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RANKL and OPG Serum Levels in Acute Coronary Syndrome

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Abstract
Background: Numerous inflammatory mediators seem to play a pathogenic role in coronary artery disease, promoting atherogenesis and plaque destabilization, leading to thrombus formation with development of this disease. The effects of the RANKL/OPG system, like modulation of cell survival and inflammation, make it an elect mediator in the progression of atherosclerotic lesions.
Objectives: To estimate the role of RANKL and OPG and their relative ratio (RANKL\OPG) in pathogenesis of acute coronary syndrome.
Subjects and Methods: Sixty patients with acute coronary syndrome were enrolled in this study, those patients were divided into two groups: 31 patients were with unstable angina (UA) and 29 patients were with myocardial infarction (MI), the latter group also subdivided into [18 with ST segment elevation myocardial infarction (STEMI) and 11 with non-ST-segment elevation myocardial infarction (NSTEMI)], their ages range from 25-83 years. Twenty apparently healthy volunteers their ages and sexes were matched with the patients were also participated in the study. ELISA was carried out for estimation the serum levels of RANKL and OPG in the studied groups.
Results: There was no significant difference ($p>0.05$) in median of serum levels of RANKL between patients and control. In addition the comparison among the three groups of patients (NSTEM1, STEM1 and UA) showed no significant differences ($p>0.05$) in RANKL level. Whereas the OPG level was significantly higher ($p<0.001$) in patients than that in healthy control, and there was significant increase in median serum level of OPG in the three patients groups as compared to control. On the other hand, there were no significant differences ($p>0.05$) in median serum levels of OPG among patients groups. The ratio of RANKL / OPG was significantly increase ($P<0.001$) in healthy control as compared to patients. The median of RANKL / OPG ratios in NSTEM1, STEM1 and UA patients groups were significantly lower ($P<0.001$) when compared to the ratio in healthy control. In contrast there were no significant differences in ratio among patients groups ($p>0.05$).
Conclusion: These findings indicated that there was high significant elevation in serum level of OPG in acute coronary syndrome, so, it enforce the clinical utility of OPG in atherosclerosis and suggested that RANKL/OPG ratio could be a biomarker for acute coronary syndrome.
Keywords: Acute coronary syndrome, RANKL, OPG.

1. Introduction

Acute coronary syndrome usually occurs as a result of one of three problems: unstable angina (UA), non–ST-segment elevation myocardial infarction (NSTEMI) or ST-segment elevation myocardial infarction (STEMI). These types are named according to the appearance of the electrocardiogram (Torres and Moayedi, 2007). Since the early 1900s cardiovascular disease (CVD) has been the leading cause of death (Dallas *et al*., 2002). Among men, risk increases with each decade of age and comparisons between men and women demonstrate that for premenopausal women the risk corresponds with that of men approximately 10 years younger (British Heart Foundation and Coronary Heart Disease Statistics, 2004).

Cytokines are released by the host myocardium to modulate tissue repairs and adaptation after injury. The osteoprotegerin (OPG)/ Receptor activator of nuclear factor-kappa B ligand (RANKL)/ Receptor activator of nuclear factor-kappa B (RANK) axis has been implicated in various inflammatory responses and has also been linked to atherogenesis (Sandberg *et al*., 2006). RANKL/RANK/OPG system has an important role in several aspects of the processes leading to calcification (Anand *et al*., 2006). RANKL binds to its membrane receptor RANK and produces several intracellular signals that regulate the fusion, development, function, and survival of the osteoclasts (Abedin *et al*., 2004; Anand *et al*., 2006).

OPG is a soluble member of the tumor necrosis factor receptor superfamily with pleiotropic effects on bone metabolism, endocrine function, and the immune system (Simonet *et al*., 1997). OPG inhibits osteoclastogenesis by binding the receptor activator of nuclear factor *R*k*R*B ligand (RANKL), acting as a decoy receptor to competitively inhibit RANKL interaction with its receptor, RANK (Boyle *et al*., 2003). OPG can be detected in atherosclerotic lesions (Sandberg *et al*., 2006), and in humans elevated circulating OPG concentrations have been associated with aortic plaque (Abedin *et al*., 2007), as well as with increased prevalence and severity of coronary artery disease (Anand *et al*., 2006).Thus, the aim of this study is to provide

insight into the potential role of this RANKL/OPG system in the pathogenesis of ACS.

