T-regulatory Cell Phenotyping Study on Patients Suffering From Systemic Lupus Erythematosus Disease in Basrah Governorate / South of Iraq

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دراسة النمط المظهري للخلايا المنظمة لمرضى داء الذئب الأحمراري في محافظة البصرة/ جنوب العراق

اطروحة مقدمة الى
مجلس كلية العلوم- جامعة البصرة
وهي جزء من متطلبات نيل شهادة
فلسفة دكتوراه في الاحياء المجهرية
(المناعة)

من قبل
ايمان طارق علي الأحمد
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بإشراف
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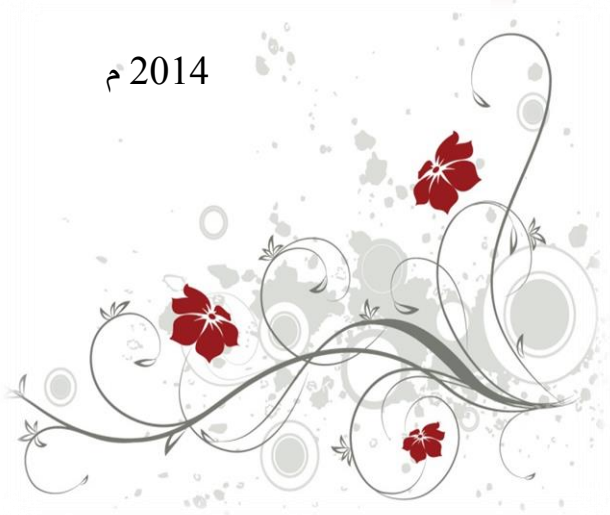

Summary

Two hundred and eighteen patients recorded from three main hospitals in Basra governorate (Al-Sadder Teaching Hospital, Basra general hospital, and Al- Mawany hospital). They were prognosis as having Connective tissue disease depending on clinical symptoms observed by physicians and Laboratory investigations from January to December of 2012. Only 50 patients suspected Systemic Lupus Erythematosus (SLE) involved in the study, SLE disease represented (22.9%) from total of six autoimmune diseases.

Thirty three (66 %) out of fifty patients with SLE diagnosed as having SLE were visiting the hospital regulatory for follow up. Seventeen (34%) out of fifty patients were new visitors to the hospital and are suspected as having SLE**.** The majority of the SLE patients were female 96%. The ratio of female to male was 24:1. The mean age of SLE patients was (32.510±9.44) year ranging from 15 to 55 and that the age group (21-40) year (whether females or male) was more affected statistically than the other age groups.

Regarding Systemic Lupus erythematosus Disease Activity Index (henceforth SLEDAI), SLE patients were divided into two groups: 42 patients (84%) belong to active disease group and 8 (16%) to inactive disease group. All of the 50 SLE patients were classified according to the involved organ into Kidney disorders (40%), Skin(16%), Lung(10%), CNS(10%), Liver (6%), Thrombocytopenia(6%) , serositis (4%), Heart(2%) ,Pancreatic (2%), Spleen (2%) and Joint pain(2%).The common clinical manifestation of SLE patients were Arthritis (100%), where as common primary symptom for SLE patients were Fever (100%), Joint pain (96%), Fatigue (86%). The least primary signs was Jaccoud`sAthropathy (4%).

The laboratory investigations such as clinical biochemistry showed increasing in concentration of both Blood urea and serum creatinine with high

concentration of protein in urine of some SLE patients during 24 hours (1.38g/24h). Moreover, study also showed significantly elevated levels in liver enzymes and a decrease in the mean concentration of calcium (7.5mg/ml). Furthermore, the haematology investigations (Complete Blood Count) have also showed a decrease in mean concentration of hemoglobin and red blood cell RBC, that 92% of SLE patients had anemia, while lymphocyte cells range between (7.2-43.2%) with increase in mean erythrocyte sedimentation rates (ESR) range (5-135mm/h).

Using ELISA technique, Positive ANA was found in 39 (78) %, positive dsDNA was found in 41 (82%), and 84% of SLE patients were positive anti-Smith. Moreover, the mean of hs-CRP concentration in SLE patients with active and inactive disease was ranging between (2.74-10.5mg/l). hs-CRP levels increase in SLE patients with all types organ, but significantly higher in SLE patients with nephritis. The mean of hs-CRP of such patients was (8.84mg/l) ranged (6.170 -10.5 mg/l), at $p<0.05$. The present Study found Strong positive correlation between hs-CRP serum levels and SLEDAI score ($r=0.437$, $p=0.001$) was observed.

The present study showed that using radial immunodiffusion method SLE patients have low C3 was 80%, low C4 was 94% and a significant elevation in concentration of IgG in serum of all SLE patients with active and inactive disease ranged (1838.7-3176.5mg/ml).

