Original Article

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Internal Medicine Section

Relationship between Serum Level of Interleukin-2 in Patients with Systemic Lupus Erythematosus and Disease Activity in Comparison with Control Group

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ABSTRACT

Background: Despite the large number of surveys, there are not any validated biomarkers for SLE disease activity till now. This study aimed to evaluate the relationship between serum level of IL-2 in patients with SLE and disease activity in comparison with control group.

Materials and Methods: In this case-control study, 73 patients with lupus and 73 healthy subjects referred to the rheumatology clinic of 5 Azar Hospital in Gorgan (North of Iran).They were studied via convenience sampling during 2011-2012. Blood samples were taken from both groups and serum levels of interleukin -2 measured by Avi Bion Human IL-2 ELISA kit. Serum Level of IL-2 greater than 15 pg/ml defined positive and lesser than this amount defined negative. Disease activity evaluated with SLE disease activity index. Score greater than or equal to three or four defined as active disease. Data analysis conducted by SPSS software (version 16) and by using descriptive statistics and statistical tests.

Results: Serum level of IL-2 was positive in 45.2% of sample studied and negative in 54.8% in case group, while in control group, serum level of IL-2 only in 11% of sample studied was positive and in 89% was negative. Statistical analysis indicated a significant relationship between serum level of IL-2 and the SLE disease activity index (p=0.025).

Conclusion: This study showed the relationship between serum levels of IL-2 and disease activity, so this biomarker can be used as a clinical indicator for assessing disease activity in patients with SLE.

Keywords: Adults, Disease activity, ELISA, Interleukin-2, Systematic lupus erythematosus

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease which affects almost every systems in the body with different degrees of severity disease [1]. In this disease, organs and cells damage by auto-antibodies binding tissue and immune complexes [2]. It is characterized by an imbalance in the cytokine network [3] and lupus T and natural killer (NK) cells fail to produce enough IL-2 and transforming growth factor (TGF-β) to induce and maintain regulatory CD4+ and CD8+ T cells that result in continuous production of auto-antibodies and immune complexes. In chronic inflammation, accumulation of growth factors and products of chronic oxidation lead to irreversible tissue damage such as fibrosis/sclerosis in glomeruli, arteries, brain, lungs, and other tissues [2]. Alcocer-Varela indicated that in SLE, T cells produce decreased amounts of IL-2 that is very obvious in cells of patients with active type of disease [4]. Campen also indicated a strong relationship between serum levels of IL-2R and clinical and laboratory indicators of disease activity in patients with lupus and rheumatoid arthritis that correlated with disease activity better than the erythrocyte sedimentation rate (ESR) and it may be a reliable serologic indicator of disease activity in inflammatory diseases characterized by immune system activation [5]. Due to the great heterogeneity of the disease and complex and difficult process to validate individual biomarkers, there are very limited numbers of consensus biomarkers to aid the treatment in SLE [6].

Despite the large number of surveys, there are not any validated biomarkers for SLE disease activity up to now. However, the vast majority of studies were not validated biomarkers abnormalities. Identification of these biomarkers may make it possible to assess disease activity and response to therapy in patients with SLE [7] and it can enhance the clinical management of patients and prepare more effective therapies for diseases that their treatment is very difficult [6]. Approving the relationship between serum levels of IL-2 and disease activity index can help produce novel drugs, more effective control of disease and reduce the disease implications in patient with SLE, so this study aimed to evaluate the relationship between serum level of IL-2 in patients with SLE and disease activity in comparison with control group.

MATERIALS AND METHODS

In this case-control study, 146 patients referred to the rheumatology clinic of 5 Azar Hospital in Gorgan (North of Iran).They were studied via convenience sampling during 2011 to 2012. Seventy three patients with the definite diagnosis of SLE based on clinical manifestations and laboratory tests and having greater than or equal to four from eleven criteria [2] classified to case group and 73 healthy subjects matched by age and sex entered to the study as control group. At the start of the study, all the patients in case group were under the treatment, however, to minimize the effect of treatment on serum level of IL-2 in case group, the patients did not receive any treatment 48 hours before the blood samples were taken.

