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Relationship between thymosin β4 & IL-10 in systemic lupus erythematous patients correlated with (Sledai) in Kerbala Governorate

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A B S T R A C T

This study included study of thirty SLE female patients that diagnosed by clinic specialists by using (SLEDAI) taken from (Al- Hussein Medical City/Kerbala/ IRAQ) During the period March/2013 to April /2014. Control group consisted of 10 healthy woman who matched in age with patients, and haven't history for this disease. The result shows a significant decreasing in concentration of thymosin-β 4 & circulating complement factors (C3&C4) in active SEL patients compare in control while IL-10 is predominantly an anti-inflammatory cytokine were significantly increased in SLE patients and to be associated with anti ANA &-dsDNA antibodies and disease activity measured using the SLE Disease Activity Index (SLEDAI)

Keywords: Thymosin-β 4, circulating complement factors (C3&C4), SLE patients, IL-10

A R T I C L E I N F O

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1. Introduction

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

is the typical autoimmune disease given its complex clinical and molecular phenomenon. Like the other rheumatic diseases, appropriate management is critically dependent upon the proper assessment of disease activity. [2] Systemic Lupus Erythematous is known to be associated with polyclonal B cell hyperre activity. Developing an understanding of the complex nature of human B cell differentiation [3] 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.(4)The cytokines may act as key players in the differentiation, maturation and activation of immune cells in the immune-pathogenesis of SLE. They are involved immune dysregulation of SLE, and local inflammatory response which substantially leads to tissue injury [5]. Pro-inflammatory cytokines, such as TGF-β, TNF-α, IFN-α, IFN-γ, IL1, IL6, IL8, IL12 and IL-17, and anti-inflammatory cytokines such as: IL4, IL5, IL10 and IL13, play crucial pathogenic roles. Basically, all these cytokines can be generated by both innate and adaptive immune response to maintain immune homeostasis. As in figure below [6].

IL-10 is an immunosuppressive cytokine produced by a variety of mammalian cell types including macrophages, monocytes, T cells, B cells and keratinocytes. This cytokine holds to effect on immune responses and soothe immune pathologies. [7] The circulating IL-10 concentrations were significantly elevated in SLE patients and correlated with the SLE score. The anti-inflammatory response is not enough to suppress the active disease. IL-10 contributes to the critical balance between inflammation and immunoregulation, thus identifying the exact contribution of the currently studied cytokines might provide future insights for targeted therapeutic strategies in SLE [8]. As IL-10 is a key immune-regulatory cytokine produced by a multiplicity of immune cells including monocytes, macrophages, mast cells, NK cells, eosinophils, and neutrophils and by adaptive immune cells such as Th1, Th2, CD8 + T and B cells. IL-10 has been shown to exert a potent suppressor effect on macrophage activity, in addition to its recognized direct inhibitory effects on the proliferation of CD4 + T cells [9].

The beta-thymosins are a family of highly conserved polar 5 kDa peptides originally thought to be thymic hormones. Further studies demonstrated that the molecule is ubiquitous; it had been found in a variety of tissues and cell lines. It is found in highest concentrations in spleen, thymus, lung, and peritoneal macrophages. 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 [10]. When individual thymosins were isolated from Fraction 5 and characterized, they were found to have extremely varied and important biological properties. However they are not truly thymic hormones in that they are not restricted in occurrence to thymus distributed throughout many different tissues.

The beta-thymosins are a family of highly conserved polar 5kDa peptides originally thought to be thymus hormones Immunological deficiencies resulting from the lack of thymic function in several animal models, as well as in humans with primary and secondary immunodeficiency diseases [11]. Increased expression of β-thymosins or even the synthesis of a β-thymosin normally not expressed might promote metastasis possibly by increasing mobility of the cells. However, poorly studies about the molecular mechanisms mediating the effects attributed to extracellular β-thymosins. Add in poorly studies in ruls of thymosin in SLE [10].

This study aimed to affirmation the role of thymosin β4 in the balance between anti-inflammatory cytokine (IL10) & some of pro inflammatory cytokine (inflammation and immune regulation).in SLE patients.

2. Materials and Methods

Patients and controls:

This study included study of thirty female patients with Systemic Lupus Erythematous that diagnosed by clinic specialists and by using (SLEDI) at female with ages ranged between (15-62) years were taken from (Al- Hussein Medical City/Kerbala/ IRAQ) during the period March/2013 to April /2014. Control group consisted of 10 healthy woman who matched in age with patients, and haven't history for this disease.

