Dyslipidemia and Xanthine Oxidoreductase Level in Jordanian Patients with Rheumatoid Arthritis

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Abstract

Context: Rheumatoid arthritis (RA) is associated with an excess mortality from cardiovascular disease which might be due to an increased prevalence of cardiovascular risk factors such as dyslipidemia, and free radical generating enzyme such as xanthine oxidoreductase (XOR). Thus, they appear to be suitable markers for clinical studies of lipid profile and XOR level in patient with RA and related cardiovascular risk.

Objectives: of the study were to assess the prevalence of blood dislipidemia and the levels of blood and synovial XOR in Jordanian patients with untreated active rheumatoid joint diseases and to investigate the clinical and biological associated factors.

Methods: Synovial fluids and blood were collected from one hundred twenty seven patients with active RA (63 male and 64 female). One hundred eleven age matched individuals were included as control. Blood lipid profile, apolipoproteins (Apo), blood and synovial XOR proteins level were determined.

Results: the levels of patient serum cholesterol (C), low density lipoprotein (LDL)-Cholesterol, Apo B, triglyceride (TG), very low density lipoprotein (VLDL-TG), LDL-TG, Apo C-III, Apo C-III/TG, Apo B/Apo A-I, and LDL-C/high density lipoprotein (HDL)HDL2-C ratios were significantly increased in RA patients. A significant reduction in the levels of HDL-, HDL2-, HDL3-C, serum Apo A-I, ApoA-II, HDL-Apo A-I, and HDL2-C/HDL3-C ratio were found in RA patients compared to healthy controls. Increased XOR in serum and synovial fluid were observed in the RA patients studied.
**Conclusions:** Abnormalities in lipids, lipoproteins, apolipoproteins and XOR were found in RA patients. Our data favor an enhanced affinity towards atherosclerosis in these patients. Management of dyslipidemia should consider as a part of cardiovascular risk management in RA patients. Attention must be paid to lipids profile for those RA patients with previous history of a cardiovascular event.

**Keywords:** Dyslipidemia, lipid profile, atherogenesis, Synovial fluid, Apolipoprotein (Apo).

1. **Introduction**

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology affecting primarily the synovium, leading to synovial inflammation, joint and bone damages, it affects 1-2% of the population, with mortality ratios ranging from 1.3 to 3.0 (Kimac et al. 2010; Helmick et al. 2008; Nurmohamed 2007; Goodson and Solomon 2006; Georgiadis et al. 2006; Watson et al. 2003; Van Doornum et al. 2002; Lems and Dijkmans 2000). Patients suffering from rheumatoid arthritis (RA) are at greater risk of developing cardiovascular disease with 70% increase in risk of death (Lourida et al. 2007; Soubrier and Dougados 2006; Sattar et al. 2003). Clinical and subclinical vacuities accelerated atherosclerosis and increases cardiovascular morbidity and mortality among RA patients (Georgiadis et al. 2006; Gabriel et al. 2003; Van Doornum et al. 2002; Goodson 2002; Bacon and Kitas 1994; Reilly et al. 1990). This increased cardiovascular risk may resulted from several factors such as dyslipidemia, accelerated atherosclerosis, diabetes mellitus, high blood pressure, higher body mass index (BMI), sex and age (Goodson and Solomon 2006; Dessein et al. 2005; Pincus and Callahan 1986) and finally, the chronic inflammatory components, and the effects of drugs used in RA treatment on the lipid profile (Ku et al. 2009; Chung et al. 2008; Shoenfeld et al. 2005). In recent years, free radicals reactions and other reactive intermediates produced in normal metabolic processes have been implicated in the pathogenic mechanism of a wide range of diseases, including acute coronary syndrome (Cheng-xing et al. 2004), in others cardiovascular diseases (Dawson and Walters 2006) and inflammatory diseases (Chung et al. 1997). XOR has generated excited widespread research result in its ability to reduce molecular oxygen, generating free radical superoxide anion (O$_2$$^-$) and hydrogen peroxide (H$_2$O$_2$) mediating ischemia-reperfusion damage in heart and joint (Almuhtaseb 2012). Studies concerning the levels of total cholesterol (TC), total triglyceride (TG), very low density lipoprotein-triglyceride (VLD-TG), low density lipoprotein-cholesterol (LDL-C) and low levels of high density lipoprotein-cholesterol (HDL-C) particularly HDL$_2$-C and inconclusive in RA patients yielded contradictory data (Arts et al. 2012; Garcia-Gomez et al. 2009; Nurmohamed 2007; Assous et al. 2007; Van halm et al. 2007; Solomon et al. 2004; Park et al. 2002; Park et al. 1999; Austin et al. 1998). Increased levels of total TC, LDL-C and low levels of HDL-C, and HDL$_2$-C are associated with an increased incidence of cardiovascular disease in general population (Choy and Sattar 2009; Castelli et al. 1986). The inflammatory environment and disturbed antioxidant mechanisms due to increased XOR in RA may promote LDL oxidation, thereby facilitating atherogenesis at low ambient lipid concentration and placing RA patients at higher cardiovascular risk (Nurmohamed 2007; Situnayake et al. 1991). Little attention has been given to apolipoproteins, the carriers of structural and functional properties of serum lipoproteins (Knowlton et al. 2012), including Apo A-1, Apo B which differ not only in their apolipoprotein composition but also in their metabolic properties and non-atherogenic and atherogenic capacities (Alaupovic 2003; Blankenhom et al. 1990). Serum apolipoproteins measurement is considered to be better discriminators than the total levels of lipoprotein (Walldius and Jungner 2006). Apo C-II and Apo C-III have also been reported to play an important role in the catabolism of TG and hence may contribute to cardiovascular disease (Havel et al. 1973). Apo C-II is activator of lipoprotein lipase (LPL) which hydrolyses TG while Apo C-III inhibits the enzyme (LaRosa et al. 1970). Apo C-III was shown to interfere with binding of Apo B containing lipoprotein to hepatic lipoprotein
receptors (Brown and Banginsky 1972) and has link with the inflammatory processes (Clavey et al. 1995; Wang et al. 1985). Recently, new data on dyslipidemia in RA patients have been published which changed our insights about the lipid profile in RA patients (Knowlton et al. 2012; Hemick et al. 2008; Solomon et al. 2003; Wolfe et al. 2003; Koopman 2001). The aim of this study is to assess the prevalence of dyslipidemia and the level of synovial and blood XOR in Jordanian patients with active RA and to investigate the clinical and biological associated factors.

