THE RELATION BETWEEN INFLAMMATORY MARKERS, LIPID PARAMETERS AND ANTIOXIDANT VITAMINS IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Background: *The association between rheumatoid arthritis and increased risk of atherosclerosis and coronary heart disease is well recognized. The role of chronic inflammation, dyslipoproteinemia, lipid peroxidation and low levels of antioxidant vitamins (vitamin A and E) in the development of atherosclerosis and coronary heart disease is being established.*

Objective: *To study the relationship between lipid profile abnormalities, antioxidant vitamins and inflammatory markers in rheumatoid arthritis patients.*

Methodology: *Thirty rheumatoid arthritis patients and twenty apparently healthy volunteers as a control group were studied .The following parameters were measured for all included subjects: markers of inflammation; CRP, RF, ESR, and VCAM (Vascular Cell Adhesion Molecule), lipid parameters; total cholesterol, triglycerides, direct HDL and LDL cholesterol and Lp (a) and antioxidant vitamins A and E.*

Results: *The inflammatory markers (CRP, ESR and VCAM) were significantly higher in patients compared to controls ($p<0.01$). Total cholesterol and LDL cholesterol in rheumatoid arthritis patients were significantly higher compared to the controls ($p<0.05$). Also Lipoprotein (a) was significantly higher in rheumatoid arthritis patients compared to the controls ($p<0.01$). Vitamins A and E were significantly lower in rheumatoid arthritis patients compared to the control group ($p<0.01$). A significant positive correlation was found between total cholesterol, LDL-C, lipoprotein (a) and VCAM*

(p<0.001, and p<0.01 and p<0.01) respectively. A significant negative correlation between the antioxidant vitamins E and A and VCAM (p<0.05 and p<0.05) in rheumatoid arthritis patients was also observed. A significant negative correlation was also found between lipoprotein (a) and both vitamins (E and A) (p<0.01 and p<0.001) respectively.

Conclusions: *The association of VCAM with high lipid parameters and low levels of antioxidant vitamins, might explain the association of chronic inflammatory processes with atherosclerosis and the high risk of cardiovascular disease in rheumatoid arthritis patients.*

INTRODUCTION

The association between rheumatoid arthritis and increased risk of atherosclerosis is well recognized *(Myllykangas et al., 1995).* RA patients are at greater risk of developing coronary heart disease which accounts for 35% to 50% of excess mortality in them. Intriguingly, most evidence suggests that classic risk factors do not explain excess vascular disease in RA *(Sattar et al., 2003).* Epidemiological observations strongly suggest that mechanisms other than classic risk factors promote accelerated atherogenesis in RA *(Sattar et al., 2003).*

The role of chronic inflammation in initiating and propagating atherosclerosis is being firmly established *(Pearson et al., 2003).* This concept of inflammatory-driven atherogenesis is consistent with the plaque composition of unstable coronary lesions with an abundance of inflammatory molecules and immune cells this appearance is similar to that of inflammatory synovitis in RA *(Pearson et al., 2003).* The causes of this association are being explored.

Rheumatoid arthritis patients have dyslipoproteinemia characterized by decreased serum concentration of total cholesterol and cholesterol in low density lipoproteins (LDL) and high density lipoproteins (HDL) *(Park et al., 1999).* Though this lipid profile is not associated with atherogenesis, it is thought that lipid peroxidation could contribute to the initiation and progression of atherogenesis *(Steinberg, 1997).* It is known that antioxidant vitamins like vitamin A and E protect LDL from oxidation leading to antiarthrogenic effect while low levels of these antioxidants are associated with increased atherogenic cardiovascular disease and RA *(Pryor 2000).*

Inflammatory markers such as c-reactive protein (CRP) and adhesion molecules (vascular cell adhesion molecule, VCAM, intercellular adhesion molecules, ICAM) have been related to the inflammatory response and the pathogenesis of RA *(Hurt et al., 2001)* and also atherosclerosis

(Hulthe et al., 2001). Our aim from this work was to study the relation of VCAM, inflammatory markers and antioxidant vitamins to Lp (a) and lipid profile abnormalities in RA patients.