2. Subjects and Methods

Patients

Sixty Patients presented with chest pain or typical symptoms suggestive for ischemic heart disease (IHD) presented to coronary care unit in Alkadhmiya teaching hospital seeking for medical help were enrolled in this study, their ages ranged from 25 to 83 years. The study was conducted during the period from January 2013 until the midst of May 2013.

The diagnosis of each case was established by clinical examination done by a cardiologist and confirmed by laboratory investigations. All the patients had no complain of other chronic or systemic diseases.

The patients were divided into two groups: myocardial infarction group: they were twenty nine patients classified clinically into STEMI (n=18) and NSTEMI (n=11), depending on the diagnosis of the cardiologist and confirmed by ECG and cardiac enzyme troponin level. The second group was unstable angina patients: they were thirty one patients presented to the emergency department with acute chest pain and ECG changes suggestive of ischemia, and then referred to CCU and thus, diagnosed as cases of unstable angina.

The ethical committee of College of Medicine/Al-Nahrain University approved this study, and all samples were obtained with informed consent in accordance with the Alkadhmiya teaching hospital declaration.

Control

Twenty (20) apparently healthy subjects whose ages and gender were matched with patients group were selected as a control group. All of them received no treatment with No complaint of other chronic or systemic diseases.

Serum samples were separated from the whole blood, a liquated and stored at -20ºC until used. The levels of RANKL and OPG were determined by using commercially available ELISA kits and performed as recommended in leaflet with kits (Human RANKL kit/ CUSABIO/ China; Human OPG kit/ CUSABIO/ China).

Statistical Analysis: Frequency distribution for selected variables was done first. The outcome quantitative variable (RANKL and OPG) was non-normally distributed. Such variable is described by median and interquartile range. The difference in median of a quantitative non-normally distributed variable between 2 groups was assessed by non-parametric test (Mann-Whitney). Among the outcome quantitative variables were normally distributed, and therefore conveniently described by mean, SD, SE and tested for statistical significance by t-test.

3. Results

The basic characteristics of patients and controls groups included in this study are presented in tables (1), no statistically significant differences ($p>0.05$) in age were existed between patients and controls (56.48±1.38 vs. 48.20±5.80). Moreover, there was male's predominance among patients, about (57%) of patients were males, while only (43%) were females, and males/females ratio was 1.3:1. Regarding the family history of disease, 22 (37%) of patients had positive family history of CAD, while 38 (63%) showed negative family history. Furthermore; 22 (37%) of patients were smokers, whereas 38 (63%) of patients were non-smokers. The current data showed that 29 (48%) were positive for troponin enzyme and the remaining patients 31 (52%) were negative.

Table-1- Descriptive characteristics of patients and controls groups

		Control group N= 20	ACS Patients group N= 60	P value
Age	Range	(21-84)	(25-83)	0.179^{NS}
	Mean± SE	48.20±5.80	56.48±1.38 [a NS]	
Gender	Female	8 (40%)	26 (43%)	0.790^{NS}
	Male	12 (60%)	34 (57%)	
	Male / Female	-	1.3:1	
Family history	Positive	-	22(37%)	
	Negative	20(100%)	38(63%)	
Smoking habit	Smokers	-	22(37%)	
	Non smoker	20(100%)	38(63%)	
Serum troponin	Positive	-	29 (48%)	
	Negative	20(100%)	31 (52%)	

a: comparison between ACS and healthy control group; NS= no statistical significant difference ($p>0.05$), SE=Standard Error

The present study showed no significant differences ($p>0.05$) in median serum level of RANKL between patients and controls (31.10 pg/ml vs. 69.25 pg/ml) and nor between patients groups (30.40 pg/ml, 19.10 pg/ml, 42.20 pg/ml) and healthy controls. In addition the comparison among three groups of patients also

showed no significant differences (p>0.05), as shown in table (2). Table (3) observed highly significant difference (p<0.001) in OPG level between patients and controls (122.00 pg/ml vs. 48.00 pg/ml). On the other hand, there was significant increase (p<0.05; p<0.001) in level of OPG in three patients groups (NSTEM1, STEM1 and UA) (122.00, 106.00 and 124.00 pg/ml respectively) as compared to healthy control group (48.00 pg/ml). Furthermore; there was no significant differences (p>0.05) in median serum level of OPG among patients groups.

Table-2- Descriptive statistics of RANKL serum level in study groups.

Study groups	RANKL (pg/ml)		
	Median	**Percentile 25**	**Percentile 75**
Healthy control	69.25	18.65	109.30
ACS patients	31.10 [a NS]	15.90	68.30
NSTEMI patients	30.40 [aNS]	15.90	65.50
STEMI patients	19.10 [aNS,bNS]	13.80	59.30
UA patients	42.20 [aNS, bNS, cNS]	17.30	73.70

a= comparison with control; b= comparison with NSTEMI; c= comparison with STEMI.

