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Association of serum osteoprotegerin and osteoprotegerin gene polymorphism with subclinical carotid artery atherosclerosis and disease activity in rheumatoid arthritis patients

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ABSTRACT

Aim of the work: To investigate the association of single nucleotide polymorphism (SNP) (rs2073618) of the OPG gene and of serum OPG with subclinical carotid atherosclerosis in RA patients.
Patients and methods: Eighty RA patients with no previous history of a cardiovascular disease were studied and forty healthy controls were enrolled in the study. Carotid atherosclerosis was evaluated by high-resolution B-mode ultrasound and the carotid intimal medial thickness (CIMT) measured. rs2073618 OPG genotyping was performed by polymerized chain reaction (PCR) and serum OPG concentrations were measured. The high sensitive C reactive protein (hs-CRP), rheumatoid factor (RF) titer and anti-cyclic citrullinated peptide (anti-CCP) were assessed. The disease activity score (DAS28) was evaluated.
Results: The patients mean age was54.1 ± 6.2 years, disease duration of 12.5 ± 8.5 years and were 72 females and 8 males. Increased IMT was found in 38 patients, 40 age and sex matched controls were included. Patients with atherosclerosis (n = 38) had longer disease duration, higherDAS28, hs-CRP, RF titer and anti-CCP. The serum OPG levels were higher in patients with atherosclerosis (1106.4 ± 1157.1 ng/l) compared to those without (658.3 ± 151.1 ng/l)(p = 0.001). Serum OPG significantly correlated with disease duration (r = 0.42, p = 0.005), DAS28 (r = 0.53, p = 0.001), hs-CRP (r = 0.41, p = 0.007), anti-CCP (r = 0.47, p = 0.003) and mean CIMT (r = 0.37, p = 0.02). The frequencies of CC, CG and GG genotypes were comparable between those with and without atherosclerosis (39.5%, 50%, 10.4% *vs* 42.9%, 47.6% and 9.5% respectively).
Conclusion: rs2073618 OPG gene may not be associated with subclinical atherosclerosis, although the serum level could be a reliable marker for disease activity and for early detection of carotid artery atherosclerosis in RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder affecting 0.5–1% of the general population with significant morbidity, disability and costs for society [1]. Premature mortality in RA patients is increased mainly due to cardiovascular disease (CVD) [2,3]. Increased aortic stiffness, carotid intima-media thickness (IMT) and serum osteoprotegerin (OPG) have been shown to be independent risk factors for cardiovascular events [4]. A high frequency of subclinical carotid atherosclerosis, recognized by ultrasound, has been reported in RA patients, and can thus help identify patients with increased CVD risk [5]. Traditional CV risk factors and subclinical atherosclerosis still do not completely clarify the higher incidence of CVD in RA suggesting that the cardiovascular risk may be associated with RA independently and may be linked to other parameters specific for the disease including systemic inflammation, disease duration and specific RA therapies [2,6]. In Egyptian RA patients, the frequency of carotid atherosclerosis was high and was significantly associated to inflammatory markers, endothelial dysfunction, antioxidant vitamins, disease duration, rheumatoid factor (RF) titer, functional disability and bone erosion

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[7]. Those with increased IMT had significantly longer disease duration, higher disease activity and insulin resistance (IR) than those with normal IMT. A remarkable association was determined between IR and subclinical atherosclerosis in RA [8].

Genetic causes represent 60% of the risk of both RA and CVD [9]. The interest to identify genetic markers of atherosclerosis in RA has been increased and the association of CVD, traditional cardiovascular risk factors and mortality with multiple genes has been increasing [10].

The human Osteoprotegerin (OPG) gene (TNFRSF11B) is located on chromosome 8q24; it can be affected by single nucleotide polymorphisms (SNPs) which have functional effects on CVD and bone homeostasis [11,12]. SNP rs2073617, rs2073618 and rs3134069 of the OPG gene were associated with atherosclerosis in non-rheumatic individuals [13,14].

The association between OPG SNP and carotid artery atherosclerosis in RA has scarcely been evaluated which could serve as a marker for detection of patients at increased risk for CVD thus help to improve RA morbidity and mortality rates.

The aim of this work was to evaluate the relation of OPGrs2073618 SNP and serum OPG with sonographic markers of subclinical carotid atherosclerosis and disease activity in RA patients.