SUBJECTS AND METHODS

Thirty rheumatoid arthritis patients (RA) and 20 apparently healthy volunteers as a control group were studied. The RA patients group consisted of 29 women and one man who fulfilled the criteria of the American College of Rheumatology (ACR) for the diagnosis of RA *(Arnett et al., 1988).* Fourteen patients (47%) were in the active stage of the disease and 16 (53%) were in the inactive stage.

The mean age of patients was 47.8 years (range 25-72 years) with a mean duration of the disease of 10.6 years (range 3 months to 28 years). The most commonly used combination therapy in our subjects was that of salazopyrine and methotrexate. The control group consisted of 19 women and one man. The mean age of control group was 51 years (range 27- 75 years). Patients with clinical signs or laboratory evidence of kidney, liver, thyroid, diabetes mellitus, infectious diseases or malignancy were excluded from the study.

Specimens: Two blood samples were taken from each subject after an overnight fast of at least 12 hours; 5 ml of whole blood in plain tube and 7 ml of whole blood in lithium heparin containing tube. The heparin tube was wrapped in aluminum paper and kept in ice water container to avoid exposure to light during transportation to the laboratory and after centrifugation till the time of analysis for vitamins A and E. All blood samples were centrifuged at 3000 rpm for 10 minutes at 4 ºC. Serum was used for CRP, RF, VCAM (Vascular Cell Adhesion Molecule), lipid profile (TC, TG and HDLD) and Lipoprotein (a) (Lp (a)). Heparinized plasma was used for vitamin A and E assay.

Methods of assay: CRP, RF and Lp (a) were determined by rate nephelometric assay on Beckman Array system according to manufacturer instructions *(Steinberg, 1977).* ESR was measured using the Westergren method. Total cholesterol, triglycerides and direct HDL cholesterol assay were measured on Beckman LX20 autoanalyzer; total cholesterol and triglycerides were measured by an enzymatic timed endpoint methods *(Allian, 1974 and Bucolo, 1973),* while HDL-c was measured by a commercial direct non precipitation method as per manufacturer instructions *(Warnick, 1995).*

LDL-c was subsequently calculated using the Friedewald's formula *(Friedwald, 1972).* VCAM assay was done using h-s VCAM –1 ELIZA kit from Roche Diagnostics *(Roche Diagnostics, GmbH, Roche Molecular*

Biochemicals Sandhofer Strasse 116 and D-68305 Mannheim, Germany). The assay is based on the quantitative sandwich enzyme- immunoassay principle using two monoclonal antibodies directed against different epitopes of human VCAM-1, the biotin labeled antibody and the peroxidase- labeled conjugated detection antibody *(Gearing,1993).*

Vitamins A and E concentrations in plasma were determined by high performance liquid Chromatography (Hewlett Packard 1050) equipped with a UV – visible detector, using retinol acetate and tocopherol acetate as internal standards. The column was a Spherisorb ODS (125X4 mm, 5 micro) and the mobile phase was methanol: water (100% for vitamin E and 98% for vitaminA) *(Catignani & Bieri, 1983).*

RESULTS

Table (1) shows the characteristics of the patient and control subjects included in the study**.** There was no significant difference between both groups for age and sex. Table (2) showed that the inflammatory markers (CRP, ESR and VCAM) were significantly higher in patients compared to controls ($p<0.01$). A highly significant positive correlation was found between VCAM and CRP ($r=0.416$ $p<0.01$) and between VCAM and ESR ($r=0.473$ $p<0.01$) in RA patients. A significant positive correlation was also observed between VCAM and RF ($r=0.36$ and $p<0.01$).

Table (1): Clinical characteristics of control group and RA patients.

