The Relationship between Interleukin-33(IL-33) and Oxidative Stress in Diabetic Patients with Cardiomyopathy

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Abstract

   Interleukin -33 (IL)-33 is among IL-1 cytokine superfamily , which shows promise as a biomarker predictive of mortality in diabetic and several cardiovascular disorders in vivo study.The objective of this study is to investigate the differences in the levels of IL-33 between healthy controls and patients with type 2 diabetes and diabetic cardiomyopathy and to investigate the correlation of IL-33 with HbA1C (biomarkers of type 2 diabetes), hs-CRP and oxidant –antioxidant status.

   One hundred and fifty individuals (age 40-55) were enrolled in this study which was divided into three groups as follows: G1:50 healthy control,G2: 50 subjects with type 2 diabetes mellitus,G3: 50 patients with diabetic cardiomyopathy. Fasting blood glucose (FBG) , glycated hemoglobin (HbA1C) ,high sensitive C-reactive protein(hs-CRP), malondialdehyde (MDA),uric acid ,total antioxidant capacity (TAC) and IL-33 were evaluated.

   The results in this study revealed highly significant increasing in FBG, HbA1C , hs-CRP ,MDA, uric acid ,and MDA/TAC ratio. while a significant decrease in TAC and IL-33 were found in patients group comparing to control group. Also a significant increase in CRP,MDA ,uric acid ,and MDA/TAC ratio were noticed for G3 compared to G2.A significant decrease in TAC and IL-33 were found for G3 compared to G2.Furthermore,a significant negative correlation between IL-33 and HbA1C, hs-CRP, and MDA/TAC ratio were found between patients groups.

   The conclusion could be drown from this study that the negative correlation of IL-33 levels with HbA1C ,hs-CRP and MDA/TAC levels in patients groups most possibly reflects the inflammatory component of diabetes and oxidant –antioxidant status.

Key words: IL-33, Diabetic Cardiomyopathy , oxidant –antioxidant status

Introduction

IL-33 is a recently described as a new member of the IL-1 family [1] and has attracted attention because, uniquely among IL-1 cytokines family, it stimulates the production of T-helper cells (Th2) cytokines such as IL-5 and IL-13, IL-33 enhanced cytokine production by invariant natural killer T cells and human natural killer cells [2], activates (Th2), and has a role in mast cell development and function [3].

IL-33 may function as an "alarmin" and have a role in signaling cellular damage and inflammatory disease pathogenesis through release from damaged or necrotic cells [4].Also, IL-33 and ST2 are widely expressed in vascular cells and tissues ,in fibroblasts and in the central nervous system[5], coronary artery endothelium [6],and in cardiac fibroblasts suggesting that IL-33 may play a role in various CV disorders[7].Moussion et al. [8] reported that IL-33 is constitutively expressed in endothelial cells from both small and large blood vessels.

Abston et. al. (2013) [9] show for the first time that IL-33 induces eosinophilic pericarditis while sST2 prevents eosinophilia and improves systolic function, and that IL-33 independently adversely affects heart function via the IL-33 receptor by using cytokine knockout mice to determine that this effect was due to IL–33 -mediated signaling but not IL-1β or IL-6. IL-33 in the inflamed arteries could act earlier in artery inflammation promoting angiogenesis, vascular leakage and tissue infiltration by inflammatory cells.IL-33 may also modulate the innate immune responses through the regulation of macrophage effecter functions [10].

Diabetes Mellitus is continuing to become a health problem since the prevalence of DM has increased dramatically over the past two decades [11].The duration of diabetes is an important factor in the pathogenesis of complication, but other factors frequently coexisting with type 2 DM [12].Cardiovascular disease is a common complication of diabetes responsible for 80% of the mortality in the diabetic population [13].

Hyperglycemia is the main pathogenesis factor, which causes abnormalities at the cardiac myocyte level, eventually leading to structural and functional abnormalities which led to diabetic cardiomyopathy that defined as the cardiovascular damage present in diabetes patients, which is characterized by myocardial dilatation and hypertrophy, as well as a decrease in the systolic and diastolic function of the left ventricle, and its presence is independent of the coexistence of ischemic heart disease or hypertension [14].

