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ORIGINAL ARTICLE

The Relationship Between Thymosin β4 and Vitamin D_3 in Systemic Lupus Erythmatosus Patients

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ABSTRACT

Objectives: The aim of the current study is to focus of the relationship between thymosin β4 and vitamin D_3 in systemic lupus erythmatosus patients.

Methods: This study included sixty of systemic lupus erythematosus patients that diagnosed by clinic specialists by using (SLEDAI) taken from (Al-Hussein Medical City/Kerbala / IRAQ). During the period from January 2015 to December 2016. Control group of 30 healthy persons who matched in gender and age with patients, and haven't history for this disease. The majority of the SLE patients were female 95% , the ratio of female male 19:1. The age of SLE patients was ranging from (7-65) compared with healthy control group as a seem in age.

Results: The result shows a significant decreasing in concentration of thymosin β4, vitamin D_3 and circulating complement factors (C3,C4) in active SLE patients compare with healthy control while significant increase in concentration of ANA and ds-DNA antibodies and disease activity measured using the SLE disease activity index (SLEDAI).

Conclusion: Positive relationship between level thymosin β4 and concentration vitamin D_3. There is an inverse relationship between Thymosin β4 and disease activity index of SLE.

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INTRODUCTION

Systemic lupus erythematosus is an autoimmune inflammatory disease characterized by the presence of incandesce of autoantibodies, particularly versus nuclear components. Although it is believed that the etiology of SLE is multifactorial, including immune dysfunction, genetic, hormonal and environmental, the molecular mechanisms implicit this systemic autoimmune response remain largely unknown[1].

Autoantibodies play an important role in the pathogenesis of SLE, and the varied clinical manifestations of the disease are caused by the precipitation of antibody-containing immune complexes in blood vessels, leading to inflammation in the kidney, brain, and skin. Direct pathogenic effects of the autoantibodies share in to hemolytic anemia and thrombocytopenia[2].

Antinuclear antibodies (ANA) positivity is usually considered as hallmark of SLE being positive in more than 95% of patients. Lupus nephritis has been associated with presence of many specific antibodies such as dsDNA which correlate with the disease activity[3].

Thymosin is a hormone concerning from thymus gland to labeled virgin T cell when can from bone marrow. There are two type of thymosin: alpha and beta. thymosin alpha is labeling virgin T cell arrive from the bone marrow and thymosin beta labeling virgin T cell in germinal sit in secondary immune organs. Thymosin β4 is detected outside of cells in blood plasma or in wound fluid. Several biological effects are attributed to thymosin β4, like induction of metallo-proteinases, chemotaxis, angiogenesis and inhibition of inflammation as well as the inhibition of bone marrow stem cell proliferation[4].

Vitamin D_3 is the common denomination of a group of sterols with a crucial role in phospho-calcic metabolism. The main source of vitamin D_3 is the conversion of 7-dehydrocholesterol to pre-vitamin D_3 in the skin, by means of solar ultraviolet B radiation. Conversion to vitamin D_3, or cholecalciferol, also takes place in the skin through a heat-mediated process[5].

The major function of $1,25(OH)2D_3$ has a regulatory in the calcium homeostasis, endocrine system, proliferation of skin keratinocytes and importantly plays a significant role in the regulation of the immune system[6].

Vitamin D_3 appears to act as an immunomodulator through its actions on the regulation and differentiation of immune cells like lymphocytes, macrophages, and natural killer cells (NK), besides interfering with the production of cytokines. Among the immunomodulatory effects demonstrated :inhibition of the production of autoantibodies by B lymphocytes and a reduction in the secretion of interleukin-2 (IL-2), gamma interferon (INFγ), and tumor necrosis factor (TNF); inhibition of the scretion of IL-6 [7].

MATERIALS AND METHODS

Patients and controls: This study has been performed on 60 patients suffering from SLE including 3 males and 57 females. Age range was (9-65) years old and apparently healthy control 30 were selected to participate as a normal group for comparison with age group and sex matching of patients. attending to the Imam Hussein Medical City in Karbala governorate during period from October 2015 to December -2016.

