Estimation of Caspase-8 in Patients with Systemic Lupus Erythematous and it is Relationship with Disease's Activity

Amna Esmaeel Al-araji[1], Sawsan M. Jabbar AL-Hasnawi[2], Ali alkazzaz[3], Shawqi Watheq Mohammed Ali[4]

[1]*Department of Medical Microbiology and Immunology, College of Medicine, University of Kerbala, Kerbala, Iraq,* [2]*Al-Hussein Medical City, Kerbala, Iraq, Al-sader Medical City and Al-Hakeem hospital, Al-Najaf, Iraq and Merjan Medical City, Babyl, Iraq*

Introduction

Many research groups studied the relationship between SLE & apoptosis as some enzymes of apoptosis considered to be the cause of relapsing of disease, such as caspases 8 & 3. So the correlation between both is still a debate issue. This study aimed to: Assess level of caspase-8 among SLE patients. This done by:

Measurment of caspase 8 level by ELISA technique.

Clarify the relationship between caspase 8 levels and SLE activity by measuring ds-DNA and ANA levels.

Method: 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 Babyl, 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-8) using ELISA technique.

Results: The level of caspase 8 show 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. = 0.001) respectively .

Keywords: *Caspases-8; systemic lupus erthymatosus; Apoptosis; ds-DNA; ANA.*

Introduction

Systemic lupus erythematosus (SLE) is an auto-immune 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). Disturbances in apoptosis and any defect in clearance of apoptotic cells, increases exposure of modified autoantigens to the immune system(4). 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(5). cells finally disintegrate into apoptotic blebs(6). These stimuli

Correspondence Author:
Amna Esmaeel Al-araji
Department of Medical Microbiology and Immunology, College of Medicine, University of Kerbala, Kerbala, Iraq
e-mail: amnaalaraji91@gmail.com

lead to activation of caspases and changes in the plasma membrane[7].SLE Disease Activity Index (SLEDAI), developedat the University of Toronto in 1992, is a global scorereflecting all aspects of disease activity[8]. SLEDAI has certainlimitations in that it does not score some life threateningmanifestations such as pulmonary haemorrhage andhaemolyticanaemia. It is heavily weighted for centralnervous system and does not take into account theseverity of manifestations. Gladman et al[9].definedthat 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 scoreof zero was remission. A change of SLEDAI score ofmore than 12 is a severe flare according to another study[10,11].The mechanism between caspase-8 and SLE is complicated,The level of caspase-8 have an inverse relationship with the activity of the disease, So the current study aimed to: Assess level of caspase- 8 among SLE patients.This done by:

Measurment of caspase 8 level by ELISA technique.

Clarify the relationship between caspase 8 levels and SLE activity by measuring ds-DNA and ANA levels .

Subject and Method

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 Babyl 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 year -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 is 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(-20C) until analyzed. Assessment of ANA, dsDNA and Assessment of apoptosis marker (caspase-8).

Laboratory Assays:

Kits	Sourse
Human CASP3 (Caspase 3) ELISA Kit	Elabscience
Human CASP8 (Caspase 8) ELISA Kit	Elabscience
dsDNA-G Kit	CHORUS
ANA- screen Kit	CHORUS
(C4) Kit	Genrui
(C3) Kit	Genrui

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 distributted variable, and Mann-Whitney U test for two independent samples was used to compare non-normally distributtedcontineous 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 $\leq$ 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 8 of SLE patients was (27.73±1.16) which was significantly lower than that

of controls which was (30.51±1.21), (P. value = 0.023), (Table 6).

Table 6. Comparison of Caspase-8 levels of SLE patients and controls

Caspase 8	SLE Patients (n = 45)	Controls (n = 45)	P. value
Mean	27.73	30.51	0.026 Mann-Whitney U test
SE of Mean	1.16	1.21	
Minimum	3.56	19.01	
Maximum	32.92	32.92	

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).