In recent years it has been shown that oxidative stress and antioxidant play an important role in DM and progression of its complication [14]. Also, data on serum IL-33 levels in human diabetic cardiomyopathy are not available. So from this point the study designed to evaluate the oxidative stress index ,IL-33 in diabetic cardiomyopathy patients and compared the parameters with those for DM patients .Also to correlate the above parameter with some inflammatory biomarkers to clarify at least in part the role of IL-33 and the oxidant - antioxidant status in such patients. Also, data on serum IL-33 levels in human diabetic cardiomyopathy are not available.

Materials and Methods

One hundred and fifty individuals with age ranged between (40-55) were enrolled in this study .They were divided into three groups; first group G1 consisted of 50 healthy individuals as a control group, the second group G2 consisted of 50 patients with T2DM as case control , the third group G3 consisted of 50 DM patients with diabetic cardiomyopathy which diagnosed by physician .The patients attended the Baghdad Teaching Hospital from September 2011 to June 2012. Patients with no other complications of DM, inflammatory or infectious disease ,autoimmune and rheumatic disease, fever ,cancer ,hematological disease,

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renal or liver failure, as well as those who under treatment with steroidal anti-inflammatory drugs were excluded.

Fasting blood samples in all studied groups were analyzed for FBG, HbA1C,hs-CRP,uric acid using standard procedures of the biochemistry laboratory of hospital.

Malondialdehyde (MDA) was determined in serum as a thiobarbituric reactive species according to the method of Buege and Aust [15].Total antioxidant capacity (TAC) was measured using Randox Total Antioxidant Capacity(cat No. Nx 2331) according to the method of Miller [16].Serum IL-33 levels was measured by enzyme-linked immuno sorbet assay (ELISA) method [17] by the using Ray Bio Human IL-33 kit according to the manufactures protocol.

Statistical Analysis

Data are presented as mean ± SD. The differences between two groups were analyzed by Student,s t-test by using SPSS. Pearson,s correlation coefficient was used to examine between IL-33 and HbA1C, hs-CRP, and MDA/TAC ratio in patients groups. P-value of < 0.05 , <0.001 considered significant.

Results& Discussion

This is the first study to document, as far to knowledge, which depict the relation between IL-33 and oxidant-antioxidant status and some inflammation and diagnostic parameters in patients with type 2 diabetes and diabetic cardiomyopathy .

Descriptive and diagnostic parameters for the three studied groups(G1,G2,G3) are shown in table (1). The results in table (1) revealed a highly significant increasing in FBG ,HbA1C, hs-CRP and uric acid in patients groups(G2,G3) comparing to control group(G1).Also a significant increase was observed for G3 compared to G2.

A well-described pathway for the development of diabetic vascular complications involves activation of the diacylglycerol (DAG)-PKC pathway which induces by hyperglycemia this results in the development of complications through altered gene expression and/or protein function, thus contributing to cellular dysfunction and damage. A well-described pathway for the development of diabetic vascular complications involves activation of the diacylglycerol (DAG)-PKC pathway [18].Evidence suggests that increases in systemic markers of inflammation, such as hs-CRP are associated with DM and its complications [19].

The results in table (2) showed a highly significant increasing in MDA and MDA/TAC ratio, while a significant decrease in TAC and IL-33 were found in patients groups comparing to control .Also a significant differences were noticed in G3 comparing to G2.Serum levels of IL-33 were significantly decreased in group G2(median 55.5 pg/ml, range 0-86) with higher reduction in IL-33 levels in G3 (median 21 pg/ml, range 0-32), p <0.001 than control group (median 85.5 pg/ml ,range 0-150) p <0.05 , <0.001.

The results in this study are compatible with reported data that strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress and higher blood glucose level had an association with free radical –mediated lipid peroxidation. Induction in TAC levels in G2 and G3 suggest the imbalance between oxidant and antioxidant systems and the depletion of the protective antioxidant system may be associated with an increase in risk of complications from DM .The increase in ROS serves to decrease the antioxidant capacity of the diabetic myocardium, contributing significantly to oxidative stress and the resultant myocardial damage [20].

Increased uric acid concentration were present in patients with DM and these correlate positively with residual antioxidant activity and risk factor for cardiovascular disease [21].