Sample's collection: Six milliliters of blood sample have been drown from each patients and healthy persons by vein puncture using disposable syringes under aseptic technique[8].

Blood of each sample has been divided to two part. one milliliters are transferred into vacuum EDTA tubes for measuring ESR . The remaining five milliliters has been transferred into vacuum gel and clot tubes left at room temperature for at least 30 minutes for clotting then centrifuge at 4000 rpm 4 minutes.

Then separated serum has been divided into four Eppendorff tubes and stored at -60° C until used to avoid repeated thawing and freezing**.** For measuring immunological test ANA ELISA kit (Generic assay/Germany) normal range (0-1 IU/ml),Anti-dsDNA ELISA kit (Generic assay/Germany) normal range (0-35 IU/ml), Complement factor (C3 , C4) , Thymosin beta 4 ELISA kit (Hcusaio/Japan) normal range (544-1234 ng/ml) , Vitamin D ELISA kit (Euroimmun/ Germany) normal range(20-30 ng/ml).

Bio-statistical Analysis: Statistical analysis was performed using statistical package for the social sciences (SPSS) statistical software for windows. The results are presented as means and standard deviation (SD). Comparison of group differences on normally distributed numerical variables were assessed by using the students' T-test to compered between patients and healthy control (group1 and 2) and ANOVA, one way to compered between classes of patients (group 1,2) depended on the least significant difference (LDS) at level less than 0.05. P-values at levels ($p<0.05$) was considered to be statically significant. Correlation between parameter were determined using the person correlation coefficients.

RESULTS

Sample distribution

Gender incidence: The majority of SLE patients were 57 female and 3 male, the ratio of female to male 19:1. In the healthy control 27 female and 3 male, the ratio of female to male 9:1.show in Figure 1.

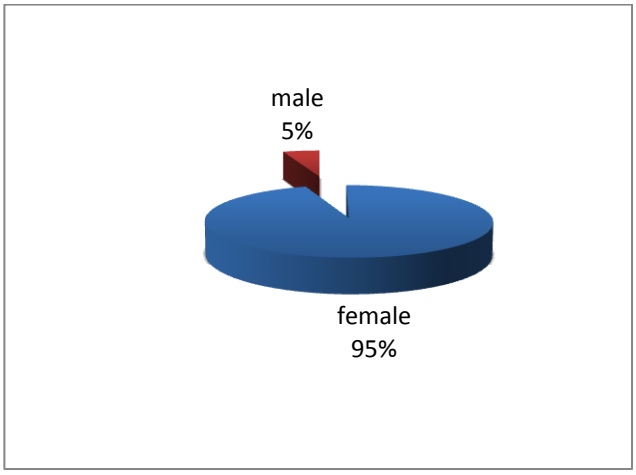

Figure 1. **Distribution of SLE patients according to gender.**

Age group: The age of patient with SLE in this study (n= 60) ranged between (7-65) years with the mean age (36.11± 1.53) compared with healthy control (n=30) ranged (19-61) years with the mean age (37.56±2.32) .

The age group of SLE patient from (1-15) years reported 1 (1.7%) while the age group (15-45) years reported 47 (78.3%) and 12 (20%) belong to (46-65) years age group, show in Figure 2.

