.05

DISCUSSION

Some sex hormones have receptors on immune cell surfaces and modulate immune responses (10). Estrogen prolongs the survival of autoimmune cells, increases the Th2-related cytokine production, and stimulates antibody production by B cells. Recent investigations have suggested that immunostimulatory action of estrogen requires the presence of prolactin (11). Estrogen stimulates prolactin secretion and prolactin increases immune responses towards antibody production (12). An association between increased prolactin levels with anti-dsDNA antibodies has been reported (13). Our study shows a significant increase in prolactin level in patients. High prolactin concentrations may in turn suppress estrogen secretion and can explain the slight nonsignificant increase we found in this study. Androgens have suppressive effects on immune responses (9, 14), and their decrease correlates with SLE activity. DHEAS has been shown to be associated with an increase in Th₁ and an inhibition of Th₂ immune responses (15). Stimulatory effects of prolactin with simultaneous androgenic decrease in SLE patients

might be responsible for the increased anti-dsDNA and anti-lymphocytic autoantibodies (16). Diminished peripheral blood cell counts are one of the characteristics of patients with SLE (17- 21), and leucopenia and lymphopenia are considered as criteria for the diagnosis of SLE (17). This study also shows a significant decrease ($p<0.05$) in the absolute number of white blood cells, lymphocytes, T lymphocytes ($CD3^+$), T helper cells ($CD3^+CD4^+$), B cells ($CD19^+$), NK cells ($CD3^-CD16^+56^+$) and CD4/CD8 ratio in SLE patients compared to control group.

Although one characteristic feature of SLE is increased activation of B cells secreting autoantibodies, however it has been shown that some SLE sera contain autoantibodies to B cells (22, 23). These antibodies might explain why B cells are decreased in our patients compared to controls ($p<0.05$). In the patients the absolute number of NK cells are decreased in comparison to controls ($p<0.05$). It has been reported that in active SLE, the absolute number of NK cells are decreased (24). This reduction might be due to the presence of antilymphocyte autoantibodies and FCRs (CD16) modulation by immune complexes (25).

$CD19^+$ B cells show a significant decrease in male patients compared to male controls (Table 3) which could be explained by the significant lower concentration of progesterone. This decrease is not significant in female patients, since the progesterone level does not show a significant difference in female patients compared to the controls.

In conclusion this study shows that increased concentration of prolactin with simultaneous decrease in DHEAS level in SLE patients is associated with anti-dsDNA elevation and a decrease in lymphocyte subsets which is probably due to the presence of antilymphocyte auto-antibodies.

ACKNOWLEDGEMENTS

We acknowledge the support of vice president for research, Mashhad University of Medical Sciences for funding this study.

REFERENCES

- Hart HH, Grigor RR, Caughey DE. Ethnic difference in the prevalence of systemic lupus erythematosus, *Ann Rheum Dis*, 1983; 42:529-32.
- Cooper GS, Dooley MA, Treadwell EL, St. Clair EW, Parks CG, Gilkeson GS. Hormonal, environment and infectious risk factors for developing systemic lupus erythematosus. *Arthritis Rheum*, 1998; 41:1714-24.
- Hochberg, MC. Epidemiology of systemic lupus erythematosus. In: Lahita RG, editor. *Systemic Lupus Erythematosus*. New York: Churchill Livingstone; 1992.p.103-17.
- Nauta AJ, Daha MR, van Kooten C, Roos A. Recognition and clearance of apoptotic cells:a role for complement and pentraxins. *Trends Immunol*. 2003; 24:148-54.
- Butler WT, Sharp JT, Rossen RD. Relationship of the clinical course of systemic lupus erythematosus to the presence of cold-reactive anti-lymphocyte antibodies with lymphopenia in systemic lupus erythematosus. *Arthritis Rheum*, 1975; 15:231-238.
- Winfield JB. Antilymphocyte Antibodies. In: Wallace DJ, Hahn BH, editors. *Dubois' Lupus Erythematosus*. Baltimore, MD;1996.p.505-15
- Rider V, Abdou NI. Gender differences in autoimmunity: molecular basis for estrogen effects in systemic lupus erythematosus. *Int Immunophar*. 2001; 1: 1009-24.
- Kaufman SH. Proteolytic cleavage during chemotherapy induced apoptosis. *Mol Med Today*. 1996; 2: 298-303
- Roubinian J, Talal N, Siiteri PK, Sadakian JA. Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum*, 1979; 22:1162-9.
- Nalbandian G, Kovats S. Estrogen, immunity and autoimmune disease. *Curr Med Chem Immune Endoc & Metab. Agents* 2005; 5: 85-91.
- Peeva E, Grimaldi C, Spatz L, Diamond B. Bromocriptine restores tolerance in estrogen-treated mice. *J Clin Invest*. 2000; 106:1373-9.
- McMurray RW. Estrogen, prolactin, and autoimmunity: actions and interactions. *Intern Immunopharm* 2001; 1:995-1008.

- 13 McMurray R, Keisler D, Izui S, Walker SE. Hyperprolactinemia in male NZB/NZW (B/W) F1 mice: accelerated autoimmune disease with normal circulating testosterone. *Clin Immunol Immunopathol.* 1994; 71: 338-43.
- 14 Verthelyi D. Sex hormones as immunomodulators in health and disease. *Int Immunopharmacol.* 2001; 1:983-93.
- 15 Suzuki T, Suzuki N, Engleman EG, Mizushima Y, Sakane T. Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol.* 1995; 99: 251-5.
- 16 Vera-Lastra O, Jara LJ, Espinoza LR. Prolactin and autoimmunity. *Autoimmun Rev.* 2002; 1: 360-4.
- 17 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum,* 1982; 25:1271-7.
- 18 Gleeson PA, Toh BH, van Driel IR. Organ-specific autoimmunity induced by lymphopenia. *Immunol Rev.* 1996; 149:97-125.
- 19 Mirzayan MJ, Schmidt RE, Witte T. Prognostic parameters for flare in systemic lupus erythematosus. *Rheumatology (Oxford).* 2000; 39:1316-9.
- 20 Starkebaum G. Chronic neutropenia associated with autoimmune disease. *Semin Hematol.* 2002; 39:121-7.
- 21 Guma M, Krakauer R. CD4+ lymphocytopenia in systemic lupus erythematosus. *Ann Intern Med.* 1994; 120:168-9.
- 22 Park MS, Terasaki PI, Bernoco D. Autoantibody against B lymphocytes. *Lancet.* 1977; 3;2:465-7.
- 23 Packer SH, Logue GL. Quantification of warm reactive IgG antilymphocyte autoantibodies in systemic lupus erythematosus. *Clin Immunol Immunopathol.* 1980;17: 515-29.
- 24 Struyf NJ, Snoeck HW, Bridts CH, De Clerck LS, Stevens WJ. Natural killer cell activity in Sjogren's syndrome and systemic lupus erythematosus: stimulation with interferons and interleukin-2 and correlation with immune complexes. *Ann Rheum Dis.* 1990; 49:690-3.
- 25 Rook AH, Tsokos GC, Quinnan GV Jr, Balow JE, Ramsey KM, Stocks N et al. Cytotoxic antibodies to natural killer cells in systemic lupus erythematosus. *Clin Immunol Immunopathol.* 1982; 24: 179- 85.