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COMPARATIVE STUDY OF PREDICTING THE RISK OF CARDIOVASCULAR DISEASES IN ACTIVE RHEUMATOID ARTHRITIS IRAQI PATIENTS BY TRADITIONAL AND NONTRADITIONAL METHOD

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ABSTRACT
Rheumatoid arthritis (RA) is associated with increased cardiovascular (CVD) mortality and morbidity. This increased in CVD risk is due to interaction between traditional (Framingham) CVD risk factors and nontraditional (biomarkers) CVD risk factors. The study was conducted to determine the relationship between the Framingham risk score (FRS) and the level of intercellular adhesion molecule 1 (ICAM-1), Tumor necrosis factor alpha (TNF-α), Interleukin 6 (IL-6), High sensitive C reactive protein (hsCRP) and erythrocyte sedimentation rate (ESR) RA and healthy subjects. This study included twenty nine active RA patients and twenty nine age and sex matched healthy controls. The levels of ICAM-1, TNF-α, IL-6, hsCRP and ESR were determined and the FRS was calculated. Then the association between these markers and the FRS was determined. in compared to the controls RA patients had increase ICAM-1, TNF-α, IL-6, hsCRP and ESR ($p<0.05$); also the FRS was higher than the controls ($p<0.05$). There was a significant direct association between FRS and (TNF-α, hsCRP and ESR). A non significant correlation between FRS and (ICAM-1and IL-6) was founded in RA patients. While there was a significant direct correlation between FRS with hsCRP, and a non significant association between FRS with ICAM-1, TNF-α, ESR and IL-6 in the controls. TNF-α, hsCRP and ESR are comparable to the FRS and might be used as a marker for future CVD event patients with RA.

KEY WORDS: Rheumatoid arthritis, Framingham risk score, TNF-α, hsCRP, ESR.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, which affects millions of people all around the world; the prevalence of the disease is ranging from 0.5 to 1% [1]. Rheumatoid arthritis has increased morbidity and mortality from premature CVD. Up to 50% of this excess mortality is secondary to ischemic heart disease (IHD) closely followed by cerebrovascular disease, with a 1.5-fold increase in the standardized mortality ratio due to CV events compared with the general population[2], this excess morbidity and mortality cannot be explained by traditional risk factors (age, gender, smoking, hypertension (HT), use of antihypertensive medications and dyslipidemia) alone [3,4,5], other nontraditional factors are hypothesized to play a role in RA are CRP, interleukin 1(IL-1), IL-6 and TNF-α [4,6].These inflammatory markers have been suggested to be independent predictors of cardiovascular events[7]. Additionally ICAM and vascular cell adhesion protein-1 (VCAM-1) are good predictors of endothelial dysfunction and accelerated atherosclerosis in RA patients[6]. The cytokines play a key role in the processes that cause inflammation, articular destruction and the comorbidities associated with RA. The major cytokines that involve in the pathogenesis of RA are TNF-α and IL-6 [8]. Intercellular adhesion molecule-1 plays an important role in the pathogenesis of RA; it's involved in the migration and activation of lymphocytes, monocytes and neutrophils [9]. High sensitive C-reactive protein may provide an adjunctive method for global assessment of cardiovascular risk. It's also shown that plasma levels of hsCRP are a strong independent predictor of risk of future myocardial infarction, stroke, peripheral arterial disease, and vascular death among individuals without known cardiovascular disease [10]. Acute phase reactants ESR and CRP provide reliable means for discrimination between drugs that provide symptomatic relief only and those with a more profound effect in RA[11]. The ESR is sensitive for most types of inflammation, but cannot distinguish if the underlying cause is infectious, inflammatory, or paraneoplastic[12]. The Framingham risk score is an extensively studied index to predict cardiovascular risk in the general population[13]. It includes age, gender, smoking, blood pressure, and cholesterol concentrations and estimates the risk of coronary events by stratifying individuals into three risk categories: low (<10% risk of an event in 10 years), intermediate (10% to 20%), and high (>20%)[14]. The aim of this study was determine the association between the FRS and the level of ICAM-1, TNF-α, IL-6, hsCRP and ESR.