2. Materials and Methods

2.1. Subjects and Clinical Evaluation

127 RA patients, diagnosed according to the ACR criteria for classification of the disease (Arnett et al 1988), and 111 control subjects, matched for age, sex, smoking status and blood pressure were enrolled after giving informed consent. The patients had active disease, as evaluated by a Disease Activity Score (DAS 28=6.5 ± 0.2) according to EULAR’s recommendations (Fransen and Riel 2005) and 63 were Rheumatoid factor positive. No subject had any symptom or laboratory finding of kidney, liver or thyroid dysfunction, infection, diabetes, malignancy, or was taking drugs affecting plasma lipid or lipoprotein levels. None had been treated with corticosteroids or disease modifying anti-rheumatic drugs prior the study. All subjects had sedentary habits and were not participating in any regular physical exercises. The present investigation was approved by the hospital ethics committee and was in accordance with the principles outlined in the declaration of Helsinki.

After overnight fast (15 h) venous blood samples of all participants (patients and controls) were collected in plain tubes (1.5 mg/ml, Monojet, Division of Sherwood Medical). The samples after their complete coagulation were centrifuged at 4°C (500 g; 15 minutes) to separate clear sera. The sera were divided into two portions, one for detection the levels of IgG and IgM and the other for immediate analysis of rheumatoid factor (RF), C-reactive protein (CRP), Uric acid (BalIs 1976), glucose (Trinder 1969), phospholipid (Takayama et al. 1977), and XOR activity (Nishino 1994; Avis et al. 1956). The ultracentrifugal fractionation for VLDL, LDL, Total HDL, HDL$_2^+$, and HDL$_3^+$ fractions (for C and TG), serum triglycerides, and total cholesterol, were determined in triplicate with standard enzymatic techniques by using an Abbott VP supersystem autoanalyser (Abbott Diagnostic Division, maidenhead, UK). Erythrocytes sedimentation rate (ESR) was determined for the first hour.

2.2. Separation of Lipoprotein

Separation of lipoproteins was performed by a modified sequential ultracentrifugation method (Havel et al. 1955) in a preparative ultracentrifuge (Sorvall OTD 75B, Bomedical products) in a fixed angle rotor (type TFT 48.6) with appropriate adaptors. The procedure was outlined in (Al-Muhtaseb et al. 1989).

Apolipoproteins A-I, A-II and B, C-II and C-III values were determined by immunoturbidimetric method using Daiichi kit (Auto N Daiichi, Daiichi pure chemicals Co. Ltd, Tokyo, Japan) and preceded according to the manufacturer recommendation.

For Apo A1 inter assay and intra assay coefficient of variation was less than 3.5% at a level of 150 mg/dl and less than 4% at a level of 100mg/dl. For Apo A-II the coefficient of variation was less than 4.5% at a level of 70mg/dl and less than 4.5% at a level of 40 mg/dl. For Apo B it was less than 4.5% at a level of 70mg/dl. Apoprotein C-II and C-III values, the Inter assay and intra assay coefficient of variation was within the recommended range (below 10%).