Furthermore, the results showed significant elevation in pro-inflammatory cytokines IL-6(541.1pg/ml), IFN-γ(434.8pg/ml) and TNF-α (330.4pg/ml) concentrations in SLE patients' sera in both active and inactive disease, while anti-inflammatory cytokine TGF-β concentrations in SLE patients sera showed significant reduction in both active and inactive groups of SLE patients ranged (45.3-291.5) pg/ml. The Lower levels of TGF-β were found in patients with Kidney involvement (125.6pg/ml) than other organs. On the other hand, study found the other anti-inflammatory cytokines IL-

10(458.1pg/ml) and IL-4(253.8pg/ml) concentrations in SLE patients' sera were increased. The Study observed strong negative correlation between TGF-and anti-dsDNA (r=-0.426p=0.002) and between total TGF-β levels and SLEDAI score (r=-0.984,p=0.000). Also found strong positive significant correlation between anti-dsDNA and IFN-γ (r=0.293, p=0.03), furthermore, strong positive correlation between SLEDAI and each of the IL-4 (r=0.330,p=0.01), IL-6(r=0.422,p=0.002) and TNF-α(r=0.335,p=0.01). Additionally, strong positive correlations were found between hs-CRP with levels of four cytokines IL-4, IL-6, IFN-γ and TNF-α, and the highest strong positive significant correlation was between IL-6 and hs-CRP (r=0.969,p=0.000), while the correlation between TGF-β and hs-CRP was strong negative correlation (r=-0.442,p=0.001). The higher level of IL-6and hs-CRP was observed in patients with Lupus nephritis.

The study showed that vitamin D_3 level was low in serum of SLE patients (4.20±5.3ng/ml) ranged (0.021-28.71 ng/ml) and serum 25(OH) D3 levels were correlated inversely with anti-dsDNA titers(r=-0.860p=0.000).

The Immunophenotyping study of $CD4^{pos}CD25^{pos}$ T cells, using flow cytometry on 11patients and 5 healthy control, showed that patients with SLE have a lower numbers of circulating T cell namely $CD4^{pos}CD25^{pos}$ conventional T-reg and $CD4^{pos}PD\text{-}1^{pos}$ T cells. Study found, the frequency of CD4 T cells (41.0%) tended to be lower in patients than in healthy (49.48%) , While the frequency of CD8 T cells was similar (27.1%) in patients and in healthy(25.6%),and it decreased the proportion of CD4/CD8 T cells in patients (1.7 ± 0.91) than healthy(2.01%). The expression of PD-1 was lower in patients (3.9%) than healthy (5.1%) and the frequency of $CD4^{pos}/PD\text{-}1^{pos}$ T cells in patients (1.8%) tended to be lower than in healthy (2.44%) , while the frequency of PD-1 expressing CD8 T cells in SLE patients was higher (1.5%) .Moreover, the study found the numbers of $CD4^{pos}CD25^{pos}$Treg was significantly lowered in SLE patients (6.6%) than in healthy(14.06%). The

frequency of T-reg, $CD4^{pos}CD25^{pos}CD127^{low}$ T cells, obtained by flow cytometry analysis gating on CD4 T cells, was similar in patients and healthy. Frequency of FOXP3positive cells amongst$CD4^{pos}$ $CD25^{pos}$ $CD127^{low/neg}$T-reg was also similar in the two groups. The present study observed strong positive correlation between $CD4^{+}CD25^{+}$Treg cells with both of PD-1 (r=0.681,p=0, 02) and CD4 (r=0.878,p=0.000). There also exists strong positive correlation between CD4 and $FOXP_3$(r=0.610,p=0.04,). The study also found strong negative correlation between expression of PD-1 and level of IFN-in SLE patients (r=0.02,p=-0.655,). Furthermore, strong negative correlation between CD25 and each of cytokines IL-4 (r=0.838,p=0.001),IL-6 (r=0.639p=0.03) and TNF-α(r=0.739,p=0.009) was found.

The study was investigated the ability of T-reg cells to suppress the T effector cell in supernatant culture by cytokines secretion to know what kind of secretion can affect the imbalance between T-reg cells and Th17. This study observed that Patients' T effectors ($CD4^{pos}CD25^{neg}$) can produce a lot of INF-γ by themselves (2359pm/ml), while about IL-10, patients T effectors can produce very little IL-10 (48 pg/ml.)T-reg suppressed T effectors or T-reg themselves produced more IL-10(99pg/ml) which decreased the IFN-γ/IL-10 ratio.

The present study tried immunomodulation of SLE disease to reduce the severity of the disease using supplementation of vitamin D for six months. Accordingly, this experiment found high significant difference between two groups of SLE patients (SLE patients before receiving Ca^{+}/Vitamin D and SLE patients after receiving Ca^{+}/Vitamin D) depending on the serum 25(OH) D_3 level and Anti-dsDNA concentrations. Study found that Serum 25(OH) D levels dramatically increased under vitamin D supplementation from (3.204ng/ml to 28.90ng/ml), while anti-dsDNA levels decreased from (243.3 to 47.40IU/ml), and this explained strong negative correlation between them.