SUBJECTS & METHODS

Subjects

This study was conducted at Rheumatology Unit, Baghdad Teaching Hospital, Baghdad, Iraq carried out over 5 months from September 2012 till March 2013 at Rheumatology Unit, Baghdad Teaching Hospital. The study include twenty nine RA patient diagnosed according to the 1987 American College of Rheumatology (ACR)

criteria [15] with moderate to highly active disease defined as disease activity score based on 28 joints and ESR (DAS28-ESR) greater than 3.2 at baseline[16]. For inclusion, patients also were required to have taken methotrexate (MTX) regularly for at least 4 previous consecutive months. A control group includes twenty nine healthy ages and sex matched individuals without systemic inflammatory diseases was recruited from the local community and evaluated in the same period. The exclusion criteria included pre-existing CVD, renal impairment, hepatic impairment, pregnancy, breast feeding, patients with mild or inactive RA, using DMARDs other than MTX , with high dose of NSAIDS, and patients with co-existent other connective tissue disease. Informed consent was obtained from all participants and this study was approved by the ethical committee of Baghdad University, College of Medicine - Medical Department.

Sample

Blood samples were obtained from the antecubital vein between 8 AM and 9 AM and after a 12-hour fast. Samples were centrifuged at 2000 rpm for 15 minutes then the serum aliquots were stored at -20 °C during the collection period in order to determine the levels of ICAM-1, TNF-α, IL-6 and hsCRP.

Clinical and laboratory evaluation

Clinical evaluation of patients for tender and swelling joints was done by specialized rheumatologist. The RA disease activity was measured using DAS28-ESR. DAS28 is calculated from the number of tender and swollen joint counts (TJC and SJC; 28-joint count), patient self-assessment of disease activity (visual analog scale, VAS), and ESR [16]. Standardized methods with quality control procedures were used to determine the serum level of the analytes. Serum total cholesterol (TC) and serum high density lipoprotein cholesterol (HDL) were measured by spectrophotometric method [17,18]. ESR was measured by Westergren method [19]. Serum levels of ICAM-1, TNF-α, IL-6 and hsCRP were measured using enzyme linked immune sorbent assay[20-23]. The Framingham risk score was calculated according to internet formula which includes age, sex, smoking status, the presence or absence of diabetes mellitus (DM), diastolic blood pressure, weather the patient take treatment for HT or not, TC and HDL [24].

Statistical analysis

Statistical package for social sciences version 16 (SPSS v. 16) was used for data input and analysis. Continuous variables presented as mean ± standard deviation (SD) and discrete variables presented as numbers and frequencies. Chi square test for independence was used to test the significance of association between discrete variables. Continuous variables were tested by both Shapiro Wilk test and by Q-Q plot to determine if they were normally or abnormally distributed. Independent sample T test was use to test the significance of difference in the mean of two independent samples in normally distributed continuous variables. Mann Whitney test was use to test the significance of difference in the mean of two independent samples in abnormally distributed continuous variables. Spearman correlation coefficient was used to assess the correlation between abnormally distributed continuous variables. For the entire test above P value of less than 0.05 was considered significance value.

RESULTS

Demographic presentation of 29 RA patients and 29 controls were elucidated in table 1. Frequency matching was successful with RA and control groups having similar age distributions (mean ages, 44.6 ± 1.17 and 46.1 ± 7.5, respectively, p=0.58), proportion of females (79.3% and 75.8% respectively, p=0.75). Table 2 showed the result of the parameters for both controls and RA patients. There was a significant increase in the serum levels of ICAM-1, IL-6, hsCRP, TC and HDL, and a highly significant difference in ESR, FRS and the serum level of TNF-α comparing with controls. Table 3 showed the correlation of FRS with ICAM-1, TNF-α, IL-6, hsCRP, and ESR in RA patients. There was a significant direct correlation between FRS with TNF-α, hsCRP, and ESR, and a non significant association between FRS with ICAM-1 and IL-6. Table (4) showed the correlation of FRS with ICAM-1, TNF-α, IL-6, hsCRP, and ESR in controls. There was a significant direct correlation between FRS with hsCRP, and a non significant association between FRS with ICAM-1, TNF-α, ESR and IL-6.