2.3. LCAT Activity Assay

The method was according to that reported by Dieplinger and Kostner in 1980.
2.4. Rheumatoid Factor Detection (RF)

Rheumatoid factor is detected by latex agglutination test using appropriate plates from Behring (Germany) according to the manufacturer recommendations. Fifty µL of latex coated with human IgG was added to different dilutions from each serum sample. Negative and positive sera were used as controls. After two minutes a clear agglutination is observed in the positive indicating the presence of RF. Sera with titer less than 20 IU/ml were considered negative according to the manufacturer recommendation.

2.5. C - Reactive Protein (CRP) Concentration Determination

CRP concentration was determined by latex agglutination test using appropriate plates from Behringer (Germany) and preceded as recommended by the manufacturer. 2.4 µl of patient serum is added to 120 µl buffer solutions (pH 8.5) and mixed with 120 µl suspension of mouse anti-human CRP monoclonal antibody that is bound to latex (2mg/ml) and incubated for 5 minutes. CRP binds to the latex-bound antibody and agglutinates. The resulting agglutination was measured spectrophotometrically at 580 nm, negative and positive control samples were included. Values higher than 9.4 mg/l for females and 8.55 mg/l for males were consider as positive.

2.6. The Erythrocyte Sedimentation Rate (ESR)

Was determined using modified Westergren method (Belin et al. 1981).

2.7. Purification of Xanthine Oxidoreductase

The purification and total protein estimation for XOR enzyme and xanthine oxidase activity assay were determined according to the previous method (Al-Muhtaseb et al. 2012).

2.8. Protein Estimation

Protein was estimated by the method of lowry et al. 1951, using Folin phenol reagent, bovine serum albumin was used as the standard.

2.9. Xanthine Oxidase Activity in the Synovial Fluid Assay

Total xanthine oxidase activity of the synovial fluid was determined by measuring the rate of oxidation of xanthine to uric acid spectrophotometrically at 295 nm in a Cary 100 spectrophotometer, using a molar absorption coefficient (ε) of 9.6 mM-1 (Avis et al. 1956).

2.10. Assays

Were performed at 25.0 ± 0.2°C in air-saturated 50 mM Na / Bicine buffer, pH 8.3, containing 100µM xanthine. Total (oxidase plus dehydrogenase) activity was determined as above but in the presence of 500 µm NAD+. Dehydrogenase content of an enzyme sample was determined from the ratio of oxidase and total activities


2.11.1. Principle of the Xanthine Oxidase Method

\[
\text{Xanthine oxidase} \\
\text{Xanthine} + \text{O}_2 + \text{H}_2\text{O} \\
\text{Uric acid} + \text{H}_2\text{O}_2
\]

The rate of formation of uric acid is determined by measuring increased absorbance at 290 nm. A unit of activity is that forming one micromole of uric acid / minute. The procedure was done according to the procedure recommended by abdelhamid et al. 2011.
2.12. Single Radial Immunodiffusion Assay (SRID)

To determine the levels of total IgG and IgM as in (Al-Muhtaseb 2012)

2.13. Statistical Analysis

Statistical analysis was carried out using Student's t test by statistical packages for social science software (SPSS). Values are expressed as mean ± SD and values of p < 0.05 were considered statistically significant. The relationships between variables were calculated using Pearson Correlation Coefficients.

3. Results

Patients positive for rheumatoid factor labeled as rheumatoid arthritis + (RA+), while seronegative patients were labeled as other joint inflammation (RA-). Latex agglutination used in determining IgM-RF factor showed that, of the 127 patients 63 (32 male, 31 female) were RA+ (Seropositive) and 64 (31 male, 33 female) were RA- (Seronegative), the Clinical and biochemical characters of the participated subjects are shown in table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RA+</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.1±9.6</td>
<td>39.9±9.6</td>
</tr>
<tr>
<td>Height cm</td>
<td>162.9±9.6</td>
<td>161.8±7.9</td>
</tr>
<tr>
<td>Weight Kg</td>
<td>68.8±8.1</td>
<td>67.3±4.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3±4.6</td>
<td>28.1±7.7</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>10.8±6.03</td>
<td>66.9±42.6</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IgM rheumatoid factor (+/-)</td>
<td>0/0</td>
<td>45/13</td>
</tr>
<tr>
<td>Glucose mg/dL</td>
<td>95.9±6.8</td>
<td>94.8±7.2</td>
</tr>
<tr>
<td>Uric acid mg/dL</td>
<td>4.2±1.6</td>
<td>7.2±1.10*</td>
</tr>
</tbody>
</table>

BMI: body mass index, ESR: erythrocytes sedimentation rate, CPR: C-reactive protein.

*P < 0.001 significantly different from the corresponding control.