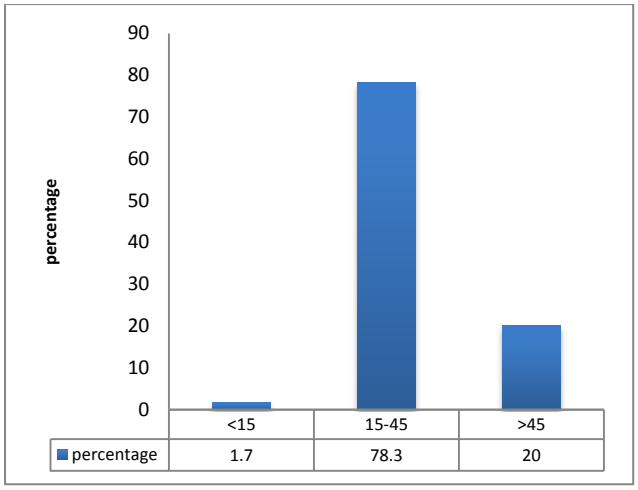

Figure 2. **Distribution of SLE patients according to age group.**

SLE Disease Activity Index (SLEDAI): According to disease activity (SLEDAI), SLE patients can be divided into three groups, mild, progressive and lupus nephritis. The current data showed that 33 (55%) of patients has mild ranged from (16-65), 14 (23.3%) had progressive which ranged from (19-58) and 13 (21.6%) had lupus nephritis ranged from (7-55) Show in Figure 3.

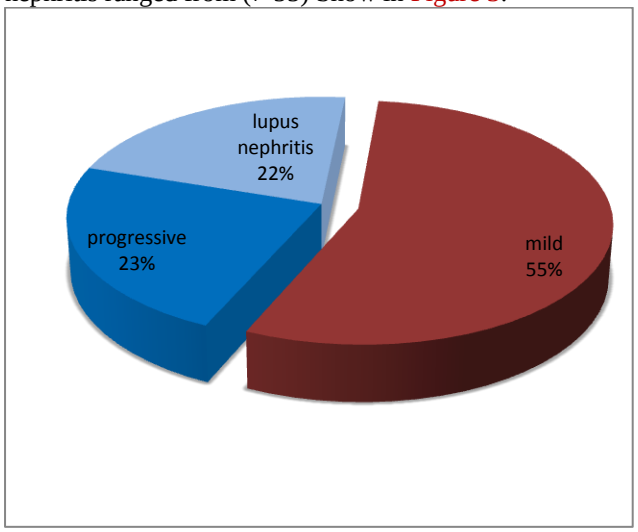

Figure 3. **The distribution of SLE patients depended on SLEDAI.**

Evolution thymosin β4 in SLE: The mean concentration of thymosin β4 for SLE patients was 195.4±384.1 range 0.001- 1727.8 compared to average of healthy control was 575.4±435.9 range 0.2 - 1290.1 , this difference was statistically high significant at p<0.05 as shown in Table 1 and Figure 4.

Table 1: **The concentration of thymosin β4 in SLE patients and healthy control.**

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	Thymosin β4 (544-1234)ng/ml				
Mean± SD	575.4±435.9	195.4±384.1	226.6±401.3	211.9±466.8	90.6±191.9
Range	0.2- 1290.1	0.001-1727.8	0.1 - 1720	0.001–1727.8	0.1 – 683.1
P-value	0.037			0.01	

P-value is significant at level 0.05 , n: number.

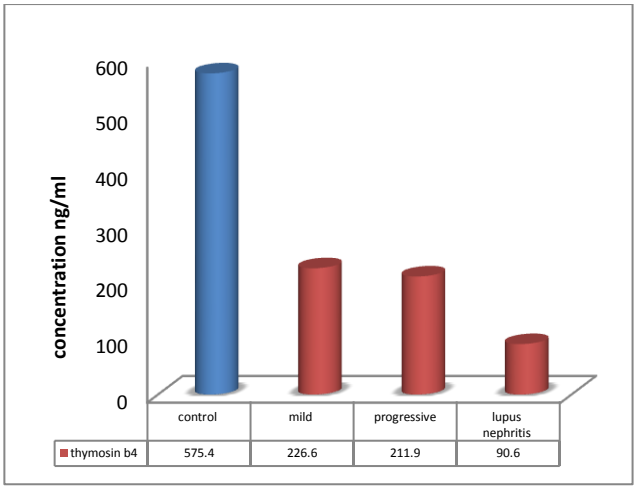

Figure 4. **Concentration of thymosin β4 in SLE patients and healthy control.**

Evaluated of vitamin D_3 levels: The mean of vitamin D3 in the SLE patients was 14.9 ± 9.7ng/ml ranged from 4.8 – 65.3ng/ml comperad with healthy control 24.8 ± 6.94 ng/ml ranged 13 -35 ng/ml. There was significant difference between the level of vitamin D3 in SLE patients and healthy control p< 0.05 , as shown in Table 2 and Figure 5.