Sample collection and assay procedure.

Venous blood sample (7ml) were taken from each patients 1ml put in anticoagulant tube to used for measurement of complete blood count & ESR ,and 6ml put in plan tube then, left at room temperature until clot and centrifuge for 15 min. at (3000 rpm).

Serum was separated in Ependwarff tubes and stored in (-20C) until time of analysis.

Estimation of, C3, C4, hs-CRP and anti-dsDNA ELISA kit (Cusabio/China) normal range (20 ng/ ml), ANA indirect Immunofluorescence test Euroimmune (Germany), Thymosinβ4 IL10 ELISA kit (Cusabio/China) normal range (100 pg /ml) in serum using commercially available and performed as recommended in leaflet with kit.

Bio-Statistical Analysis

Statistical analysis was performed using SPSS version 22. The normally distributed variables were expressed by using Kolmogorov-Smirnov test by using t-test (mean ±SD). The correlations between the parameters under study. Pearson correlation was used to analyze results by using SPSS version 22. P-value ≤ 0.05 was considered significant.

3. Results and Discussion

The result in table (1) show significant decrease in number of blood cells count 5850/ mm3 compare with control, 9190/ mm3 and lymphocyte percentage & hemoglobin (17.1%, 8.96g/dl) associated with disease progressivity in active SLE patients compare with control group (26.7 % , 14.39g/dl) respectively. .

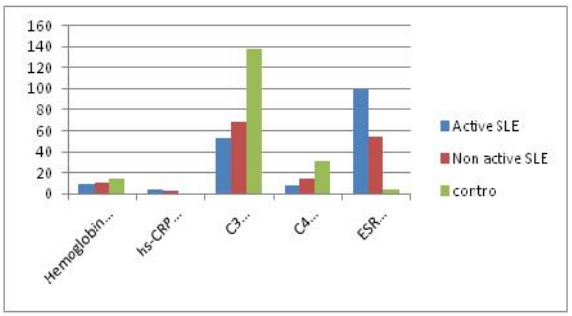

Figure 1: The result show pre inflammatory parameters in SLE patients & control. Depend on disease activity (SLEDI)**.**

The result in figure (1) and Table (2) show a Positive correlation was found between pre inflammatory with progressivity of disease compare with control group, a significant decreasing in concentration of circulating complement factors (C3 and C4) (52.86 ,7.57) in active SLE compare with control (138.04 31.62) respectively and significant increasing in ESR& non significant increase in hs –CRP, (99.9, 4.04) in active SLE compare with control (4.5,0.65) respectively associated with disease progressivity in active SLE patient compare with control group.

Table 1: Complete blood count and Hemoglobin in SLE patients & control. depend on disease activity (SLEDI)

Variable	Active SLE Mean±SD	Non active SLE Mean±SD	Health control Mean±SD
WBC (MM3)1000	1.5a± 5.850	8.02 ±1.02[c]	9.19±1.29[b]
Polymorph nuclear leucocyte %	0.97 ±75.95	71.23 ±1.23	62.12±0.78
Lymphocyte %	17.1± 4.1[a]	18.9± 4.3[c]	26.7 ±4.05[b]
Monocyte %	6.9± 2.8	9.8 ±3.2	11.14 ±1.7
Hemoglobin g/dl	8.96± 1.4[a]	10.4± 1.2 [c]	14.39 ±0.99[b]

[a]Active vs. non-active (p-value≤ 0.05),
[b]Active vs. Healthy (p-value≤ 0.05),
[c]Non-active vs. Healthy (p-value≤ 0.05).

Table 2: Pre inflammatory parameters & ESR in SLE patients & control depend on disease activity (SLEDI)

Variable	Active SLE Mean±SD	Non active SLE Mean±SD	Health control Mean±SD
hs-CRP (mg/dl)	4.04 ± 1.7	2.6 ±0.68	0.65 ±0.4
C3 (mm/dl)	52.86 ±12.6[a]	68.13 ±16.4[c]	138.04 ±29[b]
C4 (mm/dl)	7.57 ±5.6[a]	14.4 ±2.9[c]	31.62 ±7.4[b]
ESR (MM/h)	99.96± 4.6[a]	54.66 ±1.4[c]	4.5 ±3.8[b]

[a]Active vs. non-active (p-value≤ 0.05),
[b]Active vs. Healthy (p-value≤ 0.05),
[c]Non-active vs. Healthy (p-value≤ 0.05).