**P < 0.0001, between RA+ compare to RA-

Values represent the means ± standard deviation (SD)

BMI = weight < [kg/height (m)²]

The mean age and BMI for both rheumatoid arthritis RA+ and other joint inflammation RA- and their control subjects were comparable. ESR (after one hour) and CRP values were significantly elevated in patients with rheumatoid arthritis (RA+) compared with values in patients with other joint inflammation (RA-) and to those of the control values.

Latex agglutination used in determining IgM-RF showed that of the 127 patients 32 males and 31 females were RA+ (seropositive) and 31 males and 33 females were RA- (seronegative).

Concentrations of fasting plasma glucose were normal in both male and female rheumatoid arthritis (RA+, RA-) patients compared to control subjects. On the other hand, uric acid levels were significantly elevated in male and female rheumatoid arthritis RA+ and RA- patients compared with their controls (P < 0.001).

Both RA+ and RA- (male and female) rheumatoid arthritis patients had significantly increased TC, VLDL-C, LDL-C, TTG, VLDL-TG, HDL-TG, and phospholipids concentrations when compared with the corresponding control subjects (Table 2). Whereas, LDL-C, TG, VLDL-TG, LDL-TG, and phospholipids concentrations were lower in RA- female patients compared to RA+ rheumatoid arthritis female patients. Marked reduction in HDL-, HDL₂- and HDL₃-C was observed in RA+ and RA- male and female patients compared to controls. There was no significant difference in the variables studies.
Dyslipidemia and Xanthine Oxidoreductase level in Jordanian Patients with Rheumatoid Arthritis

Table 2: Serum Lipids and lipoprotein concentrations (mg/dL) in control and in rheumatoid arthritis male and female subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RA+</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Total Cholesterol TC</td>
<td>203±27.3</td>
<td>240.3±38.16*</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>30.3±10.7</td>
<td>37.6±12.3*</td>
</tr>
<tr>
<td>LDL-C</td>
<td>116.15±21.5</td>
<td>162.69±32.6*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>56.13±6.15</td>
<td>38.10±2.7**</td>
</tr>
<tr>
<td>HDL2-C</td>
<td>23.79±2.6</td>
<td>11.50±3.2*</td>
</tr>
<tr>
<td>HDL3-C</td>
<td>33.50±2.7</td>
<td>26.1±3.0**</td>
</tr>
<tr>
<td>Total triglyceride TG</td>
<td>119.2±17.6</td>
<td>186.3±20.6**</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>75.2±48.7</td>
<td>119.2±10.1*</td>
</tr>
<tr>
<td>LDL ·TG</td>
<td>28.1±5.1</td>
<td>40.6±7.2**</td>
</tr>
<tr>
<td>LDL-TG</td>
<td>17.2±6.1</td>
<td>24.9±6.2*</td>
</tr>
<tr>
<td>Phospholipid Total</td>
<td>193.3±30.3</td>
<td>260.3±32.3*</td>
</tr>
</tbody>
</table>

Values represent the means ± standard deviation (SD) in milligrams per deciliter. 

VLDL: very low density lipoprotein, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TC: total cholesterol, TG: triglyceride, TTG: total triglyceride.

*P < 0.0001, **P < 0.001 significantly different from the corresponding control.

†P < 0.01 between RA+ and RA- patients (male and female)

*P < 0.001 between RA+ male and female

bP < 0.001 between RA- male and female

cP < 0.0001 between control group (male and female) lipoprotein densities: VLDL, d < 1.006 g/ml⁻¹; LDL, d = 1.006-1.063 g/ml⁻¹; HDL2, d = 1.063-1.125 g/ml⁻¹; HDL3, d = 1.125-1.21 g/ml⁻¹.

Serum apolipoprotein A-I, A-II, B, C-II, and C-III concentrations of rheumatoid arthritis patients and controls are shown in Table 3. Significantly reduced serum levels of Apo A-I, Apo A-II and Apo A-I in HDL fraction were observed in both male and female RA+ and RA- rheumatoid arthritis patients compared with control subjects. Apolipoprotein B in serum and in LDL fraction with serum Apo C-I concentrations were significantly elevated in both male and female rheumatoid arthritis (RA+ and RA-) patients compared with their control groups (Table 3). There was no significant difference in Apo C-II in either rheumatoid arthritis group compared with their control group.