Table 2: **Concentration of vitamin D_3 in serum of SLE patients and healthy control**.

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	Vitamin D_3 (30 -50) ng/ml				
Mean± SD	24.8±6.94	14.9 ± 9.7	16.6 ± 6.8	13.5 ± 15.6	12.2 ± 7.6
Range	13 -35	4.8 – 65.3	5.5 – 39	4.8 – 65	5.5 – 29.2
P-value	0.682			0.00	

P-value is significant at level 0.05.

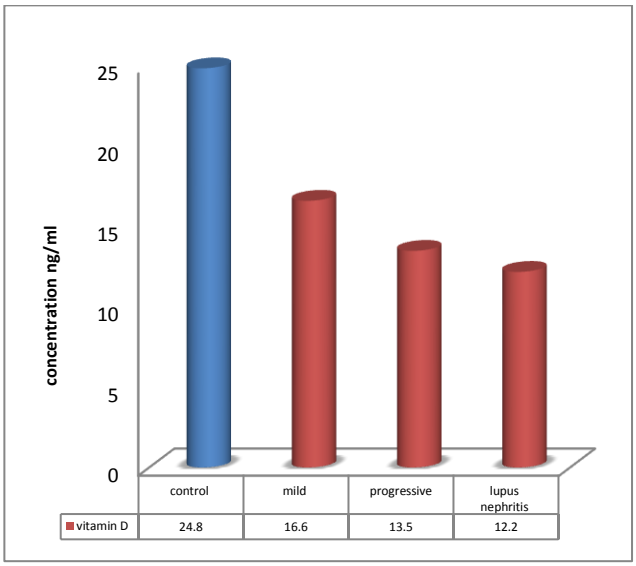

Figure 5. **Concentration of Vitamin D_3 in SLE patients and healthy control.**

Table 3: **Comparison between two groups of SLE patients before and after receiving Ca+ / vitamin D 3 depend on serum level of(ANA , ds-DNA , C3,C4) and ESR with serum level of 25-OH D3.**

Group1	ANA	ds-DNA	ESR	C3	C4	Vitamin D_3
	SLE patients before receiving Ca+/ Vitamin D_3					
Mean±SD	6.73±2.44	480.85±310	78.5±23.74	1±0.71	0.07±0.13	12.22±7.67
Rang	4.1- 10.6	77.9-1022.5	38 - 110	0.26-2.73	0.01-0.52	5.5 – 29.2
P-value	0.00	0.00	0.00	0.00	0.00	0.00
Group2	SLE patients after receiving Ca+/ Vitamin D_3					
Mean±SD	3.35±1.44	228.23±92.7	43.23±12.81	1.14±0.44	0.12±0.09	17.77±4.93
Rang	1.8- 6	60-389	20-67	0.68-1.9	0.04- 0.4	13-28
P-value	0.00	0.00	0.00	0.00	0.00	0.00

P-value is significant at level 0.05.

Anti- Nuclear Antibodies (ANA): The mean of ANA for SLE patients 2.65 ± 0.33 was statistically highly significant at $p < 0.05$ when compared to the mean of healthy controls (0.3 ± 0.02, Table 4 and Figure 6.

Table 4: **The concentration of ANA in SLE patients and healthy control.**

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	ANA (0-1)IU/ml				
Mean± SD	0.3 ± 0.14	2.65 ± 2.56	1.02 ± 0.56	2.73 ± 0.49	6.7 ± 2.4
Range	0.12 – 0.76	0.17 – 10.6	0.17 – 1.9	2.1 – 3.8	4.1 – 10.6
P-value	0.00			0.00	

P-value is significant at level 0.05 , n: number.

