Interleukin-33(IL-33) in Iraqi's Rheumatoid Arthritis Patients Prone to Atherosclerosis

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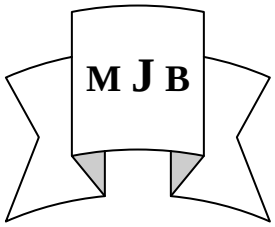

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Abstract

The aim of this study is to determine serum IL-33 levels and atherogenic index of plasma (AIP) .Forty patients with moderate activity of rheumatoid arthritis (RA) and forty healthy individuals as control group were enrolled in this study, age (25-٤5) years. Disease activity was assessed in patients by erythrocyte sedimentation rate (ESR),C-reactive protein (CRP) and rheumatoid factor(RF) .Also lipid profile (cholesterol TC, triglyceride TG, low density lipoprotein LDL-C, very low density lipoprotein VLDL and high density lipoprotein HDL-C), AIP, and IL-33 were determined in all subjects. The results revealed a significant increase in ESR,CRP and RF , TG, VLDL,AIP and IL-33,while is a significant decrease in HDL concentration in patients group compared to control group was found. No significant increase in TC and LDL-C levels were noticed between patients and control groups. Positive correlation was found between CRP and IL-33 in patients group. The conclusion could be drawn from this study that the patients presented with cardiovascular disease as concomitant of RA, which predicted by elevated of AIP value that can be calculated easily.IL-33 used as a novel parameter for assessing disease activity in patients with RA more than CRP dose alone.

Key words: Rheumatoid arthritis, Cardiovascular Disease, IL-33.

الخلاصة

الهدف من الدراسة هو تقدير مستويات الانترلوكين -٣٣ في مصل الدم كدلك تم تقدير مؤشر تصلب الشرايين العصيدي .تضمنت الدراسة اربعون مريضا مصابا بالروماتيزم الرثوي من الدرجة المتوسطة واربعون شخصا سليما كمجموعة سيطرة الدين تتراوح اعمارهم بين(٢٥-٤٥)سنة.تم تقدير فعالية المرض بواسطة معدل ترسيب كريات الدم الحمر و بروتين -س- التفاعلي والعامل الرثوي.تم تقدير الكولسترول والدهون الثلاثية والبروتين الدهني القليل الكثافة و البروتين الدهني القليل الكثافة جدا والبروتين الدهني العالي الكثافة ومؤشرتصلب الشرايين العصيدي والانترلوكين-٣٣ . بينت النتائج وجود زيادة معنوية في كل الدوال الحيوية الالتهابية والدهون الثلاثية و البروتين الدهني القليل الكثافة جدا و ومؤشرتصلب الشرايين العصيدي والانترلوكين -٣٣، بينما وجد انخفاض معنوي في البروتين الدهني العالي الكثافة في مجموعة المرضى مقارنة بمجموعة السيطرة . لم يلاحظ اي ارتفاع معنوي في مستويات كل من الكولسترول والبروتين الدهني القليل الكثافة في مجموعة المرضى مقارنة بمجموعة السيطرة.وجدت علاقة موجبة بين بروتين -س- التفاعلي والانترلوكين -٣٣ في مجموعة المرضى . تم الاستنتاج من هده الدراسة ان المرضى يعانون من المرض الوعائي القلبي كمضاعفات للروماتيزم الرثوي والدي تم التنبؤ به بواسطة ارتفاع مؤشر تصلب الشرايين العصيدي والدي يتم قياسه بسهولة.كدلك الانترلوكين-٣٣ استخدم كدالة حديثة لتعين فعالية المرض في المرضى المصابين بالروماتيزم الرثوي اكثر مما يعمله بروتين -س- التفاعلي لوحده.

