Osteoprotegerin and Soluble Receptor Activator of Nuclear Factor-Kappa B Ligand in *Exudative Age-Related Macular Degeneration*

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Abstract- Calcification and inflammation are among the important cases of exudative age-related macular degeneration (E-ARMD). The aim of the present study was to elucidate if there is any relationship between serum Osteoprotegerin (OPG), soluble receptor activator of nuclear factor-kappa B ligand (RANK-ligand) and E-ARMD. In a cross-sectional study, we compared 45 E-ARMD patients with 45 matched controls. Diagnosis was confirmed by fluorescein angiography. Serum samples were analyzed for OPG, RANK-ligand, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and triglyceride (TG). The levels of OPG and RANK-ligand were measured by ELISA methods. The mean age was 72.0±11.5 years in the E-ARMD group and 68.2±8.9 years in the control group (p=0.09). The level of serum OPG was 132.10±75.49 pg/ml in the E-ARMD group and 94.88±61.65 pg/ml in the control subjects. E-ARMD patients had significantly high levels of OPG (p=0.012), as well as significantly high levels of LDL-C and TC (p=0.001 and p=0.005, respectively). We could not find any significant difference in RANK-ligand, HDL-C, or TG between two study groups (p>0.05). To the best of our knowledge, this is the first study investigating the levels of OPG in E-ARMD patients. The present study showed that E-ARMD patients had high levels of serum OPG. It may act as a protective factor for E-ARMD or only as a secondary phenomenon of different processes of E-ARMD. Further prospective studies would be necessary for prognostic and predictive significance of OPG in patients affected by E-ARMD.

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Introduction

Age-related macular degeneration (ARMD) is the leading cause of severe loss of central visual acuity in many parts of world. It is known that exudative ARMD (E-ARMD) and its associated disabilities are prevalent in Asia. More than 10 percent of adults aged 75 years or older have E-ARMD, and both eyes are almost always affected. E-ARMD has complex risk factors. The role of oxidative stress has been proposed for many years in the pathogenesis of this condition (1,2). Inflammation, which is closely associated with oxidative stress, is another risk factor (3,4). Genetic factors and many genes have been found to be associated with ARMD. Complement system and genetic variations in this system have been shown to be associated with ARMD (5,6). Apolipoprotein E (Apo E) is also associated with the development of ARMD. Apo E, which participates in the metabolism of cholesterol and other lipids, is also found in drusen. High levels of serum cholesterol and fibrinogen have been shown to be associated with E-ARMD, and cigarette smoking, obesity, and hypertension have also been shown to be risk factors (7). Reviewing the risk factors show that cardiovascular disease and E-ARMD risk factors are similar in some patients. In addition, it has been shown that patients with carotid plaques or patients with lower extremity atherosclerosis are more at risk for developing E-ARMD (8).

Osteoprotegerin (OPG) is a soluble glycoprotein

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widely expressed in most human tissues, including bones and endothelium and smooth muscle of the vasculature (9). OPG prevents RANK-ligand RANK interaction, as well as osteoblastic activity and bone resorption. OPG is able to neutralize the pro-apoptotic action of tumor necrosis factor (TNF), which is an inflammatory cytokine associated with free radicals and oxidative stress (10,11). Clinical studies have reported that higher serum levels of OPG are associated with cardiovascular disease (12,13). Raised OPG levels have been associated with the severity of coronary arthrosclerosis in a cross-sectional study (14), and elevated serum OPG was correlated with the severity of peripheral artery disease and heart failure (15). High OPG levels are shown to be associated with coronary artery and aortic plaque. Even after correction for conventional cardiovascular risk factors, OPG remained an independent risk factor for incidence and severity of cardiovascular disease (12,16).

Vascular endothelial cells physiologically produce OPG, whereas RANK-ligand is mainly expressed by T cells and activated endothelial cell. Many studies have shown that elevated levels of OPG are associated with higher levels of inflammation (C-reactive protein, erythrocyte sedimentation rate, and fibrinogen) (17-19). The suggestion that OPG is a marker of inflammation is supported by its downregulation by anti-inflammatory agents such as immunosuppressors (20).

