Serum Pentraxin 3 and Interleukin-6 are Associated with Subclinical Atherosclerosis in Recent-onset Rheumatoid Arthritis

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Subclinical atherosclerosis is increased in patients with rheumatoid arthritis (RA), as chronic systemic inflammation leads to accelerate atherosclerosis and increase arterial stiffness in theses patients. This study aimed to evaluate the association of serum interleukin-6 (sIL-6) and serum pentraxin 3(sPTX3) with subclinical atherosclerotic in patients with recent-onset rheumatoid arthritis. Sixty patients with recent onset RA (12-24 months) and 20 controls were investigated. Carotid ultrasound examination, assays for lipid profile, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor, sPTX3 and sIL-6 were done. RA patients demonstrated significantly higher carotid intima-media thickness (cIMT) values and increased carotid plaques than the control (*P*<0.001 and *P*=0.02, respectively). Levels of ESR, CRP, sPTX 3 and sIL-6 were significantly higher in RA patients than controls. RA related risk factors (disease duration, CRP, ESR, and duration of treatment with steroids), as well as sPTX 3, sIL-6 and cIMT were significantly higher in RA with atherosclerotic carotid plaques compared to those without atherosclerotic carotid plaques (all ‧ 0.05). It is concluded that accelerated atherosclerosis in patients with recent-onset RA is associated with elevated levels of CRP, sPTX 3 and sIL-6.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting many tissues especially the synovial lining of joints that can lead to cartilage and bone destruction (Atzeni & Sarzi-Puttini 2007). Several clinical researches on early RA included patients with disease duration of 1–3 years at baseline (Machold *et al.,* 2002; Moreland *et al.,* 2006). However, this period is relatively short as duration of RA may last for decades. The main pathophysiological mechanisms of the disease, the erosion and destruction of joints, probably have become firmly established at one or more years after onset. During this preclinical phase many factors are interacting through as autoantibodies (e.g. rheumatoid factors, anticitrullinated protein/peptide antibodies) soluble factors (e.g. C reactive protein, cytokines, cytokine receptors, chemokines) and gene polymorphisms (Rantapa¨a¨-Dahlqvist *et al.,* 2009).

Many complications as infective, gastrointestinal, renal, pulmonary or cardiovascular complications are contributed to increased morbidity and mortality rates in RA patients than in the general population (Nurmohamed, 2009*)*. The most common cardiovascular complications are atherosclerosis (Del Rincon *et al,* 2003; Gonzalez-Gay *et al,* 2005). The pivotal markers of atherosclerotic disease are increased carotid artery intima-media thickness and carotid plaques. In the early stages of the disease, it is usually asymptomatic, and associated with an unfavorable prognosis, so, it is important to detect preclinical atherosclerosis in RA patients to assure adequate treatment to limit long-term morbidity and mortality (Caporali *et al,* 2009). Chronic inflammatory process in RA constitutes a specific risk factor of atherosclerosis causing endothelial

dysfunction (Hansson, 2005). So, increased level of mediators or markers of inflammation can predict subsequent atherosclerotic cardiovascular disease.

Interleukin (IL)-6 is a pleiotropic cytokine with wide ranges of biological actions. It is produced by several cells such as lymphocytes, monocytes, fibroblasts, endothelial cells and adipose tissue (Papanicolaou *et al,* 1998). IL-6 is induced by many stimuli, as viral infections (ex, HIV) lipopolysaccharide, and several cytokines (IL-1β, TNF-α, platelet-derived growth factor, IFN-γ) (Shalaby *et al,* 1989). IL-6 helps in regulation of immune reactivity, the acute phase response, inflammation, oncogenesis, and hematopoiesis (Song & Kellum, 2005). It also, plays an important role in the initiation and amplification of the inflammatory cascade in RA, and at the same time high serum level of IL-6 was found to predict cardiovascular mortality over a 5 years follow-up, independently of traditional CVD risk factors (Harris *et al,* 1999).

