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Age and gender predict OPG level and OPG/sRANKL ratio in maintenance hemodialysis patients

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ABSTRACT

Purpose: Cardiovascular disease (CVD) is a major cause of death among chronic hemodialysis (HD) patients. Gender and age belong to its classical risk factors. OPG/RANK/sRANKL (Osteoprotegerin/ Receptor Activator of Nuclear Factor κB/ soluble Receptor Activator of Nuclear Factor κB Ligand) axis constitute a system connecting bone and vascular remodeling.
Methods: We aimed to evaluate the plasma levels of OPG, sRANKL and OPG/sRANKL ratio in 21 HD patients and 16 healthy volunteers in relation to gender, age and the other clinical parameters.
Results: OPG and OPG/sRANKL ratio were significantly higher in HD patients than in controls whereas sRANKL was similar in both groups. Adjusted for gender, in controls OPG were higher in women whereas sRANKL did not differ between men and women. In HD group OPG and sRANKL were higher in women whereas OPG/sRANKL ratio was similar in both genders. Female patients compared to healthy women revealed 56% higher OPG concentration and 54% higher OPG/sRANKL ratio. Comparison of male patients and controls revealed 61% higher level of OPG and 75% higher OPG/sRANKL ratio in HD group. Interestingly, OPG and OPG/sRANKL ratio positively correlated with age only in male patients. Contrary, the association between OPG/sRANKL ratio and age was negative in HD women.
Conclusion: Higher OPG levels in HD women comparing to age matched HD men indicate the necessity of more careful screening towards the presence of CVD and bone-mineral disorders. The negative association between age and OPG/sRANKL ratio in HD women warrant in-depth study for thorough understanding of this complex interrelationship.

Key words: Osteoprotegerin, RANKL, hemodialysis, age, gender

INTRODUCTION

Cardiovascular diseases (CVD) are a major cause of death among end stage renal disease patients (ESRD) [1-4]. Gender and age belong to its classical risk factors.

OPG/RANK/RANKL (Osteoprotegerin/Receptor Activator of Nuclear Factor κB/ Receptor Activator of Nuclear Factor κB Ligand) axis compose a system connecting bone and vascular remodeling [5]. RANKL acts through its receptor RANK. After binding, RANKL induces intracellular cascade, which in consequence affects differentiation, function and survival of many cells, osteoclasts' precursors and mature osteoclasts among others. OPG, expressed in varied cells of many organs (osteoblasts, kidney, liver, spleen, bone marrow), acts as a decoy receptor which binds RANKL and therefore prevents its interaction with RANK [6].

Animal studies proved that OPG protects large vessels from medial calcification [7] and also limits calcification of atherosclerotic plaques [8]. Interestingly, human studies showed positive correlation between high serum OPG levels and the presence of cardiovascular disease [5, 9-13]. It remains uncertain whether OPG is just a marker of endothelial damage, mediates progression of vascular disease or it is a kind of protective mechanism aimed to limit vascular

injury. On the other hand, RANKL is assumed to stimulate osteogenic differentiation and calcification of vascular smooth muscle cells [9]. Because OPG directly counteract all RANKL mediated actions through RANK, imbalances in the OPG/RANKL ratio may underlie the pathology of many disorders. OPG/RANKL ratio, being an important determinant of bone mass and skeleton integrity, may also be a determinant of vascular stability.

We aim to evaluate age and sex influence on plasma OPG and soluble RANKL concentrations in the maintenance hemodialysis (HD) patients with reference to healthy subjects.

MATERIAL AND METHODS

Twenty-one chronically HD patients (10 women and 11 men) and 16 age matched healthy volunteers (10 women and 6 men) were included in the study. All examined women, regardless of the presence of kidney disease, were postmenopausal (>50 yrs.). The cause of ESRD was: glomerulonephritis (n=6), interstitial nephritis (n=5), hypertensive nephropathy (n=1), diabetic nephropathy (n=3), secondary amyloidosis (n=1), adult polycystic kidney disease (n=1), acute kidney injury (n=1) and unknown (n=3). None of these patients received immunosuppressive treatment (including steroids) or any other hormone replacement therapy. Infection with HIV 1/2, HBV and HCV was excluded. None of these patients had an evidence of liver injury. Four patients had atrial fibrillation. All patients received erythropoietin and hypotensive therapy. Eleven (52.4%) patients had previously diagnosed ischemic heart disease (IHD) class I-II according to Canadian Cardiovascular Society classification, based on one or more of typical clinical symptoms with prior ECG changes or positive exercise ECG test or past cardiac infarct, alone or in combination. There was no history of any neoplasmatic disease prior and during this study. Clinical and laboratory characteristics of the patients are presented in *Tab. 1.*