		Controls N=20	Patients N=30
Age	years	51±12.4	47.8 ± 11.5
Sex	F/M	19/1	29/1
Disease duration	years		10.6 ± 6.6
Activity of RA	Active		14 (46.6%)
	Inactive		16 (53.4%)
RF	% positive		24/30 (80%)
	value IU/L		404.8± 641
Swollen joint count, range (0-26)	active RA		9 (65%)
	inactive RA		0
Tender joint count, range (0-68)	active RA		14 (100%)
	inactive RA		2 (12.5%)

The results of the lipid profile as shown in table (2) showed significantly higher total cholesterol and LDL cholesterol in RA patients compared to the controls ($p<0.05$). On the other hand, the difference in

triglycerides and HDL cholesterol concentration between the two studied groups was not significant (p>0.05). The level of lipoprotein (a) was significantly higher in RA patients compared to the controls (p<0.01).

Table (2): Comparison of estimated parameters between RA patients and control group.

Parameter	Controls N=20	Patients N=30	Sig.
Inflammatory marker			
-CRP, mg/L	4.8 ± 1.4	17.9 ± 10.9	p<0.01
-ESR ,mm/h	12.3 ± 2.9	34.2 ± 40.8	p<0.01
-VCAM, ng/ml	331.2 ± 66.4	449.7±145.3	p<0.01
Lipids and Lipoproteins			
-TC, mmol/L	4.5 ± 0.73	5.6 ± 2.2	p<0.05
-TG, mmol/L	1.7 ± 0.4	1.3 ± 0.66	p>0.05
-HDLD, mmol/L	1.18 ± 0.23	1.3 ± 0.43	p>0.05
-LDL , mmol/L	2.9 ± 0.75	3.38 ± 0.71	p<0.0.5
-Lp(a), mg/dl	9.1 ± 5.3	32.5 ± 13.5	p<0.01
Vitamins			
-A , μg/L	733.4 ± 113.4	561.5 ± 223.9	p<0.01
-E , mg/L	15.3 ± 2.9	12.8 ± 3.01	p<0.01

The plasma level of both vitamins A and E was significantly lower in RA patients compared to the control group (p<0.01).

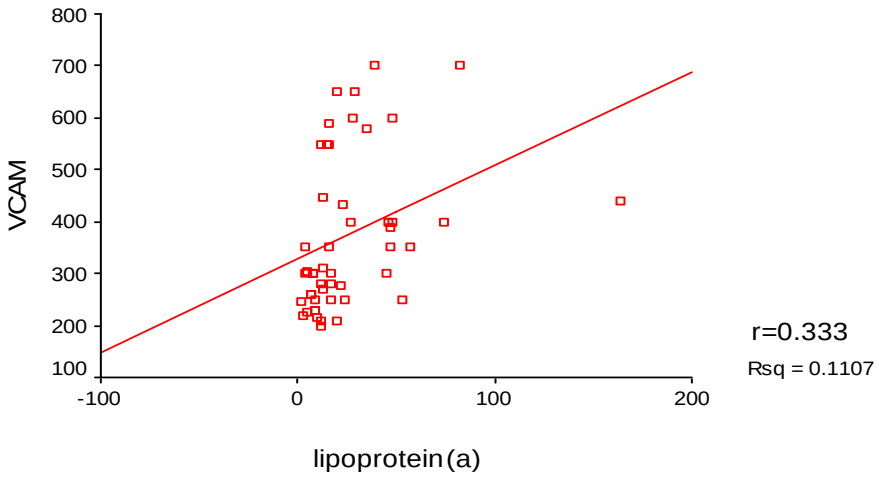
A significant positive correlation was found between some lipid parameters (total cholesterol, LDL-C, lipoprotein (a) and VCAM (r=0.477 and p<0.001, r=0.349 and p<0.01 and r=0.33 and p<0.01 respectively, as shown in fig. (1).

We observed also a significant negative correlation between the antioxidant vitamins E and A and VCAM (r=-0.31 and p<0.05 and r=-0.34 and p<0.05 respectively) in RA patients as shown in figs. (2 & 3).