Pentraxin 3 (PTX3) is a member of the long pentraxin family, which is structurally related to, but different from the classic short pentraxin: C-reactive protein (CRP) or serum amyloid proteins (Mantovani *et al,* 2008). PTX3 is produced by several cells as, dendritic cells, macrophages, fibroblasts, activated endothelial cells, neutrophils and renal cells (Ortega-Hernandez *et al,* 2009). The release of PTX3 is stimulated by lipopolysaccharide, IL-1, and TNF, but not IL-6 or IFN-γ. PTX3 plays an important role in innate immunity, female fertility and in the regulation of inflammatory reactions and autoimmunity (Breviario *et al,* 1992). It also, can interact with fibroblast growth factor 2 (FGF2) prevents its binding to endothelial cells. FGF2 plays a pivotal role in the induction of proliferation, migration and survival of vascular smooth muscle cells (SMC) and excessive growth of SMC is an important component in atherosclerosis and restenosis. Binding between PTX3 and FGF2 inhibits SMC proliferation (Camozzi *et al,* 2005). PTX3 increases tissue factor expression in mononuclear cells and endothelial cells (Napoleone *et al,* 2004), which can potentially enhance atherothrombosis. Several studies found that there is an association between the high levels of PTX3 and both of acute myocardial infarction (Peri *et al,* 2000; Latini *et al,* 2004) and an unfavorable outcome in patients with heart failure (Suzuki *et al,* 2008).

The aim of this study is to evaluate the association of sIL-6 and sPTX3 with subclinical atherosclerosis in patients with recent-onset rheumatoid arthritis.

Subjects and Methods

Patients

The study was carried out on sixty patients with recent-onset RA (symptoms 12-24 months) from in and outpatients Clinic of Internal Medicine Department, Tanta University Hospital. They were 35 females and 25 males. Their ages ranged from 23-54 years. All patients fulfilled American College of Rheumatology 1987 revised criteria for diagnosis of RA (Arnett *et al,* 1988).

All patients have no previous history of overt atherosclerosis. Patients with angina, myocardial infarction, congestive heart failure, transient ischemic attacks, or stroke, also, patients with present or past smoking habits, patients using cholesterol-lowering drugs, and obese patients (body mass index ≥30 kg/m^2) were excluded from this study.

Controls

The study also included twenty healthy subjects with matched age, sex and CVD traditional risk factors served as a control group. They were 11 females and 9 males with ages ranged from 28-55 years. All subjects gave written informed consent.

Risk factors for CVD were assessed among RA patients and controls at presentation. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or both at enrollment, or currently receiving antihypertensive treatment. Diabetes mellitus was defined as a fasting

blood glucose concentration ≥126 mg/dL at enrollment or currently receiving antidiabetic treatment. Family history of CVD attack or cerebrovascular attack before age 65 in first-degree relatives was determined by questionnaire.

Methods

All subjects in this study underwent the following:

- Complete history taking including disease duration.
- Full clinical examination.
- Laboratory Investigation
- Blood samples were collected after an overnight fast for 12–14 h. Two milliliters of blood were delivered into citrated tube for erythrocyte sedimentation rate (ESR) determination. The separated serum was used for assay of:

  1. Lipid profile {total serum cholesterol, triglycerides, and high density lipoprotein cholesterol (HDL)} were measured by standard enzymatic colorimetric methods). Low-density lipoprotein (LDL) was measured by Friedewald formula.

  2. Fasting and post prandial blood glucose by standard enzymatic colorimetric methods.

  3. Renal function tests (blood urea by standard enzymatic colorimetric methods & serum creatinine by kinetic methods)

  4. C-reactive protein using an Avitex CRP, a latex agglutination test (Omega Diagnostics).

  5. Rheumatoid factor using an Avitex RF, a latex agglutination test (Omega Diagnostics).

  6. Serum aliquot was stored at ≤ -20°C, until assayed for Pentraxin 3 and IL-6 using quantitative sandwich enzyme-linked immunoassays (Quantikine, R&D Systems, Minneapolis, MN), according to the manufacturer's instructions.

- Measurement of carotid artery intima-media thickness and plaque

  All subjects underwent B-mode carotid US examination using G.E-vivid 7 for measurement of cIMT and detection of plaque. cIMT values ≤0.9 mm indicates normal cIMT, values > 0.9 mm indicates increased cIMT, while values >1.3 mm indicates atherosclerotic plaques (Borhani *et al.,* 1996). Patients were subsequently divided according to presence of plaques into positive (+ve) or negative (-ve) plaque.