Table-3- Descriptive statistics of OPG serum level in the study groups.

Study groups	OPG (pg/ml)		
	Median	**Percentile 25**	**Percentile 75**
Healthy control	48.00	38.00	67.50
ACS patients	122.00 [a**]	87.50	198.00
NSTEMI patients	122.00 [a**]	90.00	201.00
STEMI patients	106.00 [a*,bNS]	63.00	206.00
UA patients	124.00 [a**,bNS,cNS]	98.00	168.00

* = significant difference (p≤0.05); ** = Highly significant difference (p≤0.001).

Determination the ratio of RANKL / OPG in current study revealed significant increase (P<0.001) in the ratio of RANKL / OPG in controls (1.26) as compared to patients (0.30). Also there were significant differences between three groups of patients and healthy controls. The median RANKL / OPG ratios in NSTEM1, STEM1 and UA patients groups (0.27, 0.19 and 0.41) were significantly lower (P<0.001) when compared to the ratio in healthy controls (1.26). Meanwhile there were no significant differences in ratio among patients groups (p>0.05), according to tables (4). However figure (1) showed no significant correlation between RANKL and OPG in patients (P value= 0.533).

Table -4- Descriptive statistics of RANKL /OPG ratio serum level in study groups.

Study groups	RANKL/OPG		
	Median	Percentile 25	Percentile 75
Healthy control	1.26	0.51	2.11
ACS patients	0.30	0.13	0.51
NSTEMI patients	0.27 [a**]	0.14	0.38
STEMI patients	0.19 [a*,bNS]	0.11	0.50
UA patients	0.41 [a**,bNS,cNS]	0.12	0.65

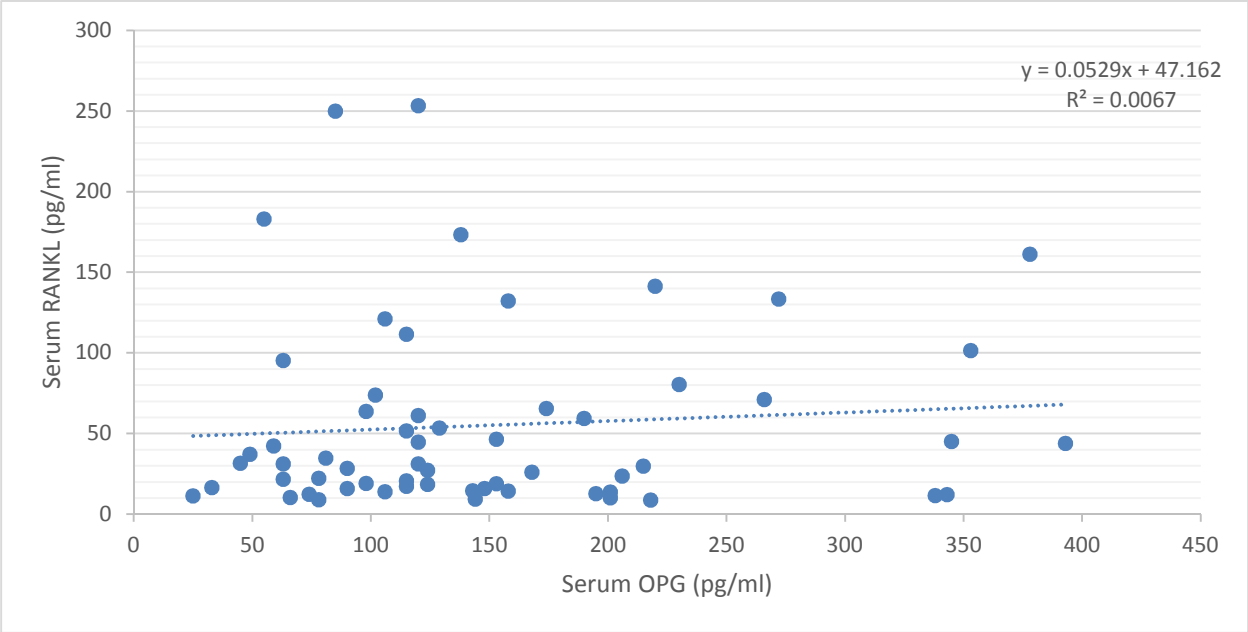

Figure-1- Correlation between RANKL and OPG in ACS

Table-5: Descriptive statistics of RANKL, OPG and RANKL / OPG ratio in ACS according to the seropositivity of troponin