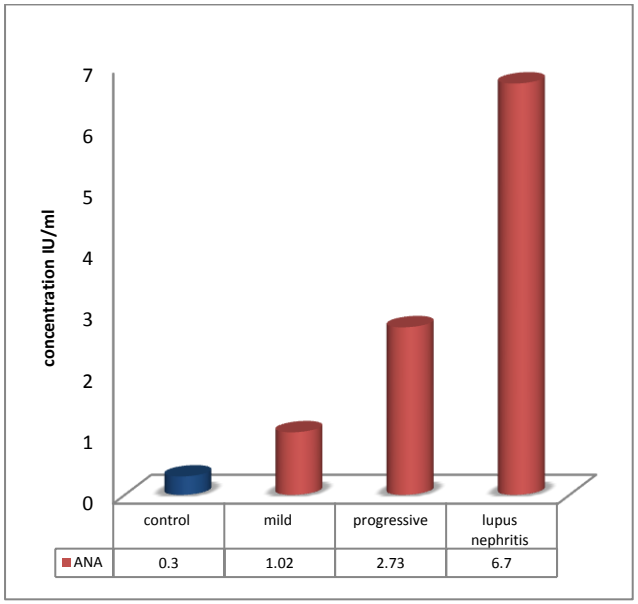

Figure 6. **The concentration of ANA in SLE patients and healthy control.**

Anti-double strand deoxyribonucleic acid (anti-dsDNA): The mean concentration of anti-dsDNA for SLE patients was 229.9 ± 243.4 IU/ml ranged 2.5 - 1020 IU/ml compared to the mean of healthy control was 10.2 ± 5.3 IU/ml ranged 2.4 -24.1 IU/ml. This difference was statistically significant at $p \leq 0.005$ as shown in Table 5 and Figure 7.

Table 5: **The concentration of anti-dsDNA in SLE patients and healthy control.**

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	Anti –dsDNA (0-30 IU/ml)				
Mean± SD	10.2 ± 5.3	229.9 ± 243.4	107.39 ± 156.57	285.90 ± 124.03	480.85±310.01
Range	2.60 -24.1	2.5 - 1020	2.50 - 876	101.40 - 580	77.90 – 1022.5
P-value	0.00			0.00	

P-value is significant at level 0.05 , n: number.

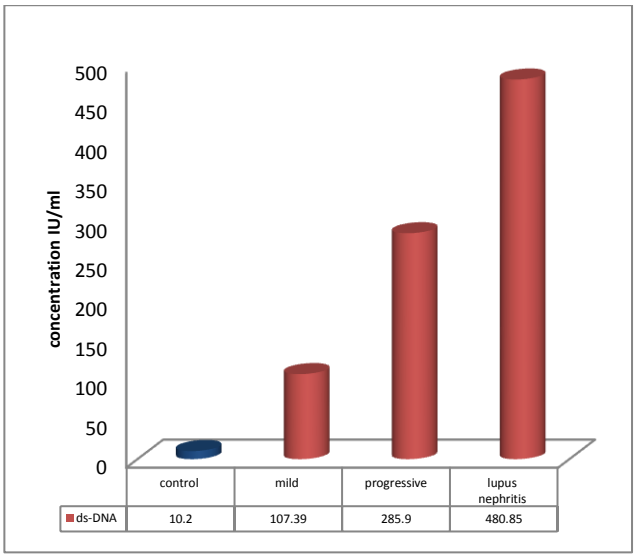

Figure 7. **Concentration of ds-DNA in SLE patients and healthy control.**

The Complements (C3, C4): The result of C3 and C4 concentration in SLE patient's sera showed significant reduction comparing to healthy control. The mean concentration of C3 for SLE patients were 1.5 ± 0.72 g/l ranged 0.26 – 3.13 while the mean concentration of C4 for SLE patients was 0.22 ± 0.24 g/l ranged 0.01- 1.5, in compared to the mean concentration of healthy controls were 1.65 ± 0.6 ; 0.36 ± 0.25 g/l ranged 0.4 – 2.64 ; o.12 – 1.4 respectively. This difference was high significant $p \leq 0.05$; Table 6 , Figure 8 and Figure 9.