Statistical Analysis

Statistical presentation and analysis of the present study was conducted, as continuous data were expressed as mean ± standard deviation while categorical data were expressed in number and percentage. Comparison of categorical data was made by chi-square test. Comparison of continuous data between two groups was made by using Student's t test. Pearson correlation between different parameter was used. Statistical significance was defined as a P value of <0.05. Analyses were performed using SPSS program, version 17 (SPSS Inc., Chicago, IL, USA) and the Graph Pad Prism software (GraphPad Prism Software Inc. San Diego, California, USA).

Result

No significant difference was found between recent-onset RA and control groups as regard, age, sex, presence of hypertension, diabetes mellitus or positive family history. Table 1.

No significant difference was found between recent-onset RA and control groups in serum levels of cholesterol, HDL, LDL, triglyceride, fasting blood glucose, blood urea or serum creatinine. On the other hand, there was significant increase in levels of ESR, CRP, rheumatoid factor, sPTX3 and sIL-6 in recent-onset RA than control group. As regard, ultrasound examination of carotid artery, it was found that the cIMT was significantly higher in RA patients compared with controls (P<0.001), and carotid plaques were 4.1-fold more prevalent in RA patients than controls (41.6% versus 10%, respectively). Table 2 & Fig.1.

Table 1. Clinical data of patients with recent-onset RA and controls

		Recent onset RA (n=60)		Controls (n=20)		*P*-value (T.test)
Age (years)	Range	23-54		28-55		NS
	Mean± SD	41.45±8.21		40.60±8.63		
SBP (mmHg)	Range	110-165		100-160		NS
	Mean± SD	130.8±12.6		128.7±8.02		
DBP (mmHg)	Range	60-100		60-95		NS
	Mean± SD	81.1± 13.2		78.5± 7.79		
		N	%	N	%	*P*-value (Chi-square)
Sex	Male	25	41.67	9	45.00	NS
	Female	35	58.33	11	55.00	
Hypertension	Positive	10	16.67	3	15.00	NS
	Negative	50	83.33	17	85.00	
Diabetes mellitus	Positive	8	13.33	2	10.00	NS
	Negative	52	86.67	18	90.00	
Family history	Positive	7	11.67	2	10.00	NS
	Negative	53	88.33	18	90.00	

SBP= systolic blood pressure, DBP= diastolic blood pressure. *P*>0.05 is not significant; NS= not significant

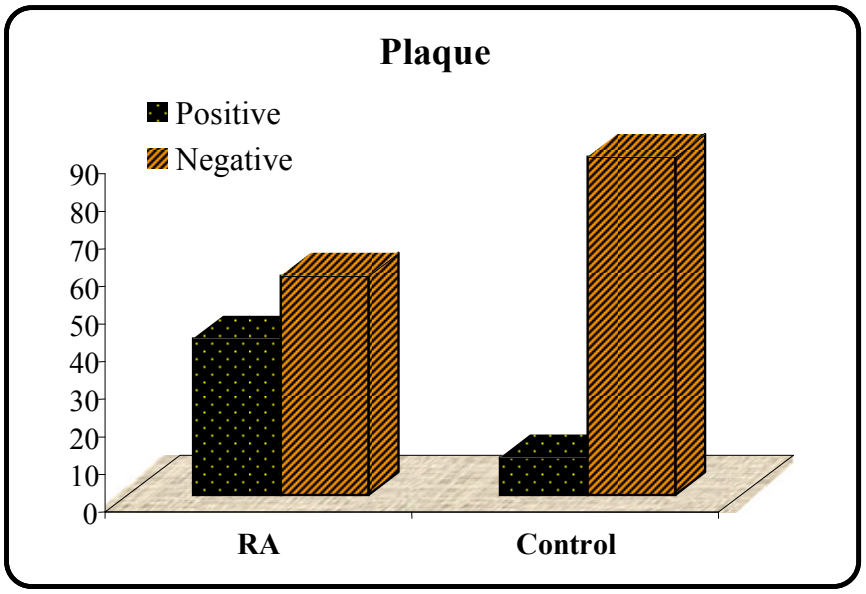

Figure 1. Comparison between the Percentage of atherosclerotic carotid plaques in recent onset RA and controls.

