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Evaluation of Caspase-3 in Patients with Systemic Lupus Erythematous and it is Connection with Disease's Activity

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Abstract

Background: Many research groups studied the relationship between SLE & apoptosis, as some enzymes of apoptosisconsidered to be the cause of relapsing of disease, such as caspases 8&3. So the correlation between both is still a debate issue.

Aim: This study aimed to: Assess level of caspase-3 among SLE patients. This done by:

• measurement of caspase-3 level by ELISA technique

• clarify the relationship between caspase-3 levels and SLE activity by measuring ds-DNA and ANA levels

Methods: Subjects enrolled in this study were categorized into two groups: patients and control groups, the patients were of both sexes with ages ranging from (10-55 year), the study carried out during the period from December, 2017 through August, 2018. This study was conducted on patients attending Al-Sadder Medical City, AL Hakeem General Hospital in Al-Najaf, AL-Hussein Medical City in Karbala and Marjan Medical City in Baby, all these provinces in Iraq from the Rheumatology and Nephrology out clinics in those hospitals. Forty Five patients (5 males & 40 females), Collect appropriate amount of blood from each patient for: Assessment of ds- DNA and ANA, Assessment of apoptosis marker (caspase-3) using ELISA technique.

Results: The level of caspase-3 show no significant correlation among patients with SLE than control (P. value = 0.023), furthermore, ANA and ds-DNA were high significant in patients compare with control (P-value <0.001 and P. value = 0.001) respectively.

Conclusion: there are important changes in the level of caspase-3 in SLE, no significant correlation had been found between SLEDAI and caspase-3.

Keywords: Caspases-3; Systemic Lupus Erythematosus; Apoptosis; ds-DNA; ANA

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Introduction

Systemic lupus Erythematous (SLE) is an autoimmune disease with a wide spectrum of clinical immunological abnormalities [1]. A characteristic hallmark of SLE is the production of autoantibodies against nuclear components [2]. To understand the pathogenesis of SLE it is important to know how self antigens become available and immunogenic to immune system, many researchers believed that apoptosis play a crucial role in autoimmunity, including SLE [3,4]. Disturbances in apoptosis and any defect in clearance of apoptotic cells, increases exposure of modified autoantigens to the immune system [5]. Apoptosis is a programmed cell death that follows characteristic biochemical and morphological features. Apoptosis can be induced by extrinsic (e.g., Fas ligand), or intrinsic factors (e.g., DNA damage) [3]. Accompanied with changes in chromatin structure and composition [6]. Cells finally disintegrate into apoptotic blebs [7]. These stimuli lead to activation of caspases and changes in the plasma membrane [8]. SLE Disease Activity Index (SLEDAI), developedat the University of Toronto in 1992, is a global scorereflecting all aspects of disease activity [9]. SLEDAI has certain limitations in that it does not score some life threateningmanifestations such as pulmonary hemorrhage and haemolyticanaemia. It is heavily weighted for centralnervous system and does not take into account theseverity of manifestations. Gladman D, et al. (1994) defined that an increase in SLEDAI score of more than threewas a flare, SLEDAI score that was within three pointsof the previous score was persistent disease and a score of zero was remission [10]. A change of SLEDAI score of more than 12 is a severe flare according to another study [11]. Serological tests are commonly used to assess thedisease activity and predict lupus flare. During activedisease, usually there is a fall in complement levels anda rise in anti-double stranded deoxyribonucleic acid (antidsDNA) levels. The pervious study suggests strong correlation between disease activity and a rise in dsDNA and fall incomplement (C3 and C4) levels [12,13]. Caspase-3 is a caspase protein that interacts with caspase-8 and caspase-9. It is encoded by the CASP3 gene. CASP3 orthologs have been identified in numerous mammals for which complete genome data are available.

Unique orthologs are also present in birds, lizards, lissamphibians, and teleosts. Caspase-3 activity might be high in SLE T cells because of their increased susceptibility to spontaneous apoptosis. caspase-3 might be involved in cleaving CD3ζ in SLE T cells, the determining the levels of expression and activity of caspase-3 in SLE T cells and exploring the direct effect of inhibition of caspase-3 activity on CD3ζ expression [14]. We report that SLE T cells display higher levels and activity of caspase-3 than normal T cells. Moreover, reversal of heightened caspase-3 activity increased the amounts of CD3ζ protein and dampened the abnormal TCR-induced calcium responses in SLE T cells [15]. The mechanism between caspase-3 and SLE is complicated, the level of caspase-8 has an inverse relationship with the activity of the disease, so the current study aimed to: Assess level of caspase-3 among SLE patients. This done by:

- Measurement of caspase-3 level by ELISA technique
- Clarify the relationship between caspase 3 levels and SLE activity by measuring ds-DNA and ANA levels

Subject and Methods

Study Population

Patients group: This study was conducted on patients attending Al-Sadder Medical City, AL Hakeem General Hospital in Al-Najaf, AL-Hussein Medical City in Karbala and Merjan Medical City in Baby these provinces in Iraq, from the Rheumatology and Nephrology out clinics in these hospitals. Forty Five patients (5 males & 40 females) with age range between 10-55 years, and duration of disease between 1-25 years included in this study who were clinically checked by Specialist and laboratory diagnosed as SLE.