In this study, medical history was taken and physical examination conducted by rheumatologist and laboratory tests asked if needed and patients with following conditions excluded from the study: history of thyroid disease (Graves' disease and Hashimoto's thyroiditis), atopic dermatitis and psoriasis, asthma and idiopathic hypereosinophilic syndrome (HES), end stage renal disease (ESRD) and hemodialysis, malignancy, repetitive abortion and early pregnancy loss, congestive heart failure (CHF) and recent myocardial infarction (MI), liver disease and autoimmune diseases such as rheumatoid arthritis (RA) and Behcet disease, chronic headache, brain injury and cerebrovascular accident (CVA),

schizophrenia, infectious diseases especially tuberculosis (TB), human immunodeficiency virus (HIV) and herpes simplex, pancreatitis, diabetes and recent consumption of Amiodarone, non steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressive drugs such as Cyclophosphamide, Cyclosporine, Tacrolimus, Syrulymus, Thalidomide and Mycophenolate Mofetil, organ transplantation and corticosteroids use within the last 48 hours [8-16].

After obtaining informed consent, 10 cc blood sample was taken from all participants and the checklist consists of age, sex, height, weight, duration of disease and patients` treatment was completed.

Serum levels of IL-2 was measured in pg/ml at a 450 nm wavelength by means of Avi Bion Human IL-2 ELISA Kit made in Orgenium Company in Fenland. The Accuracy and sensitivity of used kits were 15 pg/ml and 95%, respectively.

In laboratory, initially, 1: 100 dilution performed by 100 micro liter of ELISA diluents solution for all samples, then they were mixed by shaker and incubated at room temperature (25°C) for two hours and washed by appropriate buffer solution. After that, Avidin-Horseradish Peroxidase (Avidin-HRP) conjugated was added to samples prepared and incubated at room temperature for one hour. After rewashing them by appropriate buffer solution, *Tetramethyl-benzidine* (TMB) had been added and incubated for 30 min at room temperature in the dark environment. Finally, one molar phosphoric acid solution in proportion of one to 50 added and the serum level of IL-2 was measured in pg/ml at a 450 nm wavelength. Serum Level of IL-2 which was greater than 15 pg /ml was considered positive and less than this amount considered negative.

In this study, SLE disease activity index (SLEDAI-2000) was used to assess the disease activity and the score greater than or equal to three or four defined active disease [8]. Change in SLE-DAI score greater than or equal to four points indicates mild flare, change in SLEDAI score of five to nine points indicates moderate flare and Change in SLEDAI score greater than or equal to 10 points indicates severe flare [17].

Finally, statistical analysis conducted by SPSS software (version 16) and descriptive statistics (frequency, mean and standard deviation) and chi-square test and student t-Test were used. A p-value less than 0.05 was considered statistically significant.

RESULTS

In this case control study, 97.3% of subjects (n=71) were female and 2.7% (n=2) were male in both groups. The mean and standard deviation for age, weight and height in case and control group are shown in [Table/Fig-1]. Patients treated with Prednisone (82.2%), Hydroxychloroquine Sulfate (80.8%), Methoterexate (MTX) (34.2%) and Azathioprine (16.4%). Eighteen patients in case group (24.7%) had active disease according to score of SLE-DAI and the mean of disease duration were 38.98 ± 4.62 months in case group. SLE disease activity index (SLE-DAI) were mild in 75.3% (n=55), moderate in 15.1% (n=11) and severe in 9.6% (n=7) of patients.

	Case group	Control group
Age (year)	34.04 ± 1.40	34.45 ±1.35
Weight (kg)	62.63±1.35	62.26±1.30
Height (cm)	161.19±2.87	160.91±2.80

[Table/Fig-1]: Mean and standard deviation in case and control group for age, weight and height

In this study, serum level of IL-2 was positive in 45.2% (n=33) of sample studied and negative in 54.8% (n=40) in case group, while in control group, it only in 11% of sample studied (n=8) was positive and in 89% (n=65) was negative. Analysis with Chi-square test showed a significant relationship between serum level of IL-2 and the SLE disease activity index (DAI) (p=0.025). Moreover, a significant relationship between serum level of IL-2 and disease duration were seen (p=0.008). A strong relationship was seen between serum level of IL-2 and consumption of Prednisone, too (p=0.017).