Table 2. Laboratory and Carotid Ultrasound Data in Patients with Recent-Onset RA and Controls

		Recent onset RA (n=60)		Controls (n=20)		*P*-value T-test
Cholesterol mg/dL	Range	150-280		160-230		NS
	Mean ±SD	205.25±37.61		198.85±21.47		
HDL, mg/dL	Range	39-68		41-70		NS
	Mean ±SD	51.13±10.75		53.30±10.57		
LDL, mg/dL	Range	61-202		67-148		NS
	Mean ±SD	123.37±42.50		114.55±25.98		
TG, mg/dL	Range	120-240		115-205		Ns
	Mean ±SD	154.85±30.43		143.72±28.61		
Fasting blood glucose, mg/dL	Range	85-135		80-128		NS
	Mean ±SD	103.1±15.5		100.783±10.98		
Blood urea, mg/dL	Range	23-40		20-35		NS
	Mean ±SD	31.917±4.699		29.65±4.475		
Serum creatinine, mg/dL	Range	0.30-1.40		0.60-1.3		Ns
	Mean ±SD	1.113±0.281		1.09±0.25		
1st hour ESR mm/hour	Range	20-110		8-20		<0.001
	Mean ±SD	78.70±16.57		13.25±3.84		
CRP,mg/L	Range	24-192		6-12		<0.001
	Mean ±SD	84.40±63.03		9.00±3.08		
Rheumatoid Factor , IU/ml	Range	16-32		2-6		<0.001
	Mean ±SD	24.00±8.07		3.80±2.33		
Serum PTX3, ng/mL	Range	2-4		1-2		<0.001
	Mean ±SD	3.06±0.48		1.27±0.42		
Serum IL-6, pg/mL	Range	18-70		6-15		<0.001
	Mean ±SD	37.18±11.49		10.30±2.83		
cIMT,mm	Range	0.8-1.4		0.59-1.4		<0.001
	Mean ±SD	1.05±0.19		0.70±0.07		
Plaque						*P*-value Chi-square
	Positive	25	41.67	2	10.0	0.02
	Negative	35	58.33	18	90.0	

HDL, high density lipoprotein, LDL, low density lipoprotein, TG, triglyceride, ESR erythrocyte sedimentation rate, CRP, C-reactive protein, PTX3, pentraxin-3, IL-6, interleukin-6, cIMT, carotid intima-media thickness, *P<0.05= significant, NS= not significant.

According to presence of atherosclerotic plaques by ultrasound examination of the carotid artery, the patients with recent-onset RA were classified into two subgroups: Subgroup A. RA with positive carotid plaques (n=25). Subgroup B. RA with negative carotid plaques (n=35).

There was significant difference as regard age and hypertension between recent onset RA patients with atherosclerotic carotid plaques and those without carotid plaques, while there was no significant difference as regard, sex, diabetes mellitus, and family history. Table 3

Table 3: Comparison of Clinical data between Recent Onset RA Patients with or without Carotid Plaques.

CVD risk factors		RA with positive carotid plaque (n=25)		RA with negative carotid plaque (n=35)		*P-value (Chi-square)
Age (years)		Range	28.0-54.0	23.0-53.0		0.014
		Mean± SD	43.48±7.64	38.29±8.01		
		N	%	N	%	*P-value (Chi-square)
Sex	Male	7	28.00	18	51.43	NS
	Female	18	72.00	17	48.57	
Hypertension	Positive	8	32.00	2	5.71	0.019
	Negative	17	68.00	33	94.29	
Diabetes mellitus	Positive	5	20.00	3	8.57	NS
	Negative	20	80.00	32	91.43	
Family history	Positive	3	12.00	4	11.43	NS
	Negative	22	88.00	31	88.57	

CVD, cardiovascular disease, *P<0.05 is significant, NS= not significant.

Levels of cholesterol, LDL and RA characteristics (ESR, CRP, disease duration, and duration of steroid treatment) were significantly higher in RA patients with atherosclerotic carotid plaques compared to RA patients without atherosclerotic carotid plaques. Also, patients with atherosclerotic carotid plaques had significantly higher cIMT, sPTX3 and sIL-6 than those without atherosclerotic carotid plaques (P<0.001).