Control group: For the purpose of comparison, a group of 45 (5 males and 40 females) apparently healthy control persons were included (healthy, normal subjects with no family history of SLE, without any medical disease and chronic disease) with age range between 10-55 years. Their age ranges and gender are matched to that of patients. All groups (patients & control) have been informed about the study and its aims and their agreement were taken.

Specimen collection: Five ml of venous blood were drawn from each patients and control groups, collected in gel tubes, slow withdrawal of the blood sample via the needle of syringe to prevent hemolysis. The sample dropped into clean disposable gel tube, serum was separated after 20 minutes at room temperature the samples were then centrifuged at 3500 rpm for 5 minute and then stored in to separated three Eppendorf tubes at freeze condition (-20°C) until analyzed. Assessment of ANA, ds DNA and Assessment of apoptosis marker (caspase-3)

Laboratory assays

Kits	Source
Human CASP3(Caspase 3) ELISA Kit	Elabscience
dsDNA-G Kit	CHORUS
ANA- screen Kit	CHORUS

Statistical Analysis

Data of both studied groups were entered and analyzed using the statistical package for social sciences (SPSS) version 25. Descriptive statistics presented as mean, standard deviation, standard error, range, frequencies and proportions. All continuous variables were tested for statistical normal distribution using histogram and normal distribution curve; statistical tests were applied according to the distribution and type of variables. Student's t test for two independent samples was used to compare two means of a continuous normally distributed variable, and Mann-Whitney U test for two independent samples was used to compare non-normally distributed continuous variables. Chi-square and Fisher's exact (when chi-square inapplicable) tests used alternatively to compare frequencies. Bivariate Pearson's and Spearman's correlation test and regression curve estimation analysis were used to assess the correlations. Correlation coefficient (R) is an indicator of the strength and direction of correlations; its value ranged zero (complete no correlation) to one (perfect correlation) the higher R value close to one indicated stronger correlation, the positive (no sign) R value indicated a direct (positive) correlation and the negative signed R indicated an inverse correlation. Level of significance of ≤ 0.05 was considered as significant difference or correlation. Results and findings were presented in tables and figures with explanatory paragraphs using the Microsoft Office 2010 for windows.

Results

The mean caspase-3 of SLE patients was (9.92 ± 0.36) which was significantly higher than that of controls which was (9.60 ± 0.41), (P-value = 0.002), (Table 1).

The comparisons of mean Antinuclear Antibodies (ANA) levels of SLE patients and controls revealed that SLE patients had significantly much higher mean ANA than controls, 6.41 ± 0.69 and 0.92 ± 0.41, respectively, (P<0.001) (Table 2).

The mean Anti ds-DNA antibodies level in SLE patients was significantly higher than in controls, (74.59 ± 11.02) and (11.37 ± 4.69), respectively, (P. value = 0.001), (Table 3 and Table 4).

Table 1: Comparison of Caspase-3 levels of SLE patients and controls.

Caspase-3 ng/mL	SLE patients (n = 45)	Controls (n = 45)	P. value
Mean	9.92	9.6	0.002
SE of Mean	0.36	0.41	Mann-Whitney U test
Minimum	9.62	3.68	
Maximum	10.01	10.01	
P. value≤ 0.05			

Table 2: Comparison of ANA levels of SLE patients and controls.

ANA	SLE patients (n = 45)	Controls (n = 45)	P. value
Mean	6.41	0.92	< 0.001
SE of Mean	0.69	0.41	Mann-Whitney U test
Minimum	0.4	0.22	
Maximum	12	2.3	

Table 3: Comparison of Anti ds-DNA levels of SLE patients and controls.

ANA	SLE patients (n = 45)	Controls (n = 45)	P. value
Mean	74.59	11.37	0.001
SE of Mean	11.02	4.69	Mann-Whitney U
Minimum	7	7	
Maximum	180	35.5	

Table 4: Bivariate correlation matrix between SLEDAI and caspase-3.

	Correlations' statistics	
	R*	P-value
Caspase3	-0.099	0.518
R: Correlation coefficient		

No significant correlation had been found between SLEDAI and caspase-3, (P-value > 0.05).