2. Patients and methods

This cross-sectional study enrolled 80RA patients who were recruited from the outpatient and inpatient clinics of Rheumatology and Rehabilitation department, Faculty of Medicine, Assiut University Hospitals and diagnosed according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA [15]. 40 age and sex matched controls were included. Patients with definite diagnosis for any other systemic autoimmune disorders, patients with previous history of CVD (previous angina, myocardial infarction or stroke) were excluded from the study. RA patients were subdivided according to presence or absence of atherosclerosis based on carotid ultrasound findings. The study was approved by the Institutional Ethics Committee, Faculty of medicine, Assiut University. Written consent form was taken from all the participants.

All the patients were subjected to complete history taking including disease duration, drug intake, past and family history ofCVD, risk factors for atherosclerosis (diabetes mellitus, smoking, hypertension, dyslipidemia); thorough clinical examination and measurement of the body mass index (BMI) and disease activity score (DAS28) [16]. Laboratory assessment included complete blood picture, erythrocyte sedimentation rate (ESR), high sensitive C-reactive protein (hs-CRP), plasma glucose, liver function tests, kidney function tests, complete lipid profile, RF titer and anti-cyclic citrullinated peptide (anti-CCP).

Serum OPG was quantitatively measured by enzyme linked immunosorbent assay (ELISA) kit (OPG ELISA, SinoGeneClon Biotech Co Kit, (China) according to the instructions of manufacturer. The concentration of OPG (ng/L) was determined by comparing the optical density to the standard curve (Detection range: 50–1500 ng/L).

2.1. Preparation of genomic DNA and PCR amplification

DNA was isolated from whole blood by PureLinkR Genomic DNA Mini Kit cat no 1820-01 lot no. 1746207 (Qiagen, USA) according to manufacture instructions. Primers for the amplification of the OPG gene were designed according to the available OPG sequence in GenBank (accession no. AB008821). PCR amplifications were performed in a total volume of 25 μl with a reaction mixture containing 12.5 μl of TaqMan Universal PCR Master Mix (2X) (lot no. 1509032), 10.25 μl RNase-free water and 1.25 μl of SNP Genotyping Assay in addition to 1 μl of DNA. PCR was amplified using 7500 Fast Real-Time PCR System (Applied Biosystem) under the following conditions: 10 min at 95 °C enzyme activation followed by 40 cycles with denaturation at 92C for 15 sec, annealing and extension at 60 °C for 1 min.

2.2. Carotid artery ultrasonography

The carotid arteries were evaluated for any wall changes in all patients with the high-resolution B-mode ultrasound equipment Logic P6 PRO GE healthcare. The IMT was measured of the far wall of the common carotid arteries as the distance between the luminal-intimal interface and the medial adventitial interface. One transverse and two longitudinal measurements of IMT were obtained from 4 contiguous sites at 2 mm intervals, and the average of the 4 measurements was used for the analysis and a mean value >0.9 mm was considered indicative of thickened intima [17]. Plaques were considered as focal widening relative to adjacent segments [18]. Ultrasound evaluation was performed blindly by the same sonographer.

2.3. Statistical analysis

Data were collected and analyzed using SPSS (Statistical Package for the Social Science, version 20). Results were expressed as mean ± SD, or as frequency (percentage). Chi^2-test was used to compare the nominal data, while Mann-Whitney was used to compare the mean of two groups and ANOVA test for more than two groups. Spearman correlation was used to test the associations. Logistic regression analyses were used to investigate the association between carotid atherosclerosis in RA patients and OPG genotypes and alleles, data are presented as odds ratios and 95% confident intervals (C.I.). P value < 0.05 considered significant for all used tests. Tests for deviation from the Hardy-Weinberg equilibrium were performed using a standard $\chi 2$ test.

3. Results

The patients mean age was 54.08 ± 6.17 years with disease duration of 12.51 ± 8.52 years. They were 72 females and 8 males (9:1). Atherosclerosis was present in 38 patients.41.3% of RA patients were on methotrexate, 77.5% on hydroxychloroquine, 63.8% on leflunomide and 21.5% were on sulfasalazine. The mean age of the control was 54.25 ± 4.51 years and they were 37 (92.5%) females and 3 (7.5%) males.27 (33.75%) patients had carotid atheromatous plaques.