Patients were undergoing maintained hemodialysis for median period of 41.5 months (range 4 – 111). They were dialyzed for 4–5 h three times per week using the double-needle technique, native arteriovenous fistulas, standard bicarbonate buffer (bicarbonate + glucose in patients with diabetes), low-flux modified cellulose dialyzers, machines with controlled ultrafiltration and enoxaparin as an anticoagulation.

Fasting blood was taken from the patients and controls without the use of a tourniquet through a wide-gauge butterfly needle into monovettes containing 1.2 ml of EDTA (ethylenediaminotetraacetic acid). In ESRD patients blood was collected just before a midweek, morning HD session.

The first 1 ml of blood was discarded; then 4.5 ml of blood was collected. Tubes were centrifuged 15 min. later at 2000g for 30 min. One-third of the plasma was collected from middle region of the supernatant, aliquoted, and immediately frozen at -80°C.

Table 1. **Clinical and laboratory characteristics of the patients.**

Patients (n)	21
Male/Female (n;%)	11/10 (52/48)
Age (years) Male Female	 68.2 (44-82) 70.0 (57-78)
White blood cells (x10³/μl)	5.67 (3.5-10.1)
Red blood cells (x10⁶/μl)	3.43 (2.99-4.56)
Hematocrit (%)	33.3 (29.6-41.7)
Hemoglobin (g/dl)	10.6 (9-13.2)
Platelets (x10³/μl)	185 (104-345)
Total cholesterol (mg/dl)	185.8 (109-276)
HDL-cholesterol (mg/dl)	49.3 (29-75)
LDL-cholesterol (mg/dl)	109.2 (52-174)
Triglycerides (mg/dl)	136.2 (63-309)
Parathormon (ng/ml)	432.4 (64.3-1578)
Albumin (g/dl)	3.89 (3.0-4.2)
Erythropoietin (IU/kg/week)	62.4(0-190.5)
Hemodialysis duration (months)	41.5 (4-111)
Calcium (mmol/l)	2.13 (1.61-2.6)
Phosphate (mg/dl)	6.08 (2.95-9.8)
Alkaline phosphatase (U/L)	116 (26-249)
Clexane dose/kg per HD (mg/kg)	0.66 (0.27-0.86)

Plasma OPG and sRANKL levels were determined using ELISA kits purchased from BioVendor (Human Osteoprotegerin Elisa and Human sRANKL (Total) Elisa, respectively).

The study was performed in conformity with the Helsinki Declaration. Ethics committee approval was obtained from the local ethics committee and informed, written consent was received from each patient.

STATISTICAL ANALYSIS

The statistical analysis was performed using Statistica software (version 9.0 PL, StatSoft, Tulsa, OK, USA). Shapiro–Wilk's *W* test of normality was used for data distribution analysis. The normally distributed data were presented as means ± SD (standard deviation), and the skewed data as median (full range). Normally distributed data were tested using Student t-test for dependent and independent data. Skewed data were tested using Mann-Witney's. Bivariate correlations were assessed using Pearson's or nonparametric Spearman's regression analysis. P-values of < 0.05 were considered statistically significant.

RESULTS

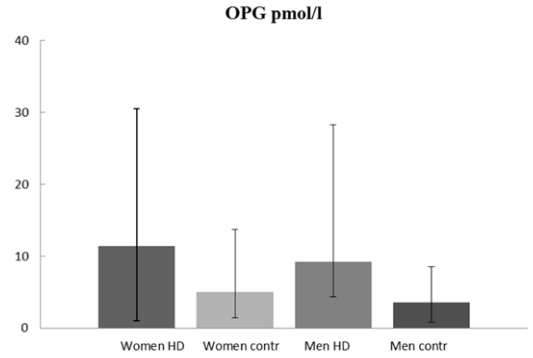
Generally, plasma OPG concentration and OPG/sRANKL ratio were significantly higher in HD patients than controls ($p<10^{-5}$) whereas sRANKL was similar in both groups (*Tab. 2*).