Another significant negative correlation was found between CRP and vitamin E (r=-0.329 and p<0.05) as shown in fig. (4), which was not found with vitamin A (p>0.05)

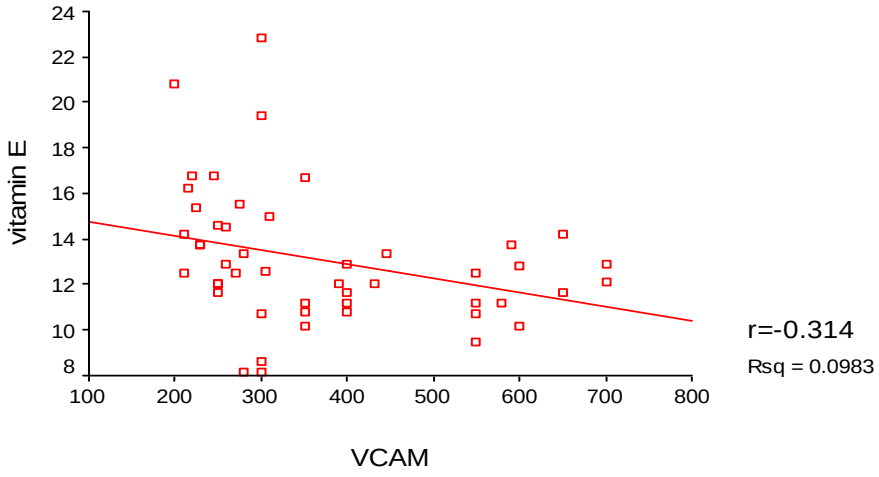
A significant negative correlation was also found between lipoprotein (a) and vitamins E and A (r= -0.414 and p<0.01 and r=-0.568 and p<0.001 respectively) in RA patients as shown in figs. (5 & 6).