According to their association with cardiovascular diseases and oxidative stress, OPG levels may be associated with E-ARMD, which has been shown to display oxidative pathogenicity, at least in some part. To the best of our knowledge there has been no report to date studying the levels of OPG in patients affected by ARMD. The aim of the present study was to determine serum OPG and RANK-ligand levels in patients with E-ARMD.

Materials and Methods

This cross-sectional study was performed in Nikookari Eye Hospital, Tabriz, Iran, which is the tertiary center for eye diseases in Northwest Iran. The study was conducted between March 2009 and September 2010.

The subjects in the case group were selected from patients in the retina clinic. All patients had a confirmed diagnosis by a vitreoretinal surgeon. Patients with history of ocular trauma, glaucoma, uveitis, complicated cataract surgery, or other ophthalmic surgical history were excluded from the case and control groups. In addition, we excluded patients affected by any other detectable macular disease, history of diabetes, hypertension, Hypercholesterolemia, cardiovascular diseases and chronic systemic disorders. Patients with a history of calcium, bisphosphonate, or steroid use and patients under replacement therapy were excluded.

Each patient underwent a primary interview, and uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) were determined with a Snellen chart. Visual acuity was calculated as the logarithm of the minimal angle of resolution. An ophthalmic examination that included fundus biomicroscopy (Haag-Streit R900 Slit lamp, Haag-Streit AG, Switzerland, Volk super-field lens) and tonometry was performed on all patients. Fundus photography and fluorescein angiography were performed on all patients. The E-ARMD diagnosis was based on presence of choroidal neovascularization and either RPE atrophy, hyperpigmentation, or break. Due to definite diagnosis and due to uniformity of the retinal lesion we choose only the patients who had classic neovascularization in fluorescein angiography for E-ARMD group. Fluorescein angiography is the gold standard for choroidal neovascularization and we included patients with classic neovascularization in fluorescein angiography in E-ARMD group.

The study was approved by the ethics committee of Tabriz University of Medical Sciences. All patients signed an informed written consent. The E-ARMD group was composed of 45 E-ARMD patients, and 45 healthy controls were matched. All participants underwent blood sampling after 8 hour fasting. All samplings were performed in a period of 4 weeks. The sera and plasma were separated immediately after sampling. Then analyzed using enzymatic assays with an automated analyzer (Abbott Analyzer, Abbott Laboratories, Abbott Park, IL, USA) for serum fasting blood sugar (FBS) and serum lipid profile, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

Serum RANK-ligand concentration was determined by commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits: sRANKL by BioVendor kit (Intra assay: CV = 8.70 %, Inter assay: CV = 9.19 %). Serum OPG level was determined by Bender Med Systems GmbH (Intra-assay: CV=7.0%, Inter assay: CV=8.0%). All tests were performed according to the manufacturer's instructions. Each plate included standards in parallel with the samples, and the limit of detection 0.4 pmol/l (RANK-ligand) and 2.5 pg/ml (OPG). All case and control samples were analyzed on the same day for each

assay kit.

The data was analyzed by SPSS software version 18 (SPSS Inc., Chicago, IL, USA), and a significance level of 0.05 was considered for the statistical tests. Independent t-test was used for comparison between groups, and linear correlation analysis was performed by calculating Pearson's linear correlation of coefficients.

Result

Ninety patients (53 females and 37 males) participated in this study. Forty-five patients, all affected by exudative ARMD (E-ARMD), were studied in the case group. The definite diagnosis was made by the same sub-specialist. Forty-five control subjects were matched from healthy individuals who had visited the hospital clinics for problems other than retinal disease. Patients with inactive end-stage disease, any other macular disease, or history of estrogen, calcium, bisphosphonate, or parathormone use in the 6 months previous to the study were excluded. In addition, patients with any history of medical or surgical/laser treatment were excluded.