Adjusted to gender, in controls, OPG were higher in women (p=0.03) (*Fig. 1*), whereas sRANKL did not differ between men and women (*Fig. 2*). In HD group OPG and sRANKL were higher in women (p=0.04, p=0.02 respectively) (*Fig. 1* and *Fig. 2*), whereas OPG/sRANKL ratio was similar in both genders (*Fig. 3*).

Female patients compared to healthy women revealed 56% higher OPG concentration ($p<10^{-4}$), (*Fig. 1*) and 54% higher OPG/sRANKL ratio (p=0.004) (*Fig. 3*). Comparison of male patients and controls revealed 61% higher level of OPG (p=0.001), (*Fig. 1*) and 75% higher OPG/sRANKL ratio (p=0.003), (*Fig. 3*) in HD group.

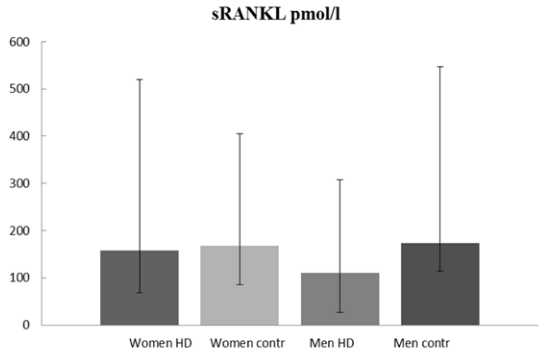
There was no statistically significant correlation between OPG and age in women (neither controls nor HD). Interestingly, OPG and OPG/sRANKL ratio positively correlated with age in male HD patients (p=0.001 R=0.836 and p=0.011 R=0.727, respectively). Contrary, the association between OPG/sRANKL ratio and age was negative in HD women (p=0.046 R=-0.640). Significant negative correlation was observed also between OPG and cholesterol levels in HD patients (data not shown). We did not find any correlations between OPG, sRANK, OPG/sRANKL ratio and another clinical characteristics pointed in *Tab. 1.*

Figure 1. **Osteoprotegerin (OPG) levels in maintenance HD patients and healthy controls adjusted for gender.**



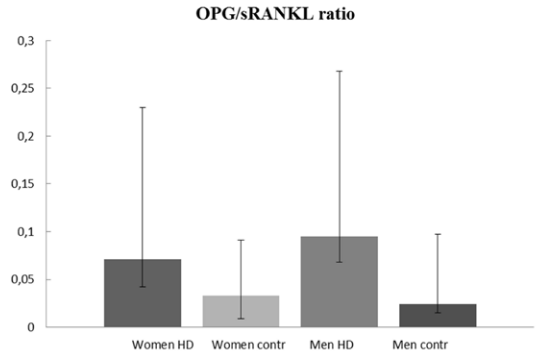
HD – hemodialyzed, contr – control group

Figure 2. **Soluble Receptor Activator of Nuclear Factor κB Ligand (sRANKL) levels in maintenance HD patients and healthy controls adjusted for gender.**



HD – hemodialyzed, contr – control group

Figure 3. **Osteoprotegerin/soluble Receptor Activator of Nuclear Factor κB Ligand (OPG/sRANKL) ratio in maintenance HD patients and healthy controls adjusted for gender.**



HD – hemodialyzed, contr – control group

Table 2. **Plasma levels of OPG, sRANKL and OPG/sRANKL ratio in maintenance hemodialysis (HD) patients and controls (healthy subjects) adjusted for gender.**

	OPG (pmol/l)		sRANKL (pmol/l)		OPG/sRANKL ratio	
	HD	Control	HD	Control	HD	Control
Group	11.1 (4.9–19.1)	4.3 [A] (2.8-8.7)	143.8 (83–361)	170.14 (60.41- 372.8)	0.091 (0.027-0.173)	0.032 [A] (0.009-0.073)
Women	11.43 [B] (10.37-19.1)	5.0 [C] (3.6-8.7)	158.1 [B] (89.7-361.1)	167.8 (82.7-237.3)	0.071 (0.029-0.159)	0.033 [C] (0.024-0.058)
Men	9.2 (4.86-19.1)	3.6 [D] (2.8-4.9)	110 (83.04-197.6)	174.2 (60.4-372.7)	0.095 (0.027-0.173)	0.024 [D] (0.009-0.073)

[A] HD vs. control $p < 10^{-5}$; [B] Women vs. Men $p < 0.05$; [C] Women vs. Women $p \leq 0.004$; [D] Men vs. Men $p \leq 0.003$

DISCUSSION

The novel finding of this study is that age and gender predict plasma OPG levels and OPG/sRANKL ratio in maintenance HD patients.