كلمات مفتاحية :الروماتيزم الرثوي ،مرض القلب الوعائي ، الانترلوكين -٣٣

Introduction

Interleukin-33 (IL-33) is the new membrane of the IL-1 cytokine superfamily.IL-33 recently was identified as a legend for the orphan IL-1 family receptor T1/T2 [1].IL-33 plays a major role in a wide range of inflammatory, infections, and autoimmune diseases [2]. IL- 33 exhibits pro inflammatory potential by inducing the production of a number of inflammatory mediators in mast cells. T/ST2 also exists as a soluble isoform (sST2) obtained by differential messenger RNA processing. Soluble ST2 is identical with the extracellular region of the long T1 / ST2 isoform except for 9 additional amino acids, and it was recently demonstrated to act as an antagonistic decoy receptor for IL-33 [3].Increased levels of sST2 have also been observed in the synovial fluid of patients with RA[4],which is a chronic inflammatory autoimmune disease characterized by synovitis, bone destruction with pannus formation, and degradation of articular cartilage [5].Mast cells have been recognized as important mediators of the pathogenesis of arthritis suggested that IL-33 mediated mast cells activation might play a role in joint inflammation [6,7].

An increase in mortality in RA is predominantly due to accelerated coronary artery and cerebrovascular atherosclerosis leading to cardio vascular disease (CVD) [8-10].The prevalence of dyslipoproteinemia and sedentary lifestyle seems to be increased in RA . However, there is limited information about the relative prevalence of CV risk factors in RA and to what extent this could account for the observed difference in CV events [11].

Atherogenic index of plasma (AIP) is the new marker of atherogenicity, since the AIP is related directly to the atherosclerosis significantly with increasing atherogenic risk (AIP from 0.24 to 0.51) [12].Existence of hypertriglyceridemia will increase the activity of hepatic lipase which results in the increase of HDL-c catabolism (degradation of HDL-c). Each degradation of one mg HDL-c will correlate with 2% increase in the risk coronary heart disease [13].AIP indicate a balance between the actual concentration of plasma total triglyceride and high density lipoprotein (HDL) , which predetermine the direction of the cholesterol transport in an intravascular pool in the flux of newly produced cholesterol esters by lecithin cholesterol acyltransferase towards atherogenic LDLs beneficial HDLs [14].

This study aimed to examine the level of IL-33 and the predictive value to atherosclerosis in RA patients using the value of AIP and correlation of IL-33 with CRP to clarify the relation of atherosclerosis development in RA patients.

Materials and Methods

Patients and Control:

Serum samples were obtained from forty Iraqis adult patients of RA with moderate disease activity (22 women and 18 men) and forty healthy individuals as a control group(24 women and 16 men) which enrolled in this study with aged range (25-45) years . The patients attended the Physical Therapy Center in Baghdad during (2011-2012).The design of the study is cross-sectional.

Disease Activity Assessment:

ESR, CRP and RF [15] were measured for patients in the first visit to the hospital and to the control group as diagnostic parameters and disease activity.

Lipid Profile Analysis:

TC, TG and HDL were determined by using ready kit from LINEAR chemicals ,S.L.-Spain. The methods in determining of Ch and TG depended on enzymatic method, while HDL determination based on the selective precipitation of apolipoprotein (VLDL and LDL) [16].

LDL and VLDL concentrations were commonly calculated by using the empirical Friedwald formula [17].

LDL (mg/dl) = TC- (HDL + TG/5)

VLDL = TG/5

Atherogenic Index of plasma (AIP):

AIP was calculated as log of ratio (TG/ HDL-C) in (mmol/L) [18].

Measurement of Serum IL-33 :

Serum IL-33 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) [16] kit (Ray Bio Human IL-33 for *in vitro* quantitative measurement of human IL-33 in serum) ,according to the manufactures protocol.

Statistical Analysis:

The data was expressed as mean ± SD. The comparison between patients group and control group were analyzed by using student t-test. Pearson's correlation coefficient was used to examine between IL-33 and CRP in patients group. P-value of < 0.001 and < 0.05 were considered highly significant and significant respectively.

Results

Descriptive and disease activity assessment (ESR, CRP, and RF) in RA patients and control groups are shown in table (1).

The results revealed significant increase in ESR, CRP and RF in RA patients group compared to healthy control group , which they are considered as patients with moderate disease activity. These results are in agreement with reported data showing that RA is associated with an increased ESR, CRP and RF [19].

The results in table (2) revealed highly significant increase in TG,VLDL,AIP and IL-33, ($P< 0.001$)and a significant decrease in HDL was found in patients group comparing to control group ($P<0.05$) .There was no significant difference in serum TC and LDL-C levels were noticed between patients and healthy individuals($P\geq 0.05$) .