The mean ± SD age was 72.0 ± 11.5 years in the E-ARMD group and 68.2 ± 8.9 years in the control group (*P*=0.09). The results of independent t-test shows no significant differences between in the mean BMI of study groups (25.3 ± 3.8 vs. 27.3 ± 5.6, *P*=0.11). BCVA in the E-ARMD group was 1.18 ± 0.36 LogMAR and 0.10 ± 0.07 LogMAR in the control group (*P*=0.001).

In the E-ARMD group 60.0% of the participants were female, and in the control group 57.8% were female. The mean ± SD of FBS was 88.1 ± 31.0 mg/dl in the E-ARMD group and 85.2 ± 14.90 mg/dl in the control group (p=0.58). The mean ± SD for LDL-C and HDL-C was 131.6 ± 40.2 mg/dl and 43.1 ± 5.0 mg/dl, respectively, in the E-ARMD group. In the control group, the mean ± SD for LDL-C and HDL-C was 105.1 ± 35.3 mg/dl and 44.2 ± 6.3 mg/dl, respectively. TC and TG were 204.0 ± 39.5 mg/dl and 144.5 ± 63.8 mg/dl, respectively in the E-ARMD group. In the control group the mean ± SD of TC and TG was 181.1 ± 36.3 mg/dl and 153.7 ± 49.5 mg/dl, respectively. Serum level of RANK-ligand was 101.33 ± 96.83 pmol/l in the E-ARMD group and 92.89 ± 58.37 pmol/l in the control group. OPG concentration was 132.10 ± 75.49 pg/ml in the E-ARMD group and 94.88 ± 61.65 pg/ml in the control group.

No significant difference in serum RANK-ligand between the two groups was found (*P*=0.79). However, the patients affected by macular degeneration had significantly higher OPG levels compared to the control group (*P*=0.012). In addition, the E-ARMD patients had significantly higher serum TC (*P*=0.005) and LDL-C (*P*=0.001) levels. There was no significant difference in serum TG (*P*=0.45), HDL-C (*P*=0.34), or FBS (*P*=0.58) between the two groups (Table 1).

The distribution of OPG and RANK-ligand levels among the E-ARMD groups and control is shown in figure 1 and figure 2. We observed a correlation between OPG levels and RANK-ligand levels in our control group (*P*=0.02, r= -0.35).

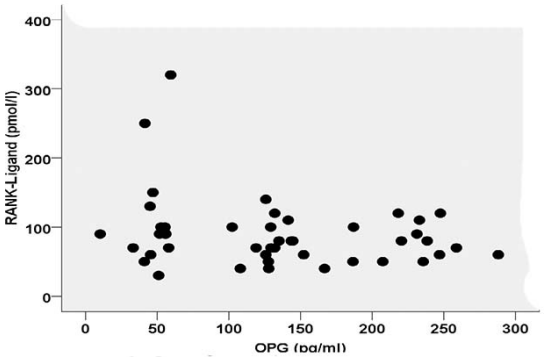

Figure 1. Correlation between the soluble receptor activator of nuclear factor kappa B ligand (RANK-ligand) and osteoprotegrin (OPG) serum level in E-ARMD (*P*=0.11, r=-0.24)

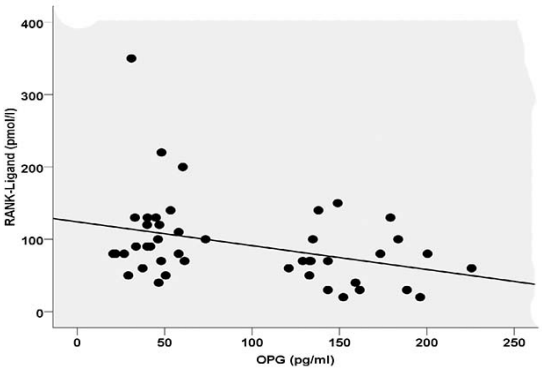

Figure 2. Correlation between the soluble receptor activator of nuclear factor kappa B ligand (RANK-ligand) and osteoprotegrin (OPG) serum level in controls (*P*=0.02, r=-0.35)

Discussion

The exact cause of ARMD remains unknown, although multiple factors have been implicated in the pathogenesis of ARMD. Smoking, atherosclerosis, increased fibrinogen, and low antioxidant levels, which may lead to increased oxidative stress, have been shown to be associated with ARMD (21-23). We found

significant high levels of serum total cholesterol and LDL-C in E-ARMD group. The data are in agreement with previous studies. Hogg *et al*. showed that neovascular AMD is associated with high serum cholesterol and significant high levels of serum total cholesterol and LDL-C in patients compared to controls were shown by Nowak *et al.* (24,25).