Table 4. Comparison between RA Patients with or without Atherosclerotic Carotid Plaques as regard Cardiovascular Disease Risk Factors and RA Characteristics.

		Carotid Plaques		*P-value
		Positive (n=25)	Negative (n=35)	(T-test)
CVD risk factors				
Cholesterol (mg/dL)	Range	189.00-280.00	150.00-225.00	0.000
	Mean ±SD	241.12±21.54	179.63±22.48	
HDL (mg/dL)	Range	39.00-66.00	41.00-68.00	NS
	Mean ±SD	50.60±11.59	51.80±10.28	
LDL (mg/dL)	Range	104.00-202.00	61.00-155.00	0.000
	Mean ±SD	163.24±22.13	94.89±28.01	
TG (mg/dL)	Range	125.00-240.00	120.00-215.00	NS
	Mean ±SD	146.00±33.86	142.09±24.58	
RA characteristics				
1st hour ESR mm/hour	Range	60.00-110.00	20.00-66.00	0.000
	Mean ±SD	83.88±5.45	47.86±12.86	
CRP mg/L	Range	24.00-192.00	24.00-192.00	0.011
	Mean ±SD	108.48±63.54	67.2±57.56	
RA disease duration (months)	Range	15.00-24.00	12.00-17.00	0.001
	Mean ±SD	18.9±0.8	14.5±0.7	
Steroid therapy duration (months)	Range	6.00-10.00	4.00-7.00	0.000
	Mean ±SD	7.80±1.38	5.49±0.95	
Others				
Serum PTX3 (ng/mL)	Range	2.3-4.0	2.0-3.00	0.001
	Mean ±SD	3.552±0.467	2.714±0.449	
Serum IL-6 (pg/mL)	Range	20.00-70.00	18.00-50.00	0.010
	Mean ±SD	41.64±11.29	34.00±10.69	
cIMT,mm	Range	1.0-1.4	0.80-1.3	0.000
	Mean ±SD	1.19±0.15	0.95±0.16	

CVD, cardiovascular disease, HDL, high density lipoprotein, LDL, low density lipoprotein, TG, triglyceride, ESR erythrocyte sedimentation rate, CRP, C-reactive protein, RA, rheumatoid arthritis,PTX3, pentraxin-3, IL-6, interleukin-6, cIMT, carotid intima-media thickness, *P<0.05*= significant

There was a positive significant correlation between carotid intima-media thickness (cIMT) and age, levels of cholesterol, LDL, ESR, CRP (Fig.2), sPTX3 (Fig.3) and sIL-6 (Fig.4). There was also a positive significant correlation between cIMT and both RA disease duration and steroid therapy duration.

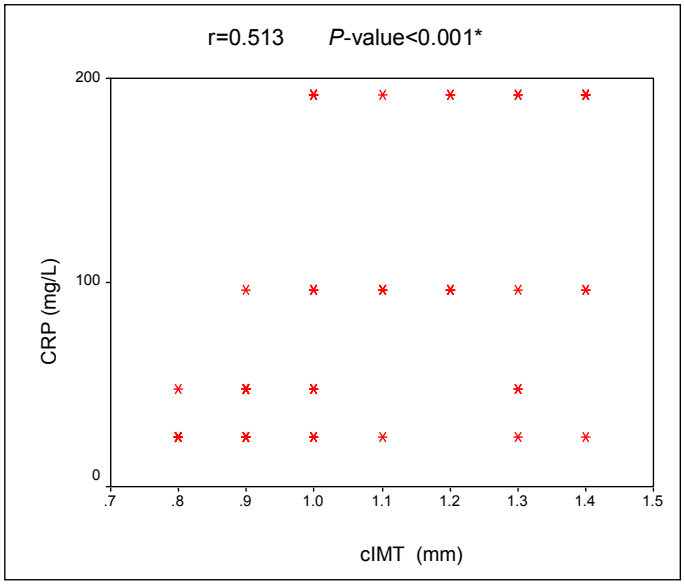

Figure 2. Correlation between Carotid Intima-Media Thickness (cIMT) and C Reactive Protein (CRP) Level.