The patients characteristics with and without atherosclerosis are presented in Table 1. Those with atherosclerosis were older while hypertension and diabetes were comparable. There was no significant difference regarding the therapeutic drug history. RA patients with atherosclerosis had significantly higher serum levels of hsCRP, RBG, low density lipoprotein (LDL), cholesterol, RF, anti-CCP and DAS28 as well as lower high density lipoprotein (HDL) in comparison to those without. RA patients had higher serum OPG levels (871.2 ± 830.4 ng/l) in comparison to control (580.1 ng/l ± 161.6 ng/l) (p = <0.001).

Patients with atherosclerosis had higher serum OPG levels (1106.4 ± 1157.1 ng/l)in comparison to those without (658.3 ± 151 .1 ng/l) (p = 0.001). Also, serum OPG levels were significantly higher in those with carotid atheroma (1125.1 ± 1208.2 ng/l) in comparison to those without (629.6 ± 155.8 ng/l) (p = 0.001) (Fig. 1).

Table 1
Demographic, Clinical and Baseline laboratory data of rheumatoid arthritis patients with and without atherosclerosis.

Variable	RA patients with/without increased IMT		*p*
	With (n = 38)	Without (n = 42)	
Age (years)	56.6 ± 6.4	51.8 ± 4.9	**0.001**
F:M	34:4 (89.5/10.5)	38:4 (9.5/90.5)	0.88
Smoking	4 (10.5)	4 (9.5)	0.88
Hypertension	7 (18.4)	10 (23.8)	0.56
Diabetes mellitus	14 (36.8)	16 (38.3)	0.91
BMI	26.01 ± 4.8	28.5 ± 6.4	0.13
Dis. duration (years)	14.5 ± 8.7	11.1 ± 7.5	**0.04**
Morning Stiffness	30 (78.9)	22 (52.4)	**0.01**
DAS28	4.8 ± 1.3	4.1 ± 1.8	**0.03**
Remission	0 (0)	5 (11.9)	**0.04**
Low	8 (21.1)	5 (11.9)	
Moderate	10 (26.3)	17 (40.5)	
High	20 (52.6)	15 (35.7)	
RBG(μmol/l)	5.8 ± 3.9	3.8 ± 1.6	**0.001**
ESR (ml/hour)	49.8 ± 22.8	39.7 ± 21.3	0.08
hsCRP (mg/dl)	19.4 ± 20.8	9.4 ± 7.2	**0.04**
LDL (mg/dl)	117.9 ± 40.9	95.9 ± 42.4	**0.01**
HDL (mg/dl)	41.2 ± 8.9	49.3 ± 10.1	**0.01**
TG (mg/dl)	131.5 ± 101.9	104.8 ± 36.1	0.53
Cholesterol (mg/dl)	190.4 ± 45.6	160.2 ± 31.6	**0.004**
RF (U/l)	201.7 ± 226.1	80.3 ± 97.1	**0.005**
Anti-CCP (U/ml)	1885.7 ± 1865.9	1022.7 ± 1310	**0.04**

RA: rheumatoid arthritis; BMI: body mass index; DAS28: disease activity score 28; RBG: random blood glucose; ESR: erythrocyte sedimentation rate; hsCRP: high sensitive CRP; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides; RF: rheumatoid factor; Anti-CCP: anti-cyclic citrullinated peptide. Data are expressed in form of mean (±SD) and frequency (percentage). Bold values are significant at $p < 0.05$.

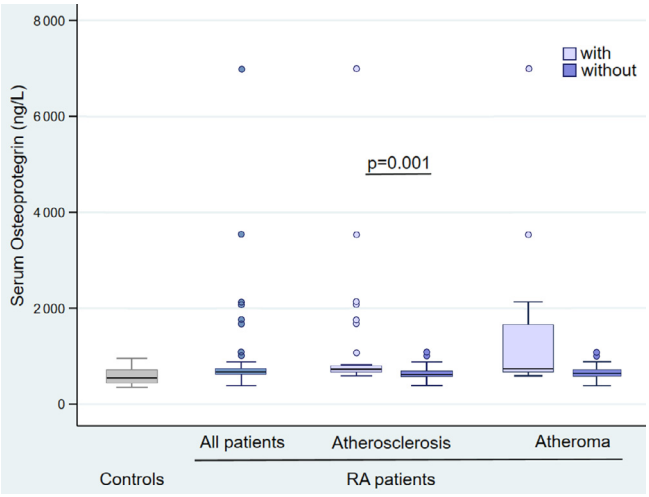

Fig. 1. Serum osteoprotegrin titres in rheumatoid arthritis (RA) patients with and without carotid atherosclerosis and atheroma as well as control.