Moreover, there was a significant relationship between serum level of IL-2 and patients age in case group (p=0.059), Moreover, analysis with t-Test showed a significant relationship between serum level of IL-2 and patients` height in case group (p=0.035). Analysis in control group showed the strong relationship between serum level of IL-2 and subjects height (p=0.039) while any significant relationship were not seen between serum level of IL-2 and patients` weight in the two groups.

DISCUSSION

Deficiency in IL-2 synthesis and activity has been proven in animal models and in humans with SLE [18] and IL-2R may be assumed as a reliable serologic indicator of disease activity in inflammatory diseases characterized by immune system activation [5]. IL-2 production of T-lymphocytes was in low levels in the majority of the patients with SLE before the administration of treatment [3] and was high in 50% of patients with active disease [19], while in this study, serum level of IL-2 was positive in 42.5% of the patients; this may be due to the patients treatment before the study which it could not be stopped based on legal and ethical regulations related to the patients. El-Shafey found that levels of soluble IL-2R alpha were significantly higher in patients with SLE compared to the control group (p<0.001) [20] and significantly higher in active than in inactive patients (p<0.05) [21]. Results of the present study also showed the significant association between serum level of IL-2 and the SLE disease activity.

Deregulation of IL-2 and IL-6 production take place during ageing process. Production of IL-2 is regulated in healthy people with the age of 60–70; however, a lower level of IL-2 was seen in the sera of not healthy people [22]. Pietschmann (2003) showed an increased proportion of T cells that were positive for interferon-γ, interleukin-2, 4, 10 and 13, in comparison of elderly to young women [23]. Present study showed the significant relationship between age of patients and serum level of IL-2. It seems that aging is one of the effective factors on IL-2 production.

Level of IL-2R elevated before the exacerbation of disease (p<0.02) and fell following the treatment (p< 0.05) and it was higher even in the absence of disease activity or during minor disease symptoms (p<0.01) and its serial measurement can be a sensitive indicator for prediction of disease exacerbations in patient with SLE [24]. While, Salaffi indicated that after 24 weeks of treatment with Methotrexate, Sulphasalazine and Dehydroxychloroquine, no differences were seen in serum sIL-2R in comparison with basal levels and the measurement of sIL-2R is not a useful marker for response to treatment with second line drugs in RA [25]. Findings of the present study showed a significant relationship between duration of disease and serum level of IL-2. Although the most studies demonstrated that serum soluble IL-2 levels can be used for evaluation of treatment in rheumatoid disorders, but it can be changed in patients received different drugs, further evaluations are needed.

Studies showed that levels of sIL-2R were higher among patients with active RA than healthy controls and decreased significantly following to corticosteroid pulse therapy [26] and Cyclosporine-A therapy in RA could significantly reduce circulating concentrations of IL-6 and sIL-2R [27] and measurement of circulating sIL-2R, p55 and IL-6 may be useful for the evaluation of disease activity and response to therapy in RA disease [28]. Results of present study also showed a significant relationship between serum level of IL-2 and consumption of Prednisone.

According to the results of current study, there was a significant relationship between serum level of IL-2 and patients height in case and control group, while any relationship between serum level of IL-2 and patients weight were not seen. Chaiamnuay indicated that an increased BMI is not associated with disease activity in patients with

SLE [29]. Garcia-Gonzalez also demonstrated that no association between leptin levels and Mex-SLEDAI score, age, duration of disease, or Prednisone doses were seen in patients with lupus [30]. Despite extensive search, any relevant articles could not have been found to approve these findings, so further research should be done to investigate the impact of weight and height of patients with SLE on serum level of IL-2.

CONCLUSION

Due to the relationship between serum level of IL-2 and disease activity in patients with SLE in this region, this biomarker can be used as a clinical indicator for disease activity in patients with SLE. Moreover, factors like age, duration of disease and height may have impact on serum level of IL-2 by unknown mechanisms, so these factors should be concerned in patient assessment and treatment.

LIMITATIONS

Complete elimination of patients` drugs was not possible due to respect for patients` rights and financial support in order to study large number of subjects.

RECOMMENDATIONS

Due to effects of age, height and duration of disease on serum level of IL-2, a study on large number of subjects with wide range of these variables is recommended.

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