Osteoprotegerin concentrations are reported to be higher in HD patients [14-19], whereas RANKL level can be higher [18, 20], lower [15, 21] or within normal range [19, 22] comparing to healthy individuals. In pre-dialysis patients, OPG increase progressively with reduction in creatinine clearance, with further increase in dialysis patients [23]. OPG monomer cannot be removed through the polysulfone hemodialysis membrane and, in physiological condition, it seems unlikely to be eliminated through the glomerular membrane because of its molecule size of 60 kDa. However, elevated OPG level returns to normal after kidney transplantation [24]. Therefore it can be assumed that kidneys play a role not only in OPG elimination but also in its metabolism regardless of renal clearance.

Most of previous studies concerning patients with [16, 22] and without [25, 26] chronic kidney disease, pointed at the positive relation between OPG and age. In our maintenance HD patients such correlation was noticed, however it reached statistical significance only in male population. The largest study evaluating relationship between OPG concentration, age and sex in general population was conducted by Khosla et al. [27]. Similarly to our data, they proved strong, positive correlation between age and OPG levels principally in male population. In our study, lack of statistical significance between OPG and age within the whole group may be explained by low diversity of examined patients' age. Another discrepancy in relevance to Khosla et al. report [27] was that postmenopausal women and men older than 50 have comparable OPG concentrations. In our study, all women, either healthy or chronically HD, were postmenopausal and had higher OPG concentrations than men (healthy or HD, respectively). Moreover, woman with ESRD had higher levels of OPG compared to healthy females. In the light of many reports concerning the relation between high OPG concentrations and cardiovascular risk, significance of discovered sex differences, despite similarity of age, may point at the rising cardiovascular risk in older HD women [5,9-13].

Physiologically, OPG production is regulated by many cytokines and hormones, among others – sex steroids. In vitro estrogens increase OPG production [28], whereas androgens have the opposite effect [29]. Nowadays it is obvious, that endogenous sex hormones show no protection against the development of vessel calcification among postmenopausal women with advanced chronic kidney disease [30]. However, there is no data about the direct relationship between OPG and estrogens/androgens levels in maintenance HD patients.

OPG increment may protect bone against its intensive loss in chronically HD patients by its accumulation in uremia and reducing of RANKL level. This mechanism may compensate any perturbations in bone turnover [15]. OPG concentrations are higher in women with a high rate of bone turnover than in those with a low rate and are substantially higher in women with the most severe degrees of osteoporosis [25]. Patient with chronic kidney disease is also at risk for development of osteoporosis. Osteoporosis can occur in both low and high-bone turnover states and bone loss. It begins much earlier in chronically HD patients than in general population. In Baretto et al. [31], OPG/RANKL ratio in osteoporosis group was higher while OPG as well as OPG/RANKL ratio correlated negatively with trabecular bone volume (BV/TV). Increase in OPG concentration, as a protective mechanism, probably limits the bone loss by decreasing bone resorption. It may be also a marker of bone remodeling disturbances. However, we did not find any correlations between plasma OPG or sRANKL levels and clinical markers of bone turnover such as parathormon, calcium, phosphates or alkaline phosphatase concentrations. Interestingly, according to the previous finding, higher OPG levels seem to identify patients with high cardiovascular risk and presence of atherosclerosis [32]. Remembering of the OPG role in vasculature, increase of OPG concentration may take effect in OPG/RANKL ratio disturbances and finally play a role in development of cardiovascular complications in chronic kidney disease.

Kerschan-Schindl et al. [33], reported higher levels of RANKL in men and its decreasing concentrations with age in general population. We did not find any of these relations in HD patients. According to our study, sRANKL concentrations were comparable in both sexes in controls whereas in HD patients unexpectedly women had higher sRANKL levels. Simultaneously, the association between OPG/sRANKL ratio and age in women was negative. This observation, though very interesting, is difficult to discuss now because of the lack of any relevant studies.

CONCLUSSIONS

Higher OPG levels in HD women compared to age matched HD men indicate the necessity of more careful screening towards the presence of CVD and bone-mineral disorders in older female population.

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The authors declare no conflict of interest.

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