Table 3: Serum Apolipoprotein A-1, A-II, B, C-II and C-III concentrations of patients with rheumatoid arthritis and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RA+</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Serum Apo A-I</td>
<td>150.3±16.3</td>
<td>110.7±13.3*</td>
</tr>
<tr>
<td>HDL Apo A-I</td>
<td>123.6±11.3</td>
<td>86.3±14.6*</td>
</tr>
<tr>
<td>Serum Apo A-II</td>
<td>59.9±3.7</td>
<td>39.1±3.6</td>
</tr>
<tr>
<td>Serum ApoB</td>
<td>110.6±9.6</td>
<td>132.3±10.8**</td>
</tr>
<tr>
<td>LDL·Apo B</td>
<td>95.9±12.8</td>
<td>115.2±10.8**</td>
</tr>
<tr>
<td>Serum apo C-I</td>
<td>4.0±0.08</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td>Serum apo C-III</td>
<td>6.8±0.09</td>
<td>8.6±0.14*</td>
</tr>
</tbody>
</table>
Values represent the means ± standard deviation (SD) in milligrams per deciliter, HDL: high-density lipoprotein, LDL: low-density lipoprotein, apolipoproteins, Apo A-I, Apo A-II, Apo B, Apo C-II, and Apo C-III.

*P< 0.001, **P< 0.001: significantly different from the corresponding control group.

†P< 0.001 between RA+ and RA- patients (male and Female).

‡P< 0.001 between RA- male and female.

§ P < 0.001 significantly different between control group (male and female).

Serum Apo A-I, Apo A-II, HDL-Apo A-I, serum Apo B, LDL-Apo B, and serum Apo C-II, levels did not differ between RA+ and RA- rheumatoid arthritis male patients, where as serum Apo A-I, Apo A-II, HDL-Apo A-I, and LDL-Apo B levels did not differ in RA+ and RA- rheumatoid arthritis female patients, but in case of serum Apo B and serum Apo C-III were significantly decreased in both RA+ and RA- female rheumatoid arthritis patients and the decrease in RA- were more significant.

In female control subjects, serum Apo A-I, Apo A-II, HDL-Apo A-I, serum Apo B and LDL-Apo B were significantly lower than in control male subjects.

Serum lecithin cholesterol acyltransferase (LCAT), serum and synovial xanthine oxidase (XO) activities of patients with rheumatoid arthritis and controls are shown in table 4.

**Table 4:** Serum lecithin cholesterol acyltransferase (LCAT), serum and synovial xanthine oxidase (XO) activities of patients with rheumatoid arthritis and controls.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RA+</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Serum LCAT mg/dl</td>
<td>127.8±26.7</td>
<td>99.9±13.3*</td>
</tr>
<tr>
<td>Serum XO units/mg protein</td>
<td>0.0090±0.003</td>
<td>0.056±0.0046**</td>
</tr>
<tr>
<td>Synovial XO, nmole.min⁻¹.mg⁻¹</td>
<td>NA</td>
<td>98.9±40.6</td>
</tr>
</tbody>
</table>

*P< 0.001, **P< 0.01 significantly different from control

†P< 0.001 between RA+ and RA- in male and female groups.

Serum LCAT was decreased significantly in all rheumatoid arthritis female and male groups compared with healthy controls, while serum xanthine oxidase concentrations were elevated significantly in male and female (RA+ and RA-) rheumatoid arthritis patients groups when compared with control groups.

Serum XO and synovial XO activity were significantly decreased in both male and female RA- rheumatoid arthritis patients compared to RA+ rheumatoid arthritis patients.

Total IgG and IgM (mg/dL) titers in sera of patients with rheumatoid arthritis (RA+), other joint inflammation (RA-), and healthy volunteers, using SRIDA are shown in (Table 5). Both blood IgM and IgG levels were significantly higher in male and female (RA+ and RA-) rheumatoid arthritis patient groups than in control groups. Similarly, the levels were higher in serum of patients with other joint inflammation (RA-) than in RA+ patients (P< 0.01). IgG levels were more elevated in serum of patients with rheumatoid arthritis (RA+) compared to other joint inflammation (RA-) in both male and female (P< 0.001).

**Table 5:** Total IgG and IgM titers in sera and synovial fluid of patients with rheumatoid arthritis (RA+), other joint inflammation (RA-), and healthy volunteers, using SRIDA**

<table>
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<tr>
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<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RA+</td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Total IgM (mg/ml) Blood</td>
<td>1.61±0.67</td>
<td>2.41±1.61**a</td>
</tr>
<tr>
<td>Synovial</td>
<td>NA</td>
<td>1.01±0.61*</td>
</tr>
<tr>
<td>Total IgG (mg/ml) Blood</td>
<td>12.55±10.36</td>
<td>25.61±10.31*</td>
</tr>
<tr>
<td>Synovial</td>
<td>NA</td>
<td>14.21±3.9*</td>
</tr>
</tbody>
</table>
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RA+= rheumatoid arthritis latex agglutination positive
**SRIDA= single radial immunodiffusion assay
*P< 0.001, significantly different from the corresponding control groups
aP<0.001, between RA+ and RA- patients (male and female)
bP<0.001, between RA- male and female patients
cP<0.01, between RA+ male and female

Total IgM and IgG (mg/dL) levels in serum were significantly higher in male and female rheumatoid arthritis (RA+) in comparison to the levels in patients with other joint inflammation (RA-) both male and female (P< 0.01).