Table 6: **The concentration of complements in serum of SLE patients and healthy control.**

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	C3 (1.85 – 0.83) g/l				
Mean±SD	1.65±0.6	1.5±0.72	1.75±0.6	1.39±0.74	1±0.71
Range	0.4-2.64	0.26-3.13	0.49-3.13	0.59-2.9	0.26-2.73
P-value	0.31			0.004	
	C4 (0.45 – 0.15) g/l				
Mean±SD	0.36±0.25	0.22±0.24	0.32±0.26	0.13±0.11	0.07±0.13
Range	0.12-1.4	0.01-1.5	0.1-1.5	0.01-0.38	0.01-0.52
P-value	0.014			0.000	

P-value is significant at level 0.05 , n: number.

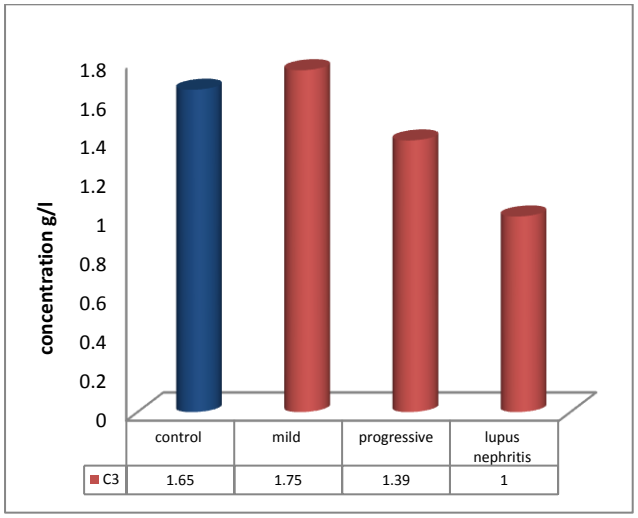

Figure 8. **Concentration of complement system (C3) in SLE patients and healthy control .**

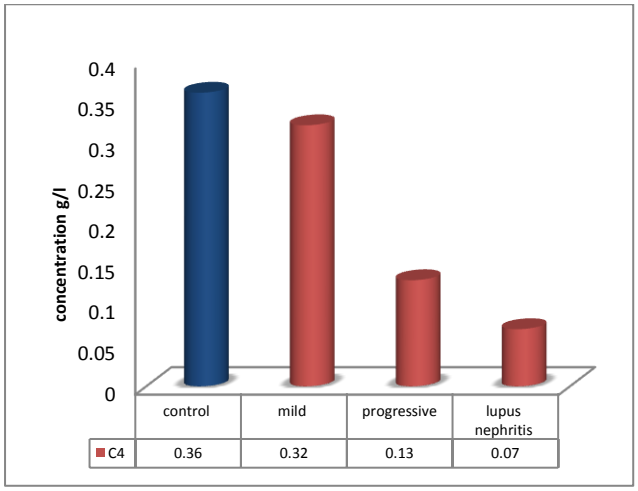

Figure 9. **Concentration of complement system (C4) in SLE patients and healthy control .**

Erythrocytes Sedimentation Rate: This study showed that SLE disease was associated with raised ESR and the mean was 61.75 ± 29.07 mm/h that range 20 -120 mm/h in SLE patients when compared to healthy controls 13.8 ± 5.76 mm/h range 5-30 mm/h the difference was statically significant $p \leq 0.05$, Table 7 and Figure 10.

Table 7: **The concentration of ESR in serum of SLE patients and healthy control.**

Parameter	Healthy control n= 30	SLE total n= 60	Mild n= 33	Progressive n=14	Lupus nephritis n= 13
	ESR (0 – 20) mm/h				
Mean± SD	13.8±5.76	61.75±29.07	50.94±26.47	73.77±29.51	78.50±23.74
Range	5-30	20 -120	20 - 120	32 -120	38 -110
P-value	0.00			0.00	

P-value is significant at level 0.05 , n: number.