Table 3: The relationship between thymosin-β-4 & pre inflammatory parameter in SLE patients & control.

Variables	NO	Thymosin β4µg /ml Mean± SD	C3 (mm/dl)	C4 (mm/dl)	hs-CRP Mg/ dl SD± mean	ESR MM/h
Total number	30	1053.6 ±9.5	Mean± SD	Mean± SD	3.3 ± 1.5	77.3 ±3.2
Active SLE	15	291.22 ± 12.5	60.5± 1.6 a	10.9 ±4b	4.04 ± 1.7	99.96 ±4.6
Inactive SLE	15	1816 ±8.17	52.86 ±1.2	5.6±7.5	2.6 ±0.68	54.6 ±1.4
Health control	10	6567.5 ± 14.6	68.13±1.64c	14.4±2.9	0.65± 0.4	4.5± 3.8

[a]THYM vs. C3 (p-value≤ 0.05, r=0.64),
[b]THYM. vs. C4 (p-value≤ 0.05, r=0.69),
[c]THYM vs. C3 (p-value ≤ 0.05, r=0.624)

Table 4: The relationship between Thymosin β4 and some immunological parameters in SLE Patients.

Variables	No	Thymosin β4 µ/ml Mean ±SD	IL-10 Pg/ML Mean±SD	IL-10 Pg/ML Mean±SD	ds-DNA Mean±SD	ANA Mean±SD
Total SLE patient	30	1053.6 ±9.5	295.4 ±3.62	295.4 ±3.62	81± 9.5[a]	17± 0.81
Active Sle Patient	15	291.22 ± 12.5	570.2 ± 3.31	570.2 ± 3.31	127.5±4.8	34 ±1.82
Inactive SLE patient	15	1816 ±8.17	20.6 ±14.3	20.6± 14.3	35±9.7	4.3±0.94
Health control	10	6567.5 ± 14.6	5.32± 2.4	5.32± 2.4	0.62±0.7	0.96-0.87

*The correlation is significant at (p<0.05, r=0.564)

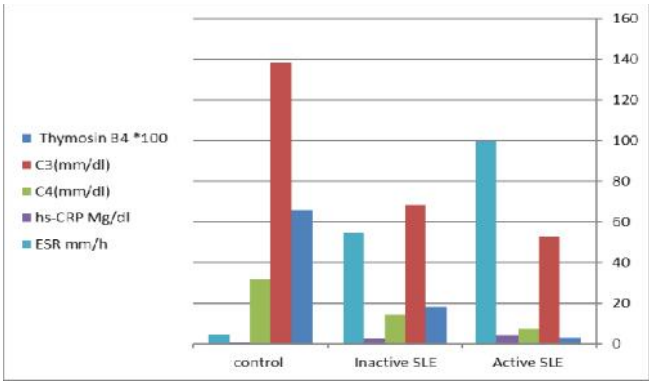

Figure 2: The relationship between thymosin -β-4 and pre inflammatory parameter in SLE patients & control.

The result showed positive correlation (p-value=0.05) which show a significant decreasing in concentration of thymosin-β 4 and circulating complement factors (C3&C4) (291.22, 52.86,7.5) respectively in active SEL compare in control (6567.5,138.4, 31.62) respectively and positive correlation (p-value=0.05) which show a significant increasing in ESR and non significant increasing in hs –CRP associated with decreasing in concentration of thymosin-β-4(99.6, 4.1, 291.22) respectively in active SLE compare with control group.(4.5, 0.65, 6567.5) respectively.

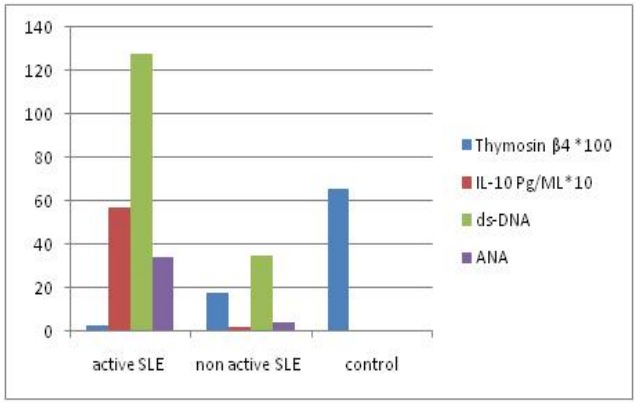

Figure 3: Show the relationship between Thymosin β4 and some immunological parameters in SLE Patients

The result show in figure three Highly significant correlation between thymosin β4 and Immunological parameters (IL10, ds-DNA , ANA ,IL10) (570.2,291.22, 127) in active SLE patients compare with control (5.32, 6567.5 ,0.62.0.96).