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 Mann-Whitney U test
SE of Mean	0.69	0.41	
Minimum	0.40	0.22	
Maximum	12.00	2.30	

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)

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

Anti ds-DNA	SLE patients (n = 45)	Controls (n = 45)	P. value
Mean	74.59	11.37	0.001 Mann-Whitney U
SE of Mean	11.02	4.69	
Minimum	7.00	7.00	
Maximum	180.0	35.5	

Discussion

Based on recent findings as described above, our work focused on the association the caspase 8 and immunological marker of SLE.

Apoptosis is a highly controlled process (12), and plays an important role in pathogenesis of SLE. In current study, we examined the level of Caspase-8 in seria patients and control using and protein levels using ELISA technique and aclarify the relationship between caspase 8 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 8 of SLE patients was significantly lower than that of controls which was, (P. value = 0.023), (Table 6) which was consistent with our results[(13)]showed that the expression level of FasL, caspase 8 was decreased in SLE patients and in female, which was in agreement with the results of some previous studies.

Another study corresponds with our study, Mass 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 8 in these patients[(14)].

Comparing the results of male and female SLE patients in present study we showed that apoptosis rate was decreased, and expression of caspase 8 in gene level was lower in female than male. The reason for this difference between male and female patients is not clear, but differences in sex hormones may be involved[(15)]. And others indicated that the level of prolactin was increased in female and male SLE patients, and the level of DHEA and progesterone as hormones having immunosuppressive effects were decreased[(16)].

Previous study that showen, Apoptotic rate is increased in patients with systemic lupus erythematosus, Interaction of death receptor (Fas) with its ligand (FasL) activates caspase-8 which is necessary for transduction of apoptosis signals in extrinsic Pathway 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[(15)].

The results in previous study[(17)] 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[(4)].

In a number of previous studies, apoptosis rate has been reported to be increased in patients with SLE[3], whereas in other studies no difference was observed between SLE patients and controls when Fas molecule was assessed instead of apoptosis[3], which was not compatible with our results.

Caspase-8 deficiency has been recently associated with human diseases, Caspase-8 also carries important non-apoptotic functions[11].

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[4]. and Caricchio et al [18] studied 13 and 25 SLE patients with similar drug dose as our study, respectively, and reported that the percentage of apoptotic cells and the expression level of Fas molecule was the same in SLE patients and healthy controls, which was comparable to our results.Different study results seem to be to someextent influenced by differences in patients' medication regimens. The differences may also be partly affected by different methodologies used in several studies.

In the current study, the mean caspase 8 of SLE patients was (27.73±1.16) which was significantly lower than that of controls which was (30.51±1.21), (P. value = 0.023)

In Iraq in Tropical-Biological Researches Unit, College of Science, University of Baghdad[19] confirmed that The sera of SLE patients were positive for ANA (100.0%) while none of the control[19].

Another study in Iraq, where explained that ANA was Positive in 90% with SLE patients and with rare precent in healthy control, Antibodies to dsDNA in patients serum are increased comparing with the control[20].

These results were confirmed in the present study, the level of caspase 8 is low wih the increase activity of disease also caspase3 in(table 7)that mean the level of extrinsic pathway correlate with serology and clinical SLE activity in our patients, the anti-dsDNA antibodies is more specific than ANA in diagnostic SLE .

Many studies did not correspond to our current study,Previous study showenthe presence of anti-ANA antibodies is not in itself diagnostic or even predictive of disease in some cases[21].

Previous study, sustained anti-dsDNA 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[22].

Other study confirmed differences in levels anti-dsDNA,This study was specifically focused on those patients with changing antids DNA levels, and it became clear that changes in anti-ds DNA content associated with the three different complement components (Clq, C4, and C3).

Some methodological limitations should be considered in the interpretation of our results: the small sample size of the groups studied,different genetic variation between contries,most our patients they take medication, also to technical failure because ELISA may fail to detect caspases, in general because of the different techniques used in the measurementcaspases, ANA & ds-DNA.

Conclusion: ther are important changes in the level of caspase-8 in SLE, The level of caspase-8 have an inverse relationship with the activity of the disease.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

Wallace D, Hahn BH. Dubois' Lupus Erythematosus and Related Syndromes E-Book: Expert Consult-Online: Elsevier Health Sciences; 2012.

Muñoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nature Reviews Rheumatology. 2010;6(5):280.

Munoz LE, van Bavel C, Franz S, Berden J, Herrmann M, Van Der Vlag J. Apoptosis in the pathogenesis of systemic lupus erythematosus. Lupus. 2008;17(5):371-5.

Xue C, Lan-lan W, Bei C, Jie C, Wei-hua F. Abnormal Fas/FasL and caspase-3-mediated apoptotic signaling pathways of T lymphocyte subset in patients with systemic lupus erythematosus. Cellular immunology. 2006;239(2):121-8.

Cohen G, Sun X, Snowden R, Dinsdale D, Skilleter Key morphological features of apoptosis may occur in the absence of internucleosomal DNA fragmentation. Biochemical Journal. 1992;286(2):331-4.

Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, Olson MF. Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK Nature cell biology. 2001;3(4):339.

Heyder P, Gaipl US, Beyer TD, Voll RE, Kern PM, Stach C, et al. Early detection of apoptosis by staining of acid-treated apoptotic cells with FITC-labeled lectin from Narcissus pseudonarcissus. Cytometry Part A. 2003;55(2):86-93.

Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH, Austin A, et al. Derivation of the SLEDAI. A disease activity index for lupus patients. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1992;35(6):630-40.

Gladman D, Goldsmith C, Urowitz M, Bacon P, Bombardier C, Isenberg D, et al. Sensitivity to change of 3 systemic lupus erythematosus disease activity indices: International validation. The Journal of rheumatology. 1994;21(8):1468-71.

Petri M, Buyon J, Skovron M, Kim M. Reliability of SELENA SLEDAI and flare as clinical trial outcome measures. Arthritis & Rheumatism. 1998;41(9):S218.

Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature. 2002;419(6905):395.

Krueger A, Fas SC, Baumann S, Krammer PH. The role of CD95 in the regulation of peripheral T-cell apoptosis. Immunological reviews. 2003; 193(1):58-69.

McNally J, Yoo D-H, Drappa J, Chu J-L, Yagita H, Friedman SM, et al. Fas ligand expression and function in systemic lupus erythematosus. The Journal of Immunology. 1997;159(9):4628-36.

Maas K, Chan S, Parker J, Slater A, Moore J, Olsen N, et al. Cutting edge: molecular portrait of human autoimmune disease. The Journal of Immunology. 2002;169(1):5-9.

Rastin M, Hatef MR, Tabasi N, Mahmoudi M. The pathway of estradiol-induced apoptosis in patients with systemic lupus erythematosus. Clinical rheumatology. 2012;31(3):417-24.

Zandman-Goddard G, Peeva E, Shoenfeld Y. Gender and autoimmunity. Autoimmunity reviews. 2007;6(6):366-72.

Rastin M, Mahmoudi M, Hatef M, Sahebari M, Tabasi N, Haghmorad D, et al. T lymphocyte apoptosis in systemic lupus erythematosus patients. Iranian journal of basic medical sciences. 2013;16(8):936.

Caricchio R, Cohen PL. Spontaneous and induced apoptosis in systemic lupus erythematosus: multiple assays fail to reveal consistent abnormalities. Cellular immunology. 1999;198(1):54-60.

Abbas AH, Melconian AK, Ad'hiah AH. Detection of anti-cytomegalovirus antibodies in sera of Iraqi systemic lupus erythematosus women patients. Iraqi Journal of Science. 2016:317-20.

Deane KD, El-Gabalawy H. Pathogenesis and prevention of rheumatic disease: focus on preclinical RA and SLE. Nature Reviews Rheumatology. 2014;10(4):212.

Olsen NJ, Karp DR. Autoantibodies and SLE— the threshold for disease. Nature Reviews Rheumatology. 2014;10(3):181.

Rekvig O. Anti-ds DNA antibodies as a classification criterion and a diagnostic marker for systemic lupus erythematosus: critical remarks. Clinical & Experimental Immunology. 2015;179(1):5-10.