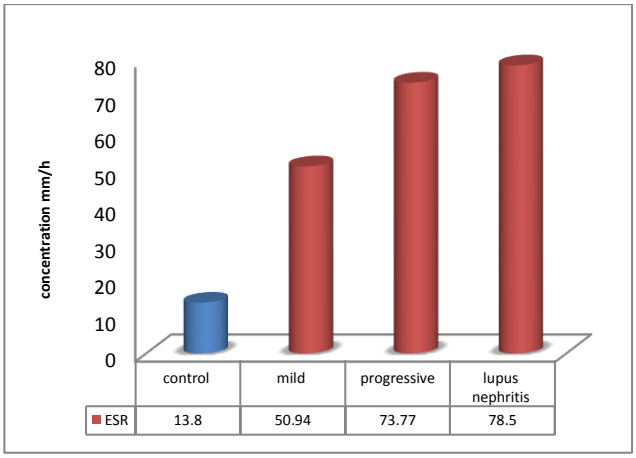

Figure 10. **Level of ESR in SLE patients and healthy control.**

Discussion

It has been found that female of all mammalian species have an increased incidence of autoimmune/inflammatory disease compared to males, and the presence of estrogen increases the severity of autoimmune disease[9].

In the current study, the demographic distribution of data showed that SLE was teenager and old age women less than women of fertile age (> 15 & < 45) year. This result agreement with the results published by many studies in Iraq and other countries[10,11,12].

Calculation of SLEDAI depended mainly on the initial primary symptom presentation that varied widely from patient to patient and essential diagnoses test to recognize active and inactive of SLE disease[13] and the third group is sub division from active group which lupus nephritis .

This result show that the level of thymosin β4 significantly decrease in SLE patients compared with healthy control, and more related with lupus nephritis. Other study suggested that thymosin β4 decrease with flare-up, this result agreed to other study[14].

The result exhibited that low vitamin D_3 level was frequent in SLE patients, and indicated that SLE patients had higher risk of insufficient vitamin D_3. The high prevalence of SLE patients who have vitamin D_3 level below normal was similar with most studies in the world[15].

The low level of vitamin D3 causes impaired immunological response that is thought to increase disease activity in SLE[16]. Vitamin D3 deficiency is worldwide problem with serious health effects such as

SLE disease and one of the most important risk factors for immune system[17].

This result show significantly decrease in SLE patients compared with healthy control, and more related with lupus nephritis[18]. After treatment receiving Ca+/Vitamin D_3 the result show increase the level of vitamin D_3 and this increasing depend on level dose, but stay the level of vitamin D_3 less than normal value[19].

This study demonstrated that high titers of ANA are most often associated with active SLE[20] said this test is a one of the most common tests used by physicians to help diagnosis lupus is the anti-nuclear antibody (ANA) are heterogeneous group of antibodies produced against variety of antigens within the cell nucleus .

Antibodies to dsDNA in serum of patients are elevated compared with the control. These results are consistent with Ter Borg *et al*.[21] who have found that increase in anti-dsDNA antibody concentration prior to disease exacerbations of SLE are part of a restricted immune response or merely the consequence of polyclonal B cell activation. Moreover Giasuddin *et al*.,[22] have mentioned that the anti dsDNA antibodies are present in 85.3% of SLE patients .

In this result show that the level of complement (C3 and C4) in SLE patients was significantly decreased compared to the healthy control , and C4 level more related with lupus nephritis ,this result is in agreement with other reported studies[23]. In this studies have found a relationship between C3 or C4 serum levels and renal flares have discovered that C4 is critical for starting a renal flare, while C3 activation is implicated in the actual tissue damage[24].

This result showed that increased of ESR in SLE patients compared with healthy control, this elevation due to inflammation causes an increase in the ESR, this result match with[25].

Conclusions

Low concentration vitamin D_3 in all patients with systemic lupus erythematosus, especially in lupus nephritis. Positive relationship between level thymosin b4 and concentration vitamin D_3. There is an inverse relationship between Thymosin β 4 and disease activity index of SLE.

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