The frequency of OPG genotypes in the patients are presented in Table 2. There were no significant differences between the CC and CG genotypes of the studied OPG gene regarding the therapeutic history. There were no significant differences when comparing RA disease characteristic including RA disease activity between the CC and CG genotypes of the studied OPG gene except for lower mHAQ in GG genotype (Table3).

The OPG genotypes and alleles had no significant differences as regard serum OPG concentrations; CC genotype had a mean level of 1059.1 ± 1236.4 ng/L, GC was 757.7 ± 301.7 ng/L and GG was 648.9 ± 76.7 ng/L. The mean level in those with C allele was 947.2 ± 997.7 ng/L and in G allele was 726.1 ± 260.9 ng/L.

Correlations of OPG and IMT with the demographic, clinical, laboratory and sonograhic findings of the patients are presented in Table 4. Association of serum OPG concentrations with disease characteristics, activity, markers of systemic inflammation, and CIMT were evaluated using linear regression model and a significant association between serum OPG with ESR and anti-CCP existed (Table 5).

4. Discussion

The risk for CVD in RA patients is substantially elevated compared with the general population [3], traditional CV risk factors and inflammatory markers do not completely clarify this expanded risk [19]. Hence, efforts to recognize other risk factors can improve our ability to identify those patients with increased risk. Both RA and atherosclerosis are complex polygenic diseases. The role of genetic factors and polymorphisms in the development of atherogenesis in RA has been confirmed [20]. OPG is a decoy receptor activator for nuclear factor κB ligand (RANKL) which plays a role in regulation of immunity, bone resorption and CV function. Both human atherosclerotic plaque and osteoblasts demonstrated OPG expression [21]. Synovial tissue from the joints of RA patients showed high OPG expression suggesting its potential role in atherosclerosis and RA pathogenesis [22]. Considering the obvious role of OPG in both RA and atherosclerosis, in this study, the association between serum OPG level, OPG (rs2073618) SNP and sonographic markers of subclinical atherosclerosis in RA patients was investigated.

Regarding the association between subclinical carotid atherosclerosis and CVD risk factors it was noticed that those with atherosclerosis were older, had longer disease duration and higher serum levels for RBG, LDL, cholesterol and lower serum levels for HDL. This in agreement with Del Rincon et al. [23] and in concordance with Chung et al. and Elshereef et al. reported that RA patients with atherosclerosis were older and had longer disease duration but in contrast there was no significant difference regarding lipid profile [10,24].

In the present study, there was a significant correlation of the IMT with age, systolic blood pressure, LDL and cholesterol. In

Table 2
Association between osteoprotegrin gene (rs2073618) and carotid atherosclerosis in rheumatoid arthritis patients.

OPG SNP (rs 2073618)	RA patients with/without increased IMT			p
	With (n = 38)	Without (n = 42)	OR (95%CI)	
CC	15 (39.5)	18 (42.9)	0.83 (0.2–3.41)	0.83
CG	19 (50)	20 (47.6)	0.95 (0.3–3.04)	0.93
GG	4 (10.5)	4 (9.5)	–	–
Alleles				
C	49/76 (64.5)	56/84 (66.7)	1 (0.41–2.43)	1
G	27/76 (35.5)	28/84 (33.3)	–	–

RA: rheumatoid arthritis; IMT: intimal medial thickness; SNPs, single nucleotide polymorphism; OR: Odds ratio; CI: confidence interval. Data are expressed as frequency (percentage) and median (interquartile range).

Table 3
Rheumatoid arthritis disease characteristics according to osteoprotegrin (OPG) rs2073618 G/C genotypes.