Fig.(1) Scatter diagram for the relation between Lipoprotein(a) & VCAM



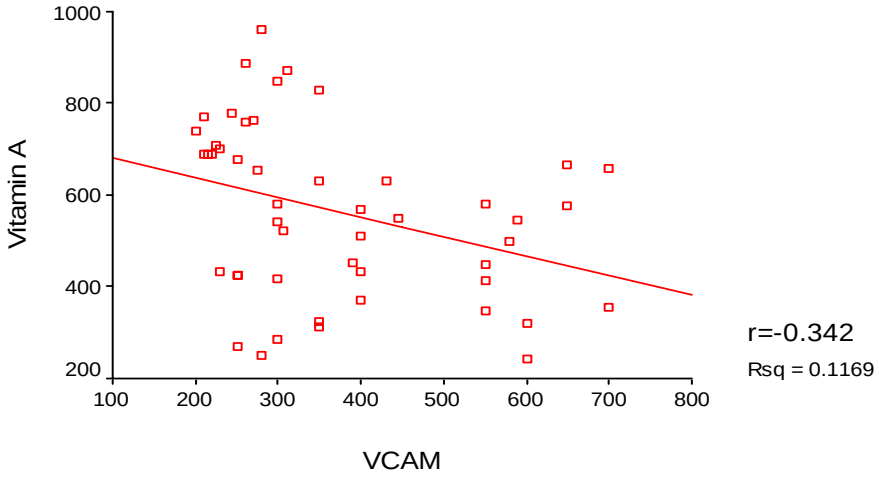
P<0.05

Fig.(2) Scatter diagram for the relation between VCAM & Vitamin E



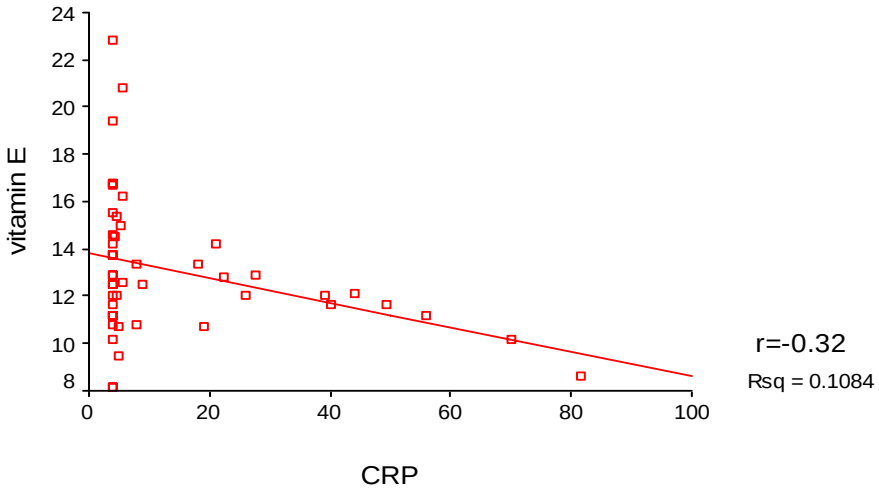
P<0.05

Fig.(3) Scatter diagram for the relation between VCAM & Vitamin A



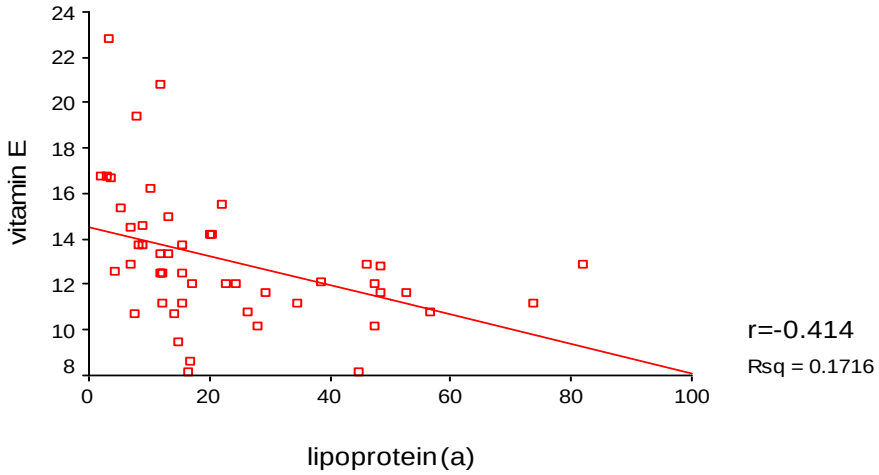
P<0.05

Fig.(4) Scatter diagram for the relation between CRP & Vitamin E



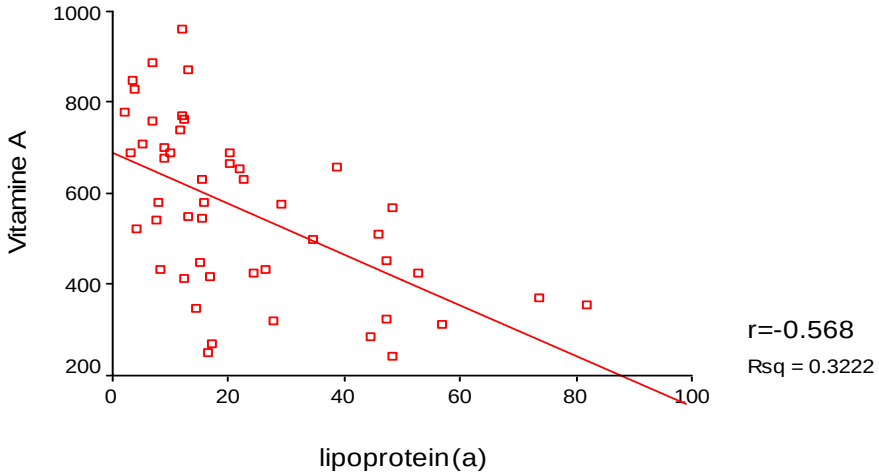
P<0.05

Fig.(5) Scatter diagram for the relation between Lipoprotein (a) & Vitamin E



P<0.01

Fig.(6) Scatter diagram for the relation between Lipoprotein (a) &Vitamin A



P<0.01

DISCUSSION

It is firmly established that systemic markers of inflammation as well as measures of endothelial function are of predictive value for atherogenesis and subsequent CHD *(Vita & Keaney, 2002).* In our study, we found a significant increase in inflammatory markers (CRP & ESR) and vascular cell adhesion molecule VCAM (a marker of endothelial activation) in RA patients as well as a significant positive correlation between these markers. Considerable existing indirect evidence that supports systemic endothelial activation in RA, reflected as increased levels of ICAM and VCAM that is correlated with the increase in inflammatory markers was found in other studies *(Paredes et al., 2002, Camejo et al., 2001 and Cogalgil & Taysi 2002).*