Based on review of literature shows the present study is the first investigation of OPG and RANK-ligand association with E-ARMD. The major finding of our investigation is that OPG is higher in patients affected by E-ARMD.

The similarities of pathogenicity and risk factors of E-ARMD and CAD have been described in numerous previous studies. OPG, a secreted glycoprotein, is a member of the TNF receptor super family, originally characterized as an osteoclast suppressor. This glycoprotein acts by binding to RANK-ligand (26). Another important ability of OPG is cell survival stimulation by acting as a receptor for TNF-related apoptosis-inducing ligand (TRAIL), which is also a member of the TNF receptor super family. This ligand is able to induce cell apoptosis; OPG is a soluble agent that binds TRAIL and prevents cellular apoptosis (27). Some studies have shown that OPG is elevated in patients affected by coronary artery disease (12). Moreover; patients with increased OPG levels have a higher risk of cardiovascular disease (28).

OPG expression has been studied by investigators and it has been suggested that it is expressed especially in the vascular endothelium and immune system. It has been shown that OPG may be able to prevent the process of vascular calcification and atherosclerosis and lower levels of OPG is shown in calcifying vascular smooth muscle cells (29). We suppose that high levels of serum OPG suggest a defect in OPG and related biomolecules and its normal action. However OPG expression in normal cellular biology is preventive of vascular damage and promotes survival of endothelial cells (30,31).

A population-based study of 915 individuals by Kiechl *et al*. suggested that high serum OPG level is an independent risk factor for cardiovascular disease (32). Rhee *et al*. assessed the association of OPG serum levels with C-reactive protein and acute phase reactants (33). Keeping in mind all of the above; we have strong evidence implicating the role of OPG as a pro-inflammatory molecule, especially in context of macrovascular and microvascular disease.

It has been showed that the OPG/RANKL/RANK system has an important role also for vascular calcification and inflammatory diseases by cytokine misbalancing (34). In spite the inflammatory role of OPG, Conflicting results have been demonstrated regarding prevention or the induction role of OPG for arterial calcification (35). Although OPG could primarily prevent arterial calcification, its secretion secondary to inflammatory processes could mediate an arterial calcification (36). It seems the later effect is due to expression and up regulation of endothelial OPG, which belongs to the TNF-a super-family. Evidence also suggests that OPG may act as a pro-inflammatory molecule and inducer of vascular calcification and atherosclerosis (37).

The concept of anti-TNF role in ARMD is proposed by some authors. However the results are not directly relevant, a matter that one may bear in mind is the present data may support the idea of anti-TNF therapy in ARMD patients.

Until now, there have been no studies on OPG levels in ARMD, and there is little data on the role of OPG in retinal disease. Regarding the similarity of mechanisms of cardiovascular diseases and ARMD, and regarding clinical data, our findings are in agreement with previously described pathophysiology and confirm the role of inflammatory regulators in ARMD.

The present study had some limitations; certainly, a larger prospective study would be needed for accurate judgment, so this might be considered as the first, preliminary study. Whether high concentration of OPG or RANK-ligand is a risk factor of ARMD or a risk factor of choroidal neovascularization is a remaining challenge for future studies. In addition our patients had high levels of LDL-C and TC. Although high LDL-C and TC is a normal finding in E-ARMD patients and the data are in accordance to previous studies, future studies would elucidate the question whether the E-ARMD patients with normal levels of LDL-C and TC have high OPG levels.

In conclusion, the present study suggests that OPG levels are higher in patients affected by E-ARMD, and high OPG levels may represent a risk factor for ARMD.

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