Parameter	OPG genotype in RA patients (n = 80)			p
	CC (n = 33)	CG (n = 39)	GG (n = 8)	
Dis. duration (years)	13.7 ± 8.9	12.2 ± 8.6	9.3 ± 5.8	0.41
Morning stiffness	24 (72.7)	23 (59)	5 (62.5)	0.47
Deformity	19 (57.6)	22 (56.4)	6 (75)	0.61
Disease activity				0.65
Remission	1 (3)	3 (7.7)	1 (12.5)	
Low	7 (21.2)	6 (15.4)	0 (0)	
Moderate	9 (27.3)	15 (38.5)	3 (37.5)	
High	16 (48.5)	15 (38.5)	4 (50)	
DAS28	4.5 ± 1.5	4.4 ± 1.8	3.9 ± 1.5	0.69
TJC	11.6 ± 10.6	9.2 ± 9.9	17.4 ± 12.1	0.12
SJC	3.1 ± 3.9	2 ± 3.3	1.8 ± 2.2	0.35
mHAQ	0.75 ± 0.6	1.03 ± 0.6	0.65 ± 0.7	**0.009**
RF (U/l)	135.1 ± 180.7	130.4 ± 170.7	186.6 ± 238.5	0.73
Anti-CCP (U/ml)	1542 ± 1828	1406.7 ± 1556.1	1107.7 ± 1405.9	0.76

OPG: osteoprotegrin; RA: rheumatoid arthritis; DAS28: disease activity score; TJC: tender joints count; SJC: swollen joints count**;** mHAQ: modified health assessment questionnaire; RF: rheumatoid factor; Anti CCP:anti-cyclic citrullinated peptide. Data are expressed as mean ± SD and frequency (percentage). Bold values are significant at $p < 0.05$.

Table 4
Correlation of serum osteoprotegrin (OPG) titre and carotid intima-media thickness (IMT) with demographic, clinical, laboratory and sonograhic data in rheumatoid arthritis patients.

Parameter r (p)	RA patients with/without increased IMT (n = 80)							
	OPG (ng/l)				IMT (mm)			
	With (n = 38)		Without (n = 42)		With (n = 38)		Without (n = 42)	
Age	0.39	**(0.02)**	0.31	**(0.04)**	0.49	**(0.001)**	0.35	**(0.02)**
Dis. duration	0.42	**(0.005)**	0.35	**(0.03)**	0.36	**(0.03)**	0.33	**(0.03)**
SBP	0.39	**(0.02)**	0.38	**(0.01)**	0.37	**(0.02)**	0.29	(0.05)
DBP	0.29	(0.07)	0.11	(0.49)	0.03	(0.84)	0.22	(0.16)
BMI	0.09	(0.61)	0.04	(0.80)	− 0.07	(0.64)	0.21	(0.16)
ESR	0.29	(0.05)	0.14	(0.40)	0.05	(0.74)	0.12	(0.45)
hsCRP	0.41	**(0.007)**	0.35	**(0.03)**	0.39	**(0.02)**	0.34	**(0.03)**
LDL	0.32	(0.04)	0.30	(0.06)	0.35	**(0.02)**	0.14	(0.39)
HDL	−0.1	(0.55)	−0.4	(0.56)	−0.22	(0.19)	−0.15	(0.32)
Triglyceride	0.36	**(0.02)**	0.27	(0.09)	0.31	(0.05)	0.19	(0.22)
Cholesterol	0.35	**(0.03)**	0.34	**(0.03)**	0.48	**(0.002)**	0.35	**(0.02)**
DAS28	0.53	**(0.001)**	0.35	**(0.03)**	0.39	**(0.01)**	0.35	**(0.02)**
RF	0.28	(0.09)	0.15	(0.36)	0.12	(0.46)	0.02	(0.89)
Anti–CCP	0.47	**(0.003)**	0.38	**(0.01)**	0.44	**(0.006)**	0.34	**(0.02)**
Carotid IMT	0.37	**(0.02)**	0.32	**(0.03)**	**–**	**–**	**–**	**–**
OPG	**–**	**–**	**–**	**–**	0.37	**(0.02)**	0.33	**(0.03)**

RA: rheumatoid arthritis; OPG: osteoprotegrin; IMT: intimal medial thickness; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; ESR: erythrocyte sedimentation rate; hsCRP: high sensitivity C-reactive protein; LDL: low density lipoprotein; HDL: high density lipoprotein; DAS28: disease activity score; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide. Bold values are significant at $p < 0.05$.

Table 5
Regression of serum osteoprotegrin (OPG) with demographic, clinical, laboratory and sonographic data in rheumatoid arthritis patients.