A significant positive correlation were found between IL-33 and CRP in patients group ($P < 0.001$, $r = 0.407$; Fig 1).

Discussion

This is the first study have been found the elevation in IL-33 levels in RA patients prone to atherosclerosis in the world.

C-reactive protein is a major inflammatory cytokine that functions as a nonspecific defense mechanism in response to tissue injury or infection [20].The results of this study are in agreement with a study stated that all patients undergoing long term RA are likely to generate an increased arterial deposit, leading to atherosclerosis, and the risk is increased about 2-3 folds in RA patients than control group [21].

The increased risk of CVD has many causes, but dyslipidemia plays a prominent role in it, commonly associated with an abnormal lipoprotein phenotype which is characterized by increased TG , decreased HDL-c and an accumulation of small dense LDL-c particles even when the level of TC and LDL-c are often normal [22,23].The exact nature of the protective effect of HDL levels is not known; however, a possible mechanism is that serum esterase which degrades oxidized lipids is found in association with HDL

.Possibly, the HDL-C associated protein destroys the oxidized LDL, accounting for HDL ability to protect against heart disease. On the other hand oxidized atherogenic lipoprotein, namely oxidized LDL-c is taken up by immune system cells(macrophages), which becomes engorged to foam cells. These foam cells become trapped in the wall of the blood vessels and contributes to the formation of atherosclerosis plaques that cause arterial narrowing and lead to heart attacks [24].

A number of primary predictors for risk of CVD such as CRP and dyslipoproteinemia have been used, also, according to Grover either the ratio of LDL-C/HDL-C or TC/HDL-C is the best related predictor of future cardiovascular disease [25], but a new biochemical reliable risk factors are still in demand. AIP is very important marker in RA patients which modifiable CV risk factors are highly prevalent and occur more frequently in RA than in age-matched controls [25].It has been reported that AIP has higher predicted value for atherosclerosis and some ratio of pre atherogenic markers when divided by HDL, will increase the odds ratio value which mean higher predictive value toward atherosclerosis, as compared to atherogenic markers alone [26].

Recent study demonstrated that elevated levels of serum sST2 level as well as IL-33 decreased following disease- modifying anti rheumatic drugs (DMARDs) therapy in treated RA patients, their observation lead them to suggest that the inflammatory state is involved in the production of sST2 in patients with RA, since they found that ST2L was expressed in the RA synovium [27] and this expression of ST2L is restricted to the surface of Th2 – cell and mast cell, and is implicated in regulating Th2- associated immune responses , although ST2L can also promote Th1-type responses under certain conditions [28]. Liew et al. [29] suggest that in early RA, IL-33 produced by synovial fibroblast and synovial endothelial cells induces a Th2-type response, and they hypothesized that the increased expression of ST2L in the RA synovium would be caused by increased in filtration of mast cells or neutrophiles. It has been reported that IL-33 enhanced auto- antibody-mediated mast cell degranulation in vitro and in synovial tissue in vivo , and these observation demonstrate that IL-33 can enhance autoantibody – mediated articular inflammation via promoting mast cell degranulation and pro-inflammatory cytokine production, this finding provides a novel mechanism whereby a host tissue- derived cytokine can regulate effecter adaptive immune response via enhancing innate cellular activation in inflammatory arthritis. They also demonstrate that IL-33 is a critical pro-inflammatory cytokine for inflammatory joint disease by promoting humeral immune responses as well as mast cell activities, therefore, IL-33 may be a potential new therapeutic target for RA [30-32].

Recently Nile et al. found that expression of IL-33 is associated with chronic inflammatory condition [33] while Palmer et al. suggest that increased IL-33 leads to up regulation of inflammatory cytokines in patients with RA [34] .

The conclusion could be drawn that increasing in IL-33 reflects the RA disease activity in patients prone to atherosclerosis which predicted by elevated of AIP value more than CRP alone does. Also more studies needed to ascertain the utility of IL-33 as a

biomarker for assessing disease activity in patient with RA.