Ratio of lipid fractions and apolipoproteins in control groups, male and female patients with rheumatoid arthritis (RA+ and RA-) are shown in (Table 6)

Table 6: Ratios of lipid and lipoprotein fractions in control groups and patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
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<tbody>
<tr>
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<td>Control RA+ RA-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>55</td>
<td>32</td>
<td>31</td>
<td>56</td>
<td>31</td>
</tr>
<tr>
<td>T-C/HDL-C</td>
<td>1.95±0.30</td>
<td>6.40±0.51*</td>
<td>5.30±0.65*</td>
<td>2.83±0.31</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>1.67±0.31</td>
<td>3.81±0.59*</td>
<td>3.31±0.90*</td>
<td>1.33±0.41</td>
</tr>
<tr>
<td>HDL-C/Apo A-I</td>
<td>0.39±0.03</td>
<td>0.41±0.06</td>
<td>0.37±0.03</td>
<td>0.40±0.021</td>
</tr>
<tr>
<td>T-C/Apo B</td>
<td>2.61±0.40</td>
<td>2.14±0.30</td>
<td>2.00±0.23</td>
<td>2.31±0.31</td>
</tr>
<tr>
<td>Apo B/Apo A-I</td>
<td>0.31±0.09</td>
<td>1.08±0.21*</td>
<td>1.03±0.17*</td>
<td>0.29±0.71</td>
</tr>
<tr>
<td>LDL-C/HDL_C²</td>
<td>4.01±0.061</td>
<td>10.46±3.01*</td>
<td>9.80±2.91*</td>
<td>3.61±0.081</td>
</tr>
<tr>
<td>HDL_C²/HDL_C³</td>
<td>0.71±0.01</td>
<td>0.47±0.03*</td>
<td>0.52±0.08*</td>
<td>0.69±0.09</td>
</tr>
<tr>
<td>Apo C-III/TG</td>
<td>0.30±0.04</td>
<td>0.43±0.036</td>
<td>0.50±0.04</td>
<td>0.27±0.033</td>
</tr>
</tbody>
</table>

Results are means ± SD
HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, T-C: total cholesterol, Apo A-I, Apo A-II and Apo B: apolipoproteins
*P < 0.001 significantly different from control subjects
aP < 0.01 between RA+ and RA- in both male and female patients

Significantly increased T-C/HDL-C, LDL-C/HDL-C, LDL-C/HDL\_C², Apo B/Apo A-I and Apo C-III/TG ratios, and reduction in HDL\_C²/HDL\_C were observed in the male and female rheumatoid arthritis patients compared with controls. Where as HDL-C/Apo A-I and TC/Apo B ratios did not differ when compared with controls (Table 6). On the other hand TC/HDL-C were significantly increased in (RA+) rheumatoid arthritis compared to RA- male and female patients. The remaining ratios did not differ between male and female RA+ with RA- rheumatoid arthritis patients.

3.1. The Correlations between Variables

For the groups combined HDL-C correlated positively with Apo A-I (r=0.50, P< 0.001), and negatively with TG (r=-.60, P< 0.001) and Apo B (r=-0.47, P< 0.001), Triglycerides correlated positively with Apo B (r=0.61, P< 0.001), and with Apo C-III (r=0.51, P< 0.001). Apo C-III correlated negatively with HDL-C (r=-.53, P< 0.001) and HDL\_C² (r=-.56 P P< 0.001).

Serum TG and VLDL-TG were correlated positively with serum Apo-B and LDL-Apo B (r=0.62, r=0.5.2, P< 0.001 for both) and Apo-CIII(r=0.43, P< 0.001) and negatively with serum HDL-C, HDL\_C² (r = -0.46, r=-0.50, P< 0.001 for both), serum Apo A-I, HDL-Apo A-I (r=-0.44, r=-0.51, P< 0.001 for both). In the RA+ and RA- both sex group patients. In contrast, serum C and LDL-C correlated positively with TG, serum Apo B, LDL-Apo B (r=0.49, r=0.69, P< 0.001 for both) and negatively with HDL-C, HDL\_C² (r = -0.53, r=-0.60, P< 0.001 for both), serum Apo A-I and HDL-Apo A-I (r=-0.51, r=-0.58, P< 0.001 for both).
4. Discussion

The elevated levels of CRP, ESR, beside IgM rheumatoid factors in both male and female patient groups reported in this study indicate inflammation. The slightly higher levels of total IgG and IgM titres in RA+ patients compared to the levels in RA- patients and to the levels in healthy control are inconsistent with other investigators (Arrar et al. 2008; Ng and Lewis 1994; Harrison et al. 1990), which is due to the autoimmune difference in the nature of the disease among individuals. On the other hand the synovial fluids of patients with RA+ and RA- showed lower levels of total IgG and IgM compared to the levels in their sera, these data were not in agreement with the finding of others (Arrar et al. 2008; Ng and Lewis 1994; Harrison et al. 1990). The low synovial levels may be attributed to bound immunoglobulins to the tissue or bound in immune complexes which need to be detected by other more sensitive techniques than SRID. In fact the pathology of RA is attributed to an Arthus like reaction in which immune complexes mediate the crucial role.