Variable	Serum OPG in RA patients (n = 80)				
	B	SE	95% CI		p
			Lower	Upper	
Age (years)	18.7	15.2	−11.5	49	(0.22)
Dis. Duration (years)	16.1	11.04	−6	38.1	(0.15)
ESR (mm/1sthr)	9.1	4.2	0.8	17.3	**(0.03)**
HsCRP titre (mg/L)	10.5	5.9	−1.3	22.3	(0.08)
LDL (mg/dl)	−0.6	2.9	−6.4	5.2	(0.85)
HDL (mg/dl)	−3.4	4.9	−13.3	6.5	(0.5)
TG (mg/dl)	−0.8	1.3	−3.3	1.7	(0.54)
Cholesterol (mg/dl)	1.4	3.3	−5.2	8	(0.77)
DAS28	−59.2	57.4	−173.9	55.4	(0.31)
RF (IU/mL)	−0.5	0.5	−1.5	0.5	(0.3)
Anti-CCP (U/ml)	0.2	0.1	0.03	0.3	**(0.01)**
CIMT (mm)	−158.02	450.1	−1056.5	740.4	(0.73)

OPG: osteoprotegrin; RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; hsCRP: high sensitivity C-reactive protein; LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides; DAS28: disease activity score; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide; CIMT: carotid intima media thickness. Bold values are significant at $p < 0.05$.

concordance, Tutoglu et al. and Saigal et al. reported positive association of IMT with the age, cholesterol and LDL [25,26].

Patients with atherosclerosis had significantly higher levels of RF and anti-CCP and IMT significantly correlated with the RF. This is similar to the results of others [27–31].

Regarding the association between the carotid IMT with markers of inflammation in the form of ESR and hs-CRP as well as with the disease activity, in this work the relations were significant. This is in agreement with findings in other Egyptian studies on RA [7,8].

In this study, serum OPG level was significantly higher in RA patients with atherosclerosis and those with atheromatous plaques compared to those without. Serum OPG significantly correlated with the mean carotid IMT. This is in harmony to the findings of other studies [32–34]. Asanuma et al. demonstrated that the serum OPG concentrations in patients with carotid plaque were significantly higher than patients without [35].

In the current study, serum OPG significantly correlated with hsCRP and DAS28. This is in agreement with the findings of other studies [36–38].

In this study regarding the association between OPG gene polymorphism (rs2073618) and subclinical carotid atherosclerosis, there were no significant differences between rs2073618 GG, GC, CC genotypes nor G, C alleles in RA patients with atherosclerosis and those without. This is in agreement with Genre et al. who studied the effect of OPG SNPs (rs2073618, rs3134069 andrs3134063) on the risk of CVD in RA and observed no association [39]. In another study, seven OPG gene polymorphisms were investigated in patients with and without coronary artery disease (CAD) and none of the studied SNPs was linked to the presence of CAD separately [11].

In RA patients, there was a significant association between CC, CG, GG genotypes of OPG (rs2073618) genetic polymorphisms and coronary artery atherosclerosis (detected by electron beam computed tomography scan) [10]. It has been stated that coronary artery atherosclerosis and score enhanced the discrimination and prediction of CVD better than carotid ultrasound. The discrepancy of results may be due to there is other loci that are probably in linkage disequilibrium and affect the susceptibility to atherosclerosis in RA patients and also the ethnic difference in the regulatory SNP allele (C and G) frequency among the studied group may help in the explanation of this discrepancy. The difference in the results may be also explained by the difference in the radiographic tools used to evaluate atherosclerosis [40].

In this study there was no significant difference of the serum OPG levels among the studied genotypes and alleles. This is in agreement with *Chung et al* who explained this finding by taking into account that this SNP causes a substitution from asparagine to lysine and might alter the OPG protein function instead of its concentration [10]. Current data support the role of serum OPG as a link between disease activity and increased CVD risk in RA.

Among the limitations to this work, the sample size was relatively small which could affect the magnitude of association; it was a hospital based so selection bias cannot be excluded and the sample could not be representative for the whole Egyptian population. The association between SNP in a single gene which could be in linkage disequilibrium with other genes on such diseases with complex genetic bases (RA and atherosclerosis) was investigated. To clarify the possible role of the OPG and the Receptor activator of nuclear factor kappa-B and its ligand (RANK/RANKL) axis in atherosclerosis, we recommend measuring not only serum OPG, but also RANK and RANKL serum levels and studying the interactions between multiple genes of the OPG/RANKL/RANK system on the risk of subclinical atherosclerosis on larger sample size and multinational based study.

In conclusion, this study supports the role of OPG in atherosclerosis pathogenesis in RA. Serum level of OPG could be used as a reliable biomarker for diagnosis of subclinical atherosclerosis and for determining the disease activity.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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