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Table 1 Descriptive and Disease Activity Assessment (ESR ,CRP, and RF) in RA patients and control groups

Groups / Parameters	Control n=40	RA patients n=40	t-test
Age (years)	22-35	22-35	_
Duration (years)	NA	6	_
Male/Female	16:24	18:22	_
ESR (mm/hr)	12.95± 1.57	32.53± 5.27	S
CRP (mg/L)	0.44± 0.19	1.75± 0.21	S
RF (IU/ ml)	22.02± 8.97	50.55± 6.57	S

S; significant (p< 0.05), NA not applicable

Table 2 Lipid profile, AIP and IL-33 in sera of RA patients and healthy control groups

Groups / parameters	Control n=40	RA patients n=40	t-test
TC (mg/dl)	127.50± 8.54	126.25± 9.35	NS
TG(mg/dl)	104.40± 22.15	211.12± 13.65	HS
LDL-C(mg/dl)	67.63± 10.20	63.54±18.33	NS
HDL-C(mg/dl)	40.10± 3.30	30.22 ± 3.42	S
VLDL(mg/dl)	19.80± 1.23	42.08± 2.80	HS
AIP	0.38 ± 0.061	0.83±0.062	HS
IL-33 (pg/ml)	150.93± 177.3	657.12±464.5	HS

S Significant (P < 0.05), HS highly significant(P < 0.001), NS no significant(P≥ 0.05)

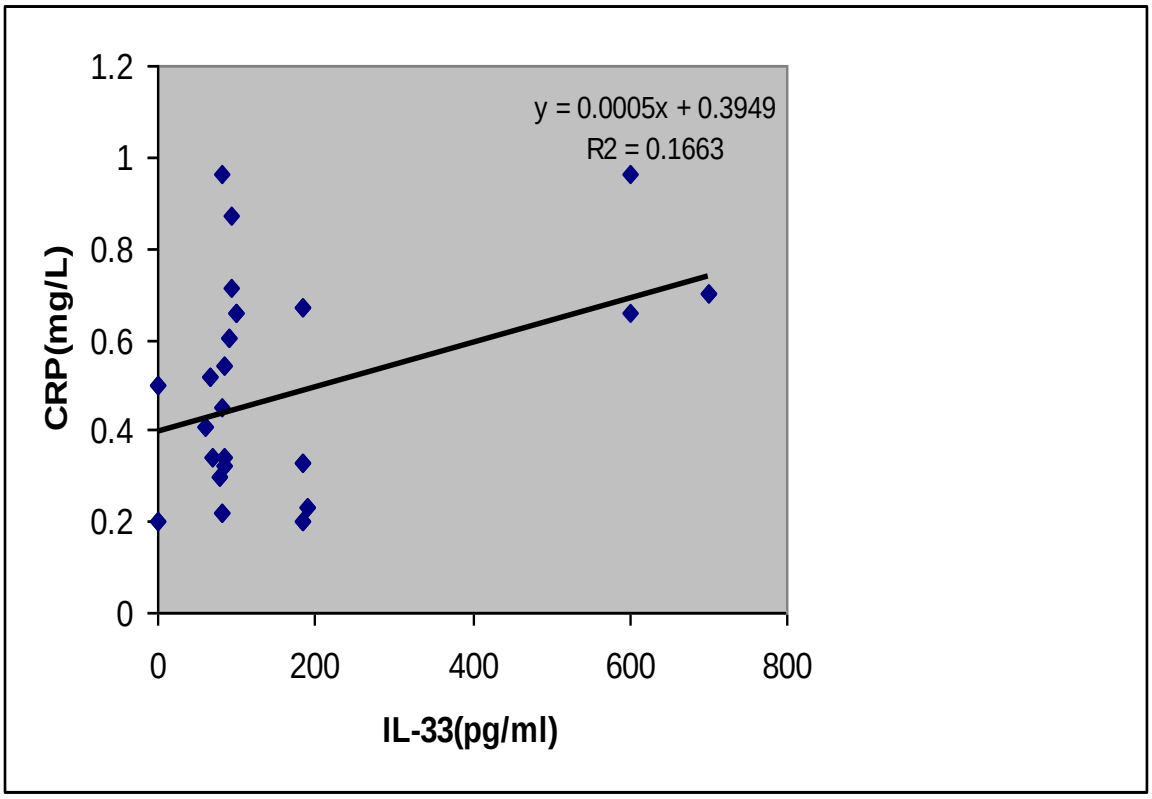

Figure 1 correlation relation between IL-33 and CRP in patients group