IL-33 is a cytokine with dual function, acting both as a traditional cytokine implicated in numerous inflammatory disorders and as a transcriptional factor .IL-33 is expressed in various

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tissues and in the heart and vascular tree and is considered to play a significant role in various cardiovascular disorders.The reduction in IL-33 levels in DM patients may due to the protective effect of IL-33 by reducing adiposity and improving glucose tolerance and insulin resistance [22].

Recent study indicates that decreased levels of IL-33 are responsible for the increased sensitivity of the myocardium to ischaemia/reperfusion(I/R) in DM which led to a chronic activation of PKCβII. I/R further enhances PKCβII activation in the diabetic myocardium which results in exaggeration of myocardial injury [23].

Other study demonstrated eosinophil activation through activation of NF-kB and the MAPK kinases by IL-33 are connected to increased oxidative stress which increased expression of endothelin 1 has been found paradoxically to restore diastolic dysfunction and endothelin 1 is a known inducer of sST2 expression and inhibitor of IL-33 signaling through p38 MAPK [24].

A significant negative correlation between IL-33 and HbA1C, hs-CRP,and MDA/TAC ratio were found which $p < 0.0001$(r= -0.58,- 0.64, -0.65 respectively , which shown in   Fig 1(A,B,C)).

The finding of the present study lead to conclude that higher reduction in IL-33 levels in the diabetic patients may be useful in detection of early cardiomyopathy events.

References

1. Milovanovic, M.; Volarevic, V.; Radosavljevic, G.; Jovanovic, I.; Pejnovic ,N.; Arsenijevic,   N.;and   Lukic,ML.(2012)IL-33/ST2   axis   in   inflammation   and immunopathology,Immunologic Research; 52(1-2):89-99.

2. Smithgal ,MD.; Comeau, MR.; Yoon, BR.; Kaufman, D.;Armtage ,R.; and Smith, DE. (2008) IL-33 allergen-reactive Th2 cells, iNKT and NK cells. Int Immunol; 20:1019-30.

3. Enoksson, M.; Westerberg, CM.; Wicher, G.; Fallon, PG.; Nilsson ,KF.; Andersson, CL.; and Nilsson, G.(2013) Neutrophils in response to IL-33 is mast cell–dependent .Blood; 121:530-536.

4. Nile ,C.J.; Barksby,E.; Jitprasertwong ,P.;Preshaw, PM.;and Taylor, JJ.(2010) Expression and regulation of interleukin-33 in human monocytes.Immunology ; 30:172-180.

5. Oboki ,K.; Ohno ,T.; Kajiwara ,N.; et al.(2010) IL-33 and IL-33 receptors in host defense and disease. Allergol Int.; 59:143-60.

6. Bartunek ,J.; Delrue, L.; Van Durme, F.; Muller, O.; Casselman, F.; De Wiest, B.; Croes, R.; Verstreken, S.; Goethals, M.;and de Raedt, H. (2008) Nonmyocardial production of ST2 protein in human hypertropy and failure is related to diastolic load.J Am Coll Cardial ;52:2166-2174.

7. Kunes, P.; Holubcova, Z.;Kolackova, M.; and Krejsek, J. (2010) Interleukin-33, a novel member of the IL-1/IL-18 cytokine family, in cardiology and cardiac surgery . Thorac Cardiovasc Surg; 58:443–449.

8.Haraldsen ,G.; Balogh, J. ;Pollheimer, J.; Sponheim ,J.; and Kuchler, AM.(2009) Interleukin -33- cytokine of dual function or novel alarmin ?.Trends Immunol.; 30:227-233.

9. Abston, ED.; Barin, JG.; Cihakova, D.; Bucek, A.; Coronado ,MJ.; Brandt, JE.; Bedja, D.; Kim ,JB.; Georgakopoulos, D.; Gabrielson, KL.; Mitzner ,W.;and Fairweather, D. (2013) IL-33 Independently Induces Eosinophilic Pericarditis and Cardiac Dilation:ST2 Improves Cardiac Function. Circ Heart Failure ,American Heart Association,Inc.;111:769v1.