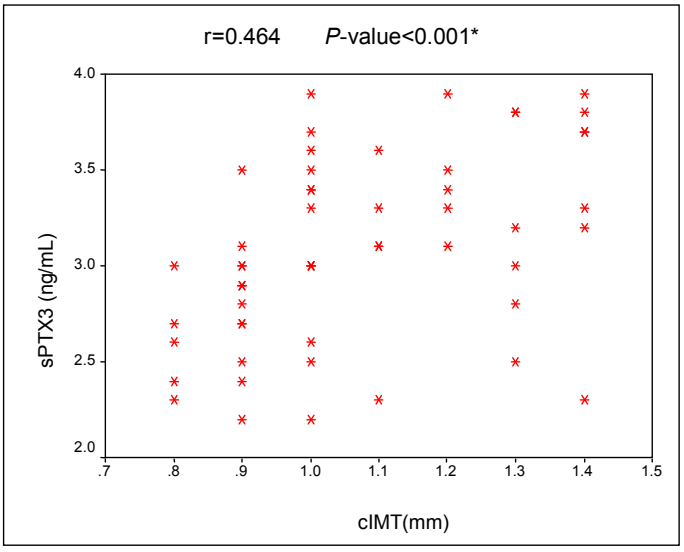

Figure 3. Correlation between Carotid Intima-Media Thickness (cIMT) and Serum Pentraxin 3(sPTX3)

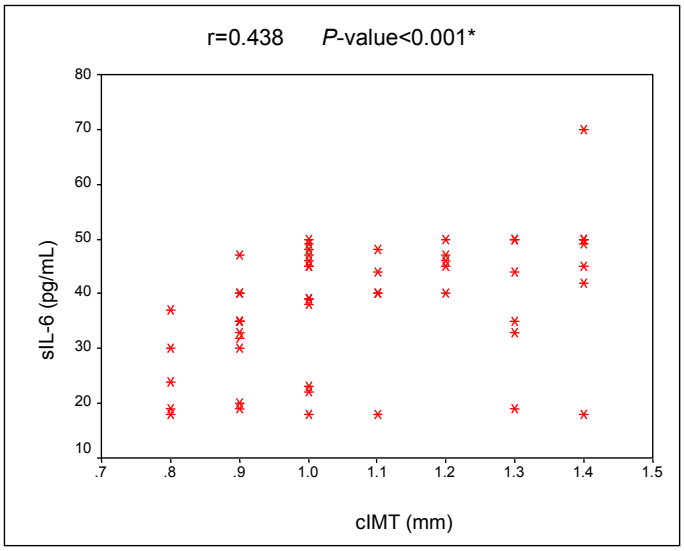

Figure 4. Correlation between Carotid Intima-Media Thickness (cIMT) and Serum Interleukin-6(sIL-6)

Discussion

Early identification of subclinical atherosclerotic cardiovascular disease allows early interference and prevent the occurrence of symptomatic CVD. Both plaque and carotid artery intima-media thickness (cIMT) are stone corner manifestations of subclinical atherosclerosis and can efficiently predict bad prognostic cardiovascular outcome (Lorenz *et al,* 2007).

In this study, in patients presenting with recent-onset RA, subclinical atherosclerosis is detected by presence of plaques or increased cIMT in carotid ultrasound examination. Carotid plaques were 4.1-fold more prevalent in RA patients than controls (41.6% versus 10%, respectively). Also, in the present study, it was found that cIMT is significantly increased in the patients than controls. In RA patients with plaques, RA-related risk factors (ESR, CRP level, disease duration and duration of steroid therapy) were significantly higher when compared to those without plaques. So, this study demonstrates that the presence of RA is a risk factor for the development of atherosclerosis. Furthermore, it was observed that there were positive significant correlations between cIMT and levels of ESR, CRP, sPTX3 and sIL-6 in patients with atherosclerotic plaques.

These findings are in consistent with the Del Rincon *et al,* (2007), who found that noninvasive measures of atherosclerosis as increased cIMT and the presence of carotid plaque are associated with markers of systemic inflammation (ESR & CRP) in patients with RA. They also found that, the cIMT and the probability of vessel plaque increased progressively with increasing levels of both the ESR and the CRP. This finding is consistent with concept which believed that the systemic inflammation has a role in pathogenesis of atherosclerosis. Also, this was in agreement with Roman *et al,* (2006) who found that in patients with rheumatoid arthritis, there was a high prevalence of preclinical atherosclerosis independent of

traditional risk factors, suggesting that chronic inflammation and, possibly, disease severity are atherogenic in this population.