Discussion

Based on recent findings as described above, our work focused on the association the caspase3 and immunological marker of SLE. Apoptosis is a highly controlled process, and plays an important role in pathogenesis of SLE [16]. In current study, we examined the level of Caspase-3 in seria patients and control using and protein levels using ELISA technique and aclarify the relationship between caspase 3 levels and SLE activity by measuring ds-DNA and ANA levels. The current study, found that the main first result is comparisons of mean Antinuclear Antibodies (ANA) levels of SLE patients and controls revealed that SLE patients had significantly much higher mean ANA than controls. The main second result is the mean Anti ds-DNA antibodies level in SLE patients was significantly higher than in controls. The main third result is the mean caspase 3 of SLE patients was higher significantly lower than that of controls which was (P. value = 0.002), Table 1, which was consistent with our results showed that the apoptosis rate has been increased in patients with SLE, which was in agreement with the results of some previous studies [15]. Another study was not compatible with the current study, Gaipl US, et al. (2007) that showed the rate of caspase3 in protein level to be decreased in SLE patients, whereas level caspase3 was not different in patients and controls, perhaps due to various factors including defects in the clearance of apoptotic cells, which could result in accumulation of apoptotic cells [17]. Many studies have reported the defects in the clearance of apoptotic cells in SLE patients. Another study disagrees with our study, Maas K, et al. (2002) assessed the expression level of a number of genes involved in apoptosis in SLE patients, and observed the reduced level of caspase 3 in these patients [18]. In the intrinsic pathway, any decrease in antiapoptotic proteins such as Bcl-2 leads to the activation of caspase-9 and the transduction of apoptosis signals [19]. The results in previous study showed that there was no significant difference in apoptosis rate in protein level neither among lupus patients and control groups nor between male and female patients with their appropriate controls, which was in accordance with results of some previous studies [20,21]. Most SLE patients enrolled in the present study were in the early stages of SLE and either took no drugs or consumed corticosteroids. Wang et al reported increased apoptosis rate in SLE patients, while nothing was mentioned in their study about the medications used for patients [22]. In the current study, the comparisons of mean Antinuclear Antibodies (ANA) levels of SLE patients and controls revealed that SLE patients had significantly much higher mean ANA than controls, 6.41 ± 0.69 and 0.92 ± 0.41, respectively, Table 2, (P<0.001). In Iraq in Tropical-Biological Researches Unit, College of Science, University of Baghdad confirmed that The sera of SLE patients were positive for ANA (100.0%) while none of the control [23,24]. Another study in Iraq, where explained that ANA was Positive in 90% with SLE patients and with rare percent in healthy control, Antibodies to dsDNA in patients serum are increased comparing with the control [25]. In a previous study done in the Fars Province of Iran, explained the level of ANA in most studies approached 100% only 93% of patients were found to be ANA positive, which is comparable with Indians, Kuwaitis and Europeans [26-29]. This may be due to technical failure because ELISA may fail to detect ANA in some cases. Rabbani M, et al. (2004) and Uthman I, et al. (1999) reported the lowest level of ANA in their series (86% and 87%, respectively) [30,31]. There was no difference in the prevalence of dsDNA as compared with Chinese, Caucasians, Africans, Hispanics and Indians [32-35]. In Department of Pathology, New York Medical College, the study confirmed that All patients had positive ANA [36]. In another study, in which ANA and anti ds DNA antibodies were detected in 97% and 74% respectively which is comparable with another study by Worrall J, et al. (1990) [37]. The present study, the mean Anti ds-DNA antibodies level in SLE patients was significantly higher than in controls, (74.59 ± 11.02) and (11.37 ± 4.69), respectively, (P. value = 0.001) (Table 3). Previous study, Titers of anti-dsDNA antibodies may shed light on the disease activity; in particularly with nephritis, titers might rise during disease flare and subside in disease quiescence [38]. These results were confirmed in the present study, the level of caspase-3 No significant correlation had been found between SLEDAI and caspase-3, P. value (0.518) (Table 4). That mean the level of extrinsic pathway correlate with serology and clinical SLE activity in our patients, the anti-dsDNA antibodies are more specific than ANA in diagnostic SLE. Many studies did not correspond to our current study, Previous study shown the presence of anti-ANA antibodies is not in itself diagnostic or even predictive of disease in some cases [39]. Previous study, sustained anti-dsRNA antibody production may appear that may relate to SLE. In the other situation, a transient antibody profile may appear that may not relate at all to SLE [40]. Some methodological limitations should be considered in the interpretation of our results: the small sample size of the groups studied, different genetic variation between countries most our patients they take medication, also to technical failure because ELISA may fail to detect caspase, in general because of the different techniques used in the measurement caspase, ANA and ds-DNA.

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