10-Ciccia ,F.;Alessandro, R.; Rizzo, A.; Raimondo, S.; Giardina, AR.; Raiata, F.;Boiardi, L.; Cavazza , A.;Guggino, G.; De Leo,G.; Salvarani, C.;and Triolo, G. (2013) IL-33 is overexpressed in the inflamed arteries of patients with giant cell arteritis .Ann Rheum Dis.;72:258–264.

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11-Susanti, E.;Donosepoetro, M.;Patellong, I.; and Arif, M. (2010): Differences between several atherogenic parameters in patients with controlled and uncontrolled type 2 diabetes mellitus. Med J Indones;19:103-8.

12-Raptis ,AE.; Markakis, KP.; Mazioti, MC.; Raptis ,SA.;and Dimitriadis ,GD. (2011) What the radiologist needs to know about the diabetic patient. European Society of Radiology; 2:193-203.

13-Voulgari ,CC.; Papadogiannis, D.;and Tentolouris ,N.(2010) Diabetic cardiomyopathy: from the pathophysiology of the cardiac myocytes to current diagnosis and management strategies. Vascular Health and Risk Management; 6 :883–903.

14- Watanabe, K.; Thandavarayan, RA.; Harima ,M.; Sari ,FR.; Gurusamy, N.; Veeraveedu, PT.; Mito, S.; Arozal, W.; Sukumaran, V.; Laksmanan ,AP.; Soetikno V.;and Aizawa ,Y.(2010) Role of Differential Signaling Pathways and Oxidative Stress in Diabetic Cardiomyopathy.Curr Cardiol Rev; 6(4): 280–290.

15-Buege,JA.; and Aust ,SD.(1978) Microsomal lipid peroxidation .Methods enzymol; 52:302- 310.

16-Miller, N.J.; Rice –Evans, C.; Davies, M.J.; Gopinathan, V.;and Milier, A.(1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates .Clin Sci.; 84 :407-412.

17-Bishop, ML.; Fody ,EP.; and Schosff, LE.(2010) Clinical chemistry techniques ,principles correlations.6ᵗʰ ed, Lippincott Williams &Wilkins:Wolters Klumer Health.

18- Connelly ,KA. ; Kelly ,DJ.; Zhang, Y.;and et al.(2009) Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. Circ Heart Fail; 2: 129–137.

19- Bloomgarden ,ZT.(2011) Diabetes and cardiovascular disease. Diabetes Care; 34:e24-e30.

20- Aydin, M.; Selcoki, Y.;Nazli, Y.;and et al.(2012)Relationship between total antioxidant capacity and the Severity of coronary artery disease.J Clin Exp Invest ; 3(1):22-8.

21- McLaren, JE.; Michael, DR .; Salter ,RC.; Ashlin ,TG.; Calder, CJ.; Miller, AM.; Liew ,FY.;and

Ramji ,DP.(2010) IL- 33 reduces macrophage foam cell formation.J Immunology; 185:1222-1229.

22--Miller ,AM.; Asquith, DL.; Hueber, AJ.; Anderson, LA.; Holmes, WM.; McKenzie, AN.; Xu, D.; Sattar, N.; McInnes, IB.;and Liew, FY.(2010) Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice.Circ Res ;185:1222-1229.

23-Rui, T.; Zhang, J.; Xu ,X.; Yao, Y.; Kao ,R.;and Martin, CM.(2012) Reduction in IL-33 expression exaggerates ischaemia/reperfusion-induced myocardial injury in mice with diabetes mellitus. Cardiovasc Res; 94(2):370-8.

24-Vignon-Zellweger, N.; Relle, K.; Kienten, E.; Seider, P.; Sharkovska, J.; Heiden ,S.; Kalk, P.; Schwab, K.;Albrecht-Kupper, B.; Theuring, F; Stasch, JP.;and Hocher ,B.(2011) Endothelin-1 over expression restores diastolic function in Enosolate knockout mice. J. Hypertens; 29:961-970.

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Table (1): Descriptive and diagnostic parameters for the three studied groups(G1,G2,G3).