TABLE1: Demographic presentation of RA patients and control.

Parameter	Control	RA	P- value
Age (year)	46.1 ± 7.5	44.6 ± 1.17	0.58
Female: Male n (female) %	22:7(75.8%)	23:6 (79.3%)	0.75
HT n (%)	3	9 (31%)	0.07
DM n (%)	-	3 (10%)	0.15
Smoker n (%)	2(7%)	2 (7%)	1
Disease duration (year)	-	9.6 ± 8.3	
Family history of RA n	2 (7%)	6 (20%)	0.12
Family history of CVD n	3 (10%)	7 (24%)	0.16

Continuous variables presented as Mean ± Standard deviation; and discrete variables as numbers and frequencies.

TABLE 2: Parameters of the patients and controls

Parameter	MTX	Control	P value
ICAM-1 pg/ml	7002 ± 3508*	4485 ± 2953	0.014
IL-6 pg/ml	18.9 ± 7.6*	11.8 ± 6.7	0.02
TNF-α pg/ml	36.2 ± 1.9***	11.8 ± 1.14	0.000
hsCRP µg/ml	7.1 ± 4.5**	2.8 ± 3.1	0.003
HDL mg/ml	37.71 ± 8.77*	44.5 ± 9.57	0.043
TC mg/ml	208.36 ± 41.1*	180.8 ± 33.9	0.04
ESR mm/hr	57.05 ± 3.11***	15.6 ± 8.9	0.000
Framingham risk score	8.6 ± 6.3***	1.3 ± 0.57	0.000

Value were presented as mean ± standard deviation of mean; (*) = significance difference, (P<0.05) with respect to control group and RA group, (**) = highly significant (P<0.01) difference with respect to control group and RA group, (***) = highly significant (P<0.001) difference with respect to control group and RA group.

TABLE 3: Spearman correlation between Framingham risk score and other parameters in RA patients.

Parameter	R value	P value
ICAM-1 pg/ml	0.024	P = 0.902
IL-6 pg/ml	0.038	P = 0.845
TNF-α pg/ml	0.412	P = 0.026*
HsCRP µg/ml	0.398	P = 0.033*
ESR mm/hr	0.386	P = 0.038*

Value were presented as mean ± standard deviation of mean; (*) = significance correlation, (P<0.05)

TABLE 4: Spearman correlation between Framingham risk score and other parameters in the controls.m

Parameter	R value	P value
ICAM-1 pg/ml	-3.16	P = 0.174
IL-6 pg/ml	-1.99	P = 0.401
TNF-α pg/ml	-0.338	P = 0.145
hsCRP µg/ml	0.552	P = 0.012*
ESR mm/hr	0.228	P = 0.334

Value were presented as mean ± standard deviation of mean; (*) = significance correlation, (P<0.05)