Hannawi *et al,* (2007) observed that the patients with RA had significantly higher average cIMT values and more plaque than the control group and that was contributed to the accelerated atherogenesis related to inflammatory process in RA. These findings agree with Mahajan *et al,* (2008*)* who found that, patients with RA exhibit premature atherosclerosis by way of increased cIMT and carotid plaques when compared to age and sex matched controls.

In the current study, we found that patients with subclinical atherosclerosis have significantly longer disease duration than those without atherosclerosis. This was also observed by Del Rincon *et al,* (2007) who found that RA patients with longer disease duration seem to have more atherosclerotic than patients of the same age, with RA of more recent onset and this suggests that atherogenesis is accelerated after the onset of RA.

In the this study, it was observed that, serum cholesterol, LDL cholesterol levels are significantly higher in recent-onset RA patients with atherosclerotic plaques than those without plaques. These findings are in agreement with Steiner and Urowitz *(2009)* who found that, there is an association between abnormal lipoprotein profile (decreased HDL level and increased LDL level) and RA. This can be explained by the fact that inflammation is a risk factor for cardiovascular disease in patients with RA, and that lipoprotein abnormalities may contribute to the increased risk. Early efficient management is recommended for patients with RA to minimize the long-term risk of CVD.

In this study, IL-6 level was significantly increased in recent-onset RA than control, was significantly increased in RA patients with plaques than those without plaques. This finding is in agreement with Ingegnoli *et al,* (2008) who found that, level of circulating IL-6 increased in RA patients than in normal control. They also, observed the overproduction of the proinflammatory cytokines, such as IL-6, induces a procoagulant shift in the hemostatic balance that promotes fibrin generation and thrombosis in inflammatory states.

Dessein *et al,* (2005) found that, there is an association between biomarkers of endothelial dysfunction (vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1 and endothelial leucocyte adhesion molecule [ELAM]-1, and both cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. They also observed that, IL-6 is more strongly associated with these markers as chronic cytokine release from inflamed joints is implicated in the increased production of adhesion molecules by endothelial cells in RA. In addition, sIL-6 associated with unfavorable traditional cardiovascular risk factor profiles in RA patients and it can predicted endothelial dysfunction, as assessed by these biomarkers, independent of traditional cardiovascular risk factor.

Rho *et al,* (2009) observed that, sIL-6 was significantly associated with the severity of subclinical atherosclerosis as it was associated with higher amounts of coronary calcium level.

In the present study, serum pentraxin 3 level was significantly increased in recent-onset RA than control and was significantly increased in RA patients with plaques than those without plaques. This was previously observed by van Doornum *et al,* (2006) who found that, elevated serum PTX3 levels in patients with inflammatory rheumatic diseases (IRD) may indicate bad cardiovascular

prognosis and patients with RA have both a higher incidence and severity of acute coronary syndromes compared with patients without RA. Similarly, Jenny *et al,* (2009) found that, circulating PTX3 is a more powerful predictor of cardiovascular outcome in the general population than CRP level, and might also have similar importance in IRD.

Bassi *et al,* (2009) observed that, PTX3 is directly involved in the pathogenesis of atherosclerosis. Using immunohistochemical staining, pentraxins were found within the atherosclerotic plaques where they could play an important role by interacting with atherogenic-modified lipoproteins, leading to the formation of foam cells, and production of proinflammatory action. They also found that increased circulating pentraxin level is associated with clinical and subclinical atherosclerosis in general population.

In conclusion; there is increased frequency of subclinical carotid atherosclerosis in patients with recent-onset RA. sIL-6 and sPTX3 can predict subclinical atherosclerosis in recent-onset rheumatoid arthritis patients. The association of atherosclerotic plaques with elevated levels of CRP, sIL-6 and sPTX3 support the concept that chronic systemic autoimmune inflammatory process is implicated in premature atherosclerosis pathogenesis. More attention should given to RA patients with elevated sIL-6 or sPTX3 to avoid long term adverse cardiovascular complications.

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