Groups parameters	G1 n=50	G2 n=50	G3 n=50	P*	p**	p***
FBG(mg/dl)	89.3±19.7	206.8±29.8	404.1±45.9	<0.0001	<0.0001	<0.001
HbA1C%	5.83±0.97	8.90±0.82	10.2±0.54	<0.0001	<0.05	<0.001
hs-CRP(g/L)	2.56±0.38	4.72±0.49	6.94±0.63	<0.0001	<0.0001	<0.001
uric acid(mg/dl)	5.6±0.63	7.47±0.89	9.93±0.71	<0.001	0.012	<0.001

P*: P-value between G1&G2, p** between G1&G3,p*** between G2&G3

Table (2): MDA, TAC and MDA/TAC and IL-33   levels in three studied groups

Groups Parameters	G1 n=50	G2 n=50	G3 n=50	P*	p**	p***
MDA(µmol/L)	1.4±0.34	3.63±1.16	4.98±0.62	<0.001	<0.05	<0.0001
TAC(mmol/L)	0.81±0.06	0.57±0.08	0.36±0.02	<0.05	<0.001	<0.0001
MDA/TAC	1.87±0.61	6.40±2.1	13.75±2.72	<0.001	<0.001	<0.0001
IL-33(pg/ml)	85.5(0-150)	55.5(0-86)	21(0-32)	<0.05	<0.0001	<0.0001

P*: P-value between G1&G2, p** between G1&G3,p*** between G2&G3
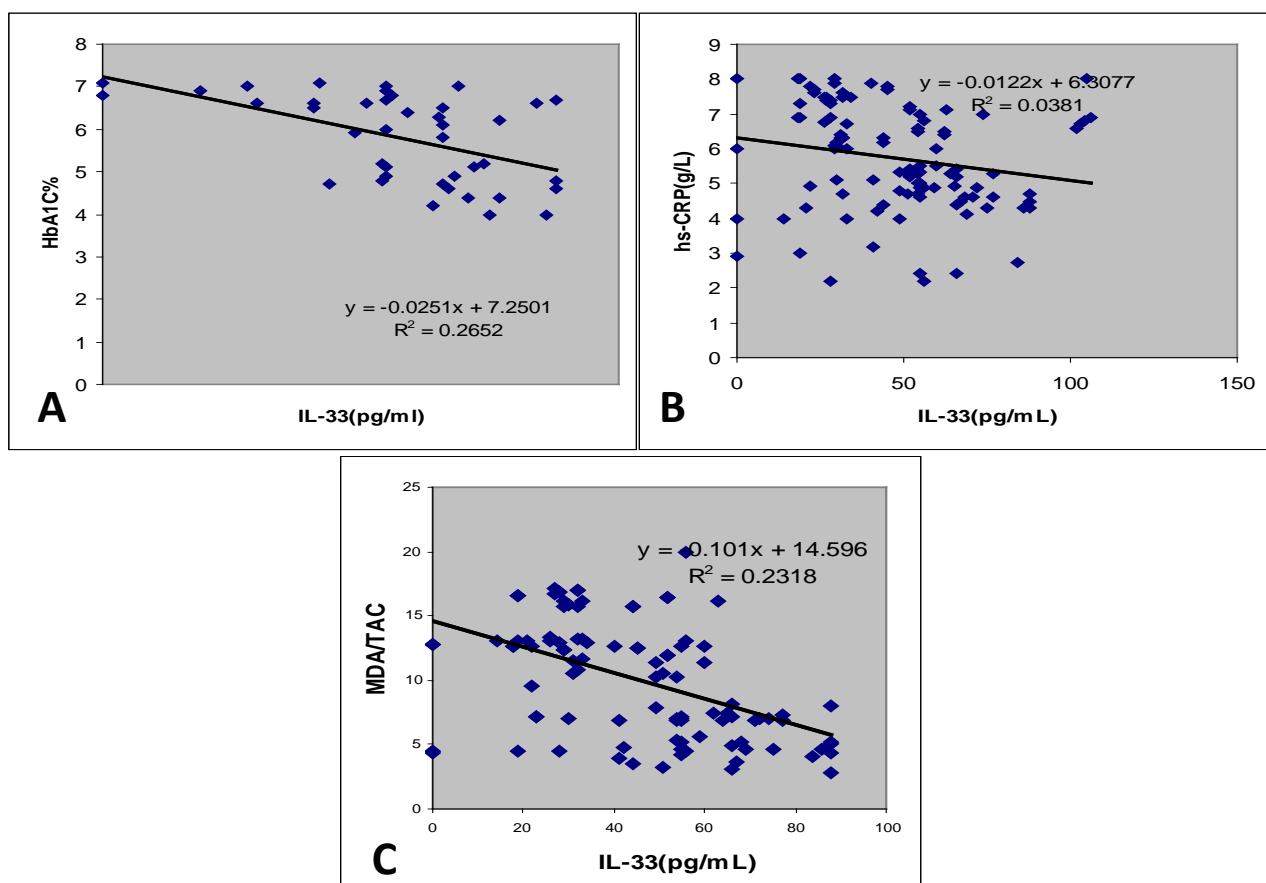
Figure(1):correlation between IL-33 and (A)HbA1C, (B)hs-CRP, and   (C)MDA/TAC