Recent research has shown that systemic inflammation plays a pivotal role in development of atherosclerosis (Nurmohamed 2007; Soubrier and Dougados 2006; Rohrer et al. 2004; Saito et al. 2003; Biondi-Zoccai et al. 2003; Lind 2003). Hence, inflammation might explain the increased cardiovascular risk in RA patients (Sattar et al. 2003). In our study, RA+ patients showed dyslipidaemia and mark atherogenic lipid profile compared with the healthy controls. This atherogenic lipid profile includes elevated TC, VLDL-C, LDL-C, TTG, VLDL-TG, LDL-TG and phospholipid levels observed in male RA+ and RA- patients, and in female RA+ patients only is in agreement with some of the previously reported data (Zrour et al. 2011; Yoo 2004; Park et al. 1999), and disagrees with others (Myasoedova et al. 2010; Oliviero 2009). The observations of significant differences found in female RA+ and RA- patients may suggests that the inflammation was more sever in RA+ women or due to a decrease in endogenous estrogen (Magkos et al. 2010; Atsma et al. 2006; Krauss et al. 1979).

Elevated total VLDL-C and LDL-C observed in this study have been previously reported (Walldius and Jungner 2004), this elevation may be due to changes in the inter composition of LDL particles as a consequence of increased flux of VLDL, or due to diet or physical exercise (Metsios et al. 2010). Alternatively, a decreased rate of LDL catabolism arising from increase LDL receptor uptake could explain this observation (Walldius and Jungner 2004).

Our RA patients had high levels of total, VLDL and LDL-TG than the control group, which agrees with others (Tian et al. 2011; Aberg et al. 1985). The high level of TG may caused by increased production of VLDL-TG or decrease in TG catabolic rate as a result of impairment of LPL activity associated with inflammation (Tian et al. 2011). Alternatively, elevated VLDL-TG in these patients could be due to influx of free fatty acids into the liver as excess substrate for hepatic TG synthesis. And because VLDL-C, LDL-TG levels were elevated, such abnormalities may reflect an elevated concentration of remnant lipoprotein metabolism.

Low levels of total HDL-C, HDL₂-C, HDL₃-C, and a high LDL-C, found in our study, is strongly associated with an increased risk of atherosclerosis, which is inconsistent with previous study (Arts et al. 2012; Regnstron et al. 1992), furthermore, the reduction in HDL-C occurs predominantly in the HDL₂ subfraction which is conforms with previous reports (Arts et al. 2012; Regnstron et al. 1992). The inverse correlation seen between HDL-C and HDL₂-C with VLDL-TG could lead to decreased production of HDL particularly HDL₂ from TG-rich lipoproteins (Tian et al. 2011; Nikkila et al. 1987).

Apolipoprotein A-1 is the predominant apolipoprotein in HDL, and apolipoprotein B is the predominant apolipoprotein in LDL (Walldius and Jungner 2004; Jones et al. 2000; Gibbons 1986). The observed high levels of apolipoprotein B and its correlation with other blood lipids reported in male RA+, RA- and female RA+ patients may be associated with the increased production and/ or impaired catabolism of LDL, possibly apolipoprotein B due to inflammation (Van Doornum et al. 2002). In contrast to Apo B, the reduction in serum Apo A-I and Apo A-II levels are in agreement with other studies (Yamada et al. 1998). ApoA-1 is reported to inhibit the production of pro-inflammatory cytokines including IL-1, TNF-α and interactions between T-cell and monocytes (Zhang et al. 2010;
This suggests that a less favorable lipid profile stimulates a pro-inflammatory status which might be related to RA disease activity or susceptibility. Our finding is in line with the researchers who believed that serum Apo A-I concentrations should be declined during active phase of RA, and has played an important role as anti-inflammation (Zrour 2011; Zhang et al. 2010; Barry et al. 2004; Burger and Dayer 2002). Chronic inflammation such as RA was also associated with low HDL-C and altered HDL composition (Rohrer et al. 2004). Other researchers reported that Apo A-I and Apo B levels are decreased (Chenaud et al. 2004; Jonas 2000). The reported Low HDL, HDL₂-C and apolipoprotein A-I levels and their inverse correlation with TG, VLDL-TG, phospholipids and apolipoprotein B levels in our study may be due to an increased production of acute phase proteins by the inflamed liver at the expense of lipoprotein production, thereby tending to reduce lipoprotein levels (London et al. 1963).