Discussion:

The result show in table (1) a significant decrease in number of leukocyte (Leukopenia) and lymphocyte % and hemoglobin level associated with disease progressivity in active SLE patient comber with control this result agree with a observed decreasing PMNs and macrophages result apoptotic macrophages from patients with SLE may indicateed unclear mechanism [12]. A diagnosis of anemia with jaundice throughout acute hemolysis, trans aminasemia and SLE. [13] The result in figure (1 & 2) show positive correlation (p-value=0.05) which show a significant decreasing in concentration of hemoglobin & circulating complement factors (C3&C4) in active SEL compare with control group , associated with the decreasing of thymosin-β-4 and positive correlation(p-value=0.05) significant increasing in ESR , & non significant increasing in hs –CRP associated with decreasing in concentration of thymosin-β-4 in active SLE compare with control group. This result agree with different studies that suggested Both C3 and C4 levels were decreased In lupus nephritis .All of lupus's patients with low C3 or C4 concentrations [14]. A defect apoptotic cells in complement-dependent clearance were reveled increase susceptibility to the development of autoimmune disease [15] In SLE patients without anti-dsDNA and low complement levels, ESR was positively associated with, renal and joint.[16] Erythrocyte sedimentation rate (ESR) may be elevated with active disease or with concomitant infection. It is a non-specific inflammatory marker that is useful for therapeutic response to treatment. CRP response in SLE has led to the hypothesis that relatively low CRP levels contribute to the pathogenesis of this disease. Recent studies raise the possibility that type I IFN -γ, led to inhibits CRP expression. [17]. Renal infections, and low albumin were associated with having elevated ESR/low CRP; low albumin predicted elevated CRP/low ESR and elevated ESR/low CRP discordance. Rheumatoid arthritis patients were less likely to have elevated ESR/depressed CRP. ESR as indicator for inflammation in systemic rheumatic disease. [18] Figure.3 Positive correlation (p-value=0.05) between thymosin β 4 and immunological parameters, the result show a significant increasing in, (IL10, ANA, ds-DNA) associated with a significant decreasing in concentration of thymosin- β-4 in active SLE patients compare with control group. The concentrations of all studied immunological parameters are significant increasing with progressive of SLE while the concentrations of thymosin β4 is decreased significantly. Immunological deficiencies resulting from the lack of thymic function in animal models, so in humans with primary and secondary immunodeficiency diseases. [11] Increased expression of β-thymosins may be promote metastasis possibly by increasing mobility of the cells. However, weakly studies about the molecular mechanisms about extracellular β-thymosins. (10 Tβ4 may be contribute in stimulating human pancreatic cancer progression by promoting proinflammatory cytokine environment. Tβ4 and related molecules may be a novel therapeutic strategy for pancreatic cancer. [19] Interleukin-10 also play a central role in overriding inflammatory processes.(20) IFN-α in SLE patients was considered a primed and persistently exposed to immune complexes, responses to IL-10 are abnormal, limiting the anti inflammatory effect of this cytokine. IL-10 production was may play a role in the development of autoimmune and malignant in SLE patients as immunoregulatory mechanisms. [21] pro- -inflammatory cytokine and anti-inflammatory cytokines especially IL10 play crucial pathogenic roles in lupus patients. [6] Modulation of the level of IL-10 may be of potential therapeutic benefit for human lupus through inhibition of pathogenic Th1 responses [22] IL-18 and IL-10 play a critical role in keeping the balance Th1 and Th2 activities and between inflammatory and anti-inflammatory responses

in both cellular and humoral immunity. IL-10 and IL-18 are involved in the pathogenesis of SLE disease that could result in new therapeutic approaches for preventing the immune alteration in SLE patients.[8]

4. Conclusion

Thymosin-β 4 & pro-inflammatory parameters significantly decreased while IL-10 are significantly increased in SLE patients depend on disease activity measured using the SLE Disease Activity Index (SLEDAI).

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