مستويات الانترلوكين-33 في النوع الثاني من داء السكري والاعتلال القلبي السكري وعلاقته بحالة الاكسدة- مضادات الاكسدة

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الخلاصة

الانترلوكين-33 هو من عائلة الانترلوكين -1 كدالة حيوية للتنبؤ في التطور في داء السكرمن النوع الثاني وانواع متعددة من امراض القلب . الهدف من الدراسة هو ان من المهم بحث الاختلافات في مستويات الانترلوكين-33 بين الاصحاء والمصابين بداء السكرمن النوع الثاني والدين يعانون من اضطرابات قلبية كمضاعفات لداء السكرو كدلك بحث العلاقة بين الانترلوكين -33 والهيموكلوبين المسكر (الدالة الحيوية لداء السكر) و بروتين سي التفاعلي ونسبة المالون ثنائي الالديهايد/سعة مضادات الاكسدة الكلي.

تضمنت الدراسة مائة وخمسون شخصا تتراوح اعمارهم بين 40-55 سنة والدين تم تقسيمهم الى ثلاث مجاميع كمايلي:المجموعة الاولى وعددهم خمسون شخصا من الاصحاء كمجموعة سيطرة.والمجموعة الثانية وعددهم خمسون مريضا من المصابين بداء السكري والمجموعة الثالثة وعددهم خمسون مريضا من مرضى الاعتلال القلبي السكري .تم قياس كل من السكر الصائم والهيموكلوبين المسكر و بروتين سي التفاعلي والمالون ثنائي الالديهايد وحامض اليوريك و والانترلوكين -33 في كل المجاميع .

بينت النتائج وجود زيادة معنوية في السكر الصائم والهيموكلوبين المسكر و بروتين سي التفاعلي والمالون ثنائي الالديهايد وحامض اليوريك و نسبة المالون ثنائي الالديهايد/سعة مضادات الاكسدة الكلي. بينما وجد انخفاض معنوي في سعة مضادات الاكسدة الكلي والانترلوكين -33 في مجاميع المرضى مقارنة مع مجموعة الاصحاء.كدلك لوحظ وجود زيادة معنوية في السكر الصائم والهيموكلوبين المسكر و بروتين سي التفاعلي والمالون ثنائي الالديهايد وحامض اليوريك و نسبة المالون ثنائي الالديهايد/سعة مضادات الاكسدة الكلي في المجموعة الثالثة مقارنة بالمجموعة الثانية. كدلك وجد انخفاض معنوي في سعة مضادات الاكسدة الكلي والانترلوكين -33 في المجموعة الثالثة مقارنة بالمجموعة الثانية.كدلك وجدت علاقة سالبة بين الانترلوكين -33 والهيموكلوبين المسكر وبروتين سي التفاعلي ونسبة المالون ثنائي الالديهايد/سعة مضادات الاكسدة الكلي في مجاميع المرضى.

تم الاستنتاج من هده الدراسة وجود علاقة سالبة بين الانترلوكين-33 و والهيموكلوبين المسكر وبروتين سي التفاعلي ونسبة المالون ثنائي الالديهايد/سعة مضادات الاكسدة الكلي تعكس وجود الالتهاب عند المصابين بداء السكري و مرضى الاعتلال القلبي السكري .

الكلمات المفتاحية: انترلوكين-33، الاعتلال القلبي السكري،حالة-الاكسدة-مضادات الاكسدة