In the previous studies in autoimmune diseases as RA it were shown that Apo C-III and TG levels are increased (Knowlton 2012; Nicolay et al. 2006; Walldius and Jungner 2004). In our study we found increased levels in total-TG and VLDL-TG in males, females RA+ and RA- patients with increase in Apo C-III but not Apo C-II levels. Furthermore, our finding of high Apo B and lower Apo A-I, HDL-C and HDL₂-C with their correlations to Apo C-III, may provide additional information in that Apo C-III level probably reflects an independent risk factor in RA patients (Knowlton 2012). The fact that Apo C-III alone or as one of the protein components of the Apo B-containing lipoprotein subclasses is linked to inflammatory processes and atherogenesis, underscores its great potential as a new marker and diagnostic test for cardiovascular diseases in RA patients (Knowlton 2012). In a recent study it was shown that only those LDL particles that contain Apo C-III are atherogenic and those particles without Apo C-III are not (Mendivil et al. 2011).

The plasma LCAT, which is activated by Apo A-I, plays role in the metabolism of lipoproteins as co-factor for esterification of free cholesterol (Tian et al. 2011; Walldius and Jungner 2004). The finding of low LCAT activity, low Apo A-I and high level phospholipids in both RA+, RA- male and female patients compared to healthy control may suggest the influence of LCAT on HDL-C level.

The increased ratio of LDL-C/HDL-C, LDL-C/HDL₂-C, TC/HDL₂-C, Apo B/Apo A-I and Apo C-III/TG as observed in RA+, RA- patients was considered a predisposing factor to increased risk of cardiovascular diseases in RA patients. The higher the value of the Apo B/Apo A-I ratio, the more circulating cholesterol in the plasma compartment (Tian et al. 2011; Walldius and Jungner 2004), this cholesterol is likely to be deposited in the arterial wall, provoking atherogenesis and risk of coronary vascular events.

Assessing the Apo B/ Apo A-1 ratio and total TG levels was recommended to predict high cardiovascular risk patients in the general population (Tian et al. 2011; Walldius and Jungner 2006) in our study the ratio was higher in male, female RA+ and RA- patients in comparison to controls, these data in support of cardiovascular risk.

Studies suggest that free radical mediated oxidative stress can influence the plasma lipid concentrations and generate potent proatherogenic mediators (Kowsalya et al. 2011). These free radicals also play an important role in the development of atherosclerosis vascular disease and other complications seen in RA (McCord 2000; Ross 1999). Our study showed increased activity of serum and synovial XOR in male, female RA+ patients compared to RA- patients, may reflect the severity of the inflammation in RA+ patients. Therefore XOR can not be used as marker for detection of myocardial infarction as recommended by other (Abdelhamid et al. 2011). XOR is an important free radical generator (Hearse et al. 1986; Chambers et al. 1985), acts on hypoxanthine and xanthine with the resultant production of oxygen free radicals (Abdelhamid et al. 2011; Raghuvanshi et al. 2007). Free radical generation result in damage to vascular endothelium (Kundalic et al. 2003), Damage to extracellular matrix, and that changes in matrix structure can play a key role in the regulation of cellular adhesion, proliferation, migration, and cell signaling; furthermore, the extracellular matrix is widely recognized as being a key site of cytokine and growth factor binding, and modification of matrix structure might be expected to alter such behavior (Rees et al. 2008).
As inflammation only explained a small part of the observed differences in lipids between the persons who later develop RA and the controls we recommend that a less favorable lipid profile might be related to the development of RA by a common or linked background, as socio-economic, ethnic, racial and geographic differences. Both genetic and dietary factors may contribute to high lipoprotein levels, especially increases fat and cholesterol consumption (van Wietmarschen and van der Greef 2012; Cesur et al. 2007). Alternatively, lipids might modulate the susceptibility to RA.

5. Conclusion

Our finding support the following finding

a. Lipid profile particularly the Apo B/ Apo A-1 ratio and TG levels, HDL$_2$-C assessments and CRP was recommended to predict high cardiovascular risk patients in the general population.

b. Management of dyslipidemia should considered as a part of cardiovascular risk management in RA patients.

c. Cardiovascular risk factor screening and appropriate treatment in form of antioxidant supplementation or antihyperlipidemic agents may be necessary for reducing the morbidity and mortality in these patients.

Further research to examine the relation between dyslipidemia, the effects of chronic inflammation on lipids, atherosclerosis and cardiovascular mortality may be of importance to both rheumatologists and cardiologists.

References


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**Declaration of Interest**

The authors report no declaration of interest.