RA patients have dyslipidemia *(Park et al., 1999).* In our study TC and LDLC were significantly increased in RA patients while the difference in TG and HDLC between patients and controls was not significant. Lp (a) was significantly increased in our RA patients. There is controversy about the dyslipidemic pattern found in RA patients. In some studies, they reported a dyslipidemic pattern similar to our findings *(Lakatos & Harsagyi 1988 and Park et al., 1999).* Other studies showed either a lipid pattern similar to the control group *(Camejo et al., 2001)* or a more common pattern of decreased TC, LDLC and HDLC with increased TG *(Paredes et al., 2002 and Sallar et al., 2003).* They attributed the atherogenic risk of this latter pattern to the increase in lipid peroxidation caused by the oxidative stress in RA patient. Noteworthy, *Lazarevic et al.* reported normalization of this dyslipidemic pattern with treatment that regulates the disease activity. Regardless of this controversy about the dyslipidemic pattern, Lp (a), a well established independent risk predictor for cardiovascular disease *(Dahlen,1994),* was significantly increased in our RA patients and in many other studies *(Rantapaa et al., 1991, Park et al. 1999, Asanuma et al., 1999 and Paredes et al., 2002).*

In our study Lp (a) was positively correlated with VCAM and negatively correlated with the antioxidant vitamins A & E . These correlations in addition to the positive correlation found between VCAM and TC & LDLC confirms the relation between chronic inflammation and the increased risk for atherogenesis with subsequent CHD. Other studies reported the same correlation between elevated Lp (a) and inflammatory markers in RA patients and explained the increase in Lp (a) to be secondary to the inflammatory activity *(Myllykangas et al., 1995).*

The plasma level of antioxidant vitamins E and A was lower in RA patients included in our study and this was also observed in the study of

Paredes et al. (2002). These vitamins protect LDL from oxidation, leading to an antiarthrogenic effect due to their antioxidant capacity. In our study, CRP showed significant negative correlation with vitamin E and VCAM showed significant negative correlation with both antioxidant vitamins. These findings again confirm the association of chronic inflammation with atherosclerosis and high risk of CHD. Similar findings were reported by other studies *(Honkanen et al., 1989 and Paredes et al., 2002)* and were explained by the hypothesis that chronic inflammation affects antioxidant vitamin levels in RA due to the increase in oxidative stress.

It was reported in the study of *(Yoshikawa et al., 1983)* that treatment in RA patients can modify their lipid profile and antioxidant status. Administration of indomethacin was found to inhibit lipid peroxidation in rat adjuvant arthritis. Also the altered lipid parameters were normalized with treatment that decreased the disease activity *(Lazarevic et al., 1992).* Moreover, improved endothelial function after anti-TNF-alpha therapy was demonstrated. These data directly implicate TNF-alpha as a mediator of endothelial dysfunction in RA *(Hurlimann et al., 2002).* It is thought, that cytokines are potent upregulators of cellular adhesion molecule expression on endothelial cells, and thus their role in endothelial activation is unambiguous. TNF- alpha could mediate endothelial dysfunction via diminished expression of endothelial nitric oxide synthesis and cyclo-oxygenase-1, as reported by *Sattar et al. (2003).*

From the findings of our study, we can conclude that, atherogenesis in RA is a chronic inflammatory process in which dyslipidemia, endothelial dysfunction and antioxidant vitamins play an important role. Furthermore, they can contribute to an explanation of the high risk of cardiovascular disease in RA patients. Also the intention to treat those patients early and aggressively would not only improve disease outcome and decrease joint damage, but it will also improve long term survival by decreasing the risk of cardiovascular disease.

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