DISCUSSION

The present study demonstrated an increase in the prevalence of the traditional modifiable CVD among RA patient comparing to control subjects. Similar findings were shown by Cecilia *et al.*, 2012 [25]. This study showed a significant increase in the level of ICAM-1, IL-6, and TC, a significant decrease in HDL, a highly significant increase in TNF-α and a very highly significant increase ESR hsCRP in the RA patient comparing to the control group. These result agreed with previous studies which showed similar result to the present study [26-30]. There is a highly significant increase in FRS in the MTX group comparing to the control group. The same result was obtained Cecilia *et al.*, 2006 [31]. This increase in the FRS is due to that RA is associated with an increased risk for CV disease[32]. Poor disease control in RA due to persistent chronic active inflammation in addition to increased burden of traditional (Framingham) cardiovascular risk has been attributed to CVD-related death in RA [33]. Determination of the CV risk has become the accepted way of targeting CV preventions toward asymptomatic individuals. This approach has been supported by the significant improvements in CV survival in the general population. This is reached using CV risk scores, such as the FRS[34]. The result of this study showed that the increase in the FRS is associated increase in the serum level of IL-6; however the correlation was not significant in both RA patients and control group. Philip *et al.*, 2009 found a non-significant correlation between IL-6 and some variables of the Framingham risk score (systolic blood pressure and age) in both controls and RA patients [35]. Young *et al.*, 2009 found that the level of IL-6 was increased in RA patient and significantly associated with the severity of subclinical atherosclerosis but this independent of the FRS [36]. The inhibition of IL-6 by the anti IL-6 tocilizumab is associated with adverse effect on lipid profile and absence of human studies on the effect of blocking IL-6 on the CV outcome may give support to the present study [37]. The result of this study showed that the increase in the Framingham risk score is associated with increased in the serum level of ICAM-1; however there is no significant correlation in both RA patients and control group. Similar finding was shown by Young *et al.*, 2009 [36]. Several studies demonstrate an increase in ICAM-1 in coronary heart disease; however the relation of ICAM with age, TC, HDL and other traditional CV was not significant except with smoking [38].

Also there is a significant positive correlation between the serum levels of TNF-α and Framingham risk score in RA patients and a non significant correlation between FRS and TNF-α in control subjects. Nels *et al.*, 2012 showed a positive correlation between the serum levels of TNF-α and age, systolic blood pressure and TC and negative correlation with HDL which are variables of FRS [39]. The use of anti TNF-α is accompanied by improvement in endothelial dysfunction and reduction in carotid artery intima-media thickness which may indicate that TNF-α play a fundamental role in the pathogenesis of atherosclerosis [40, 41]. Additionally TNF-α antagonist use was associated with the lowest risk of CV events[42]. Therefore TNF-α is comparable to FRS and can be as marker for future CVD in RA patient.

The result of this study showed a significant positive correlation between the serum levels of hsCRP and Framingham risk score in both RA patients and controls. Similar finding also showed by Sudha *et al.*, 2012 study [43]. Also Nader *et al.*, 2001, showed a direct relation between hsCRP and the risk for future CVD[44]. High sensitive C-reactive protein is the most promising inflammatory biomarker for future CVD. The hsCRP classify patients in a global risk prediction algorithm who are classified as low-, moderate- or moderately high risk by the (FRS)[45]. Serum CRP levels in RA patients frequently are above the 3 mg/L and 10 mg/L cutoffs associated with high and very high risk for CVD in the general population. A cross sectional data from a recent observational cohort of 767 RA patients showed the median CRP level to be 11 mg/L, indicating that 50% of those RA patients had CRP levels associated with very high CV risk. For comparison, less than 5% of individuals in the Women's Health Study had CRP levels 10 mg/L [46].

The result of this study showed a significant positive correlation between the ESR and Framingham risk score in RA patients and a non significant correlation between FRS and ESR in control subjects. A highly significant positive correlation between the ESR and FRS was shown by Sudha *et al.*, 2012 study[43]. Also Pilar *et al.*, 2013 showed a significant correlation between ESR and comorbidities in RA[47]. A large number of evidence suggesting that controlling inflammation in RA has been associated with a decrease in CVD. A study evaluated the effect of methotrexate in 1,240 patients with RA found a significant decrease in the number of cardiovascular deaths, is associated with the adjustment for ESR, sex, age, RF, year, duration of disease, smoking [48]. Other study in 141 RA patients, severe coronary calcification was associated with ESR and smoking [49]. In conclusion, the levels of hcCRP, TNF-α and ESR are associated with increased risk for future CV event and are comparable to FRS. These bio markers might be promising markers for determination of CVD in RA patient.

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