		troponin		p value
		Negative	Positive	
OPG (pg/ml)	Median	126.50	120.00	0.734 NS
	Percentile 25	98.00	81.50	
	Percentile 75	181.50	201.00	
RANKL (pg/ml)	Median	38.40	27.70	0.398 NS
	Percentile 25	16.60	14.85	
	Percentile 75	84.50	52.20	
RANKL/OPG	Median	0.36	0.27	0.546 NS
	Percentile 25	0.12	0.14	
	Percentile 75	0.65	0.38	

4. Discussion

The findings of the present study indicate that the OPG levels in sera of ACS patients were increased compared to controls, whereas the serum RANKL levels were decreased. These results are consistent with other studies (Omland *et al*., 2008; Jono *et al.,* 2010). In this regard, Venuraju and colleagues reported that the OPG production has been demonstrated in many different tissues, including bone (osteoblasts), heart and vasculature (endothelial and vascular smooth muscle cells). In addition to its presence in connective tissues, OPG also circulates in blood at considerably lower concentrations than in tissues (Venuraju *et al*., 2010). In numerous clinical studies, OPG has been consistently reported as a strong independent risk factor and a predictor for the onset and severity of both atherosclerosis and cardiovascular disease (Omland *et al*., 2008).

On the other hand, Shamsara and co-workers observed that OPG concentration also increases in unstable vascular calcifications and other vascular disorders, and they concluded that although OPG can prevent the effects of RANKL by anatomization, may be this increment in OPG serum level, as a protective, is not enough to neutralize the RANKL effects (Shamsara *et al*., 2009). At various with present results Rackley *et al*., stated that the serum concentration of RANKL was in the highest level in acute vascular syndromes such as MI and ischemic cerebral vascular attacks (Rackley *et al.,* 2009). In addition Giaginis and colleagues documented that OPG, but not RANKL levels, were reduced in patients with history of CAD (Giaginis *et al*., 2012).

Circulating OPG levels are increased in patients with ACS (Omland *et al*., 2008), and enhanced expression has been found within symptomatic carotid plaques (Golledge *et al*., 2004). Elevated OPG levels have also been associated with the degree of coronary calcification in the general population as a marker of coronary atherosclerosis (Abedin *et al*., 2007). OPG has been reported to predict survival in patients with heart failure after acute myocardial infarction (Ueland *et al*., 2005), to predict heart failure hospitalization and

mortality in patients with acute coronary syndrome (Omland *et al*., 2008), and to be associated with long-term mortality in patients with ischemic stroke (Jensen *et al*., 2010). There are also a few studies that show a relationship between OPG and CVD and related mortality in the general population (Semb *et al*., 2009).

Moreover, because OPG circulates at much higher levels than RANKL, it may be a more stable overall measure of RANKL/RANK activity than soluble RANKL. Therefore, the role of OPG as a marker in CVD may not be related to its role as a mediator but reflect its role as a stable marker of activity in the RANKL/OPG/RANK axis, as well as the activities in inflammatory pathways that are involved in atherogenesis. Moreover, Golledge *et al* reported OPG to be expressed at higher levels in symptomatic carotid plaques than in asymptomatic carotid plaques, and it is possible that the relation between circulating OPG levels and CVD may, at least in part, reflect shedding from atherosclerotic lesions. (Golledge *et al*., 2004).

The results of present study revealed that there is a significant difference between ACS patients groups (UA, NSTEMI and STEMI) and healthy controls according to RANKL /OPG ratio. There was significant increase in the ratio of RANKL / OPG in healthy controls as compared to ACS patients. Mohammadpour and collegues determined the changes in RANKL and OPG levels concomitantly and reported the results as RANKL/OPG ratio. They reported that there was no significant relation between RANKL serum levels and coronary artery calcification (CAC), but there was significant negative relation between OPG serum and CAC and significant positive relation between RANKL/OPG ratio and CAC. In those patients, the less serum OPG levels are, the more calcification intensity occurred; this indicates the protective effects of OPG in vascular calcification. According to these facts, they concluded that determination of the RANKl/OPG ratio in compare with each of these two factors alone is a better diagnostic indicator for intensity of vascular calcification that leads to coronary disorders such as CAC (Mohammadpour *et al.,* 2012).

Furthermor; Gerber noticed that RANKL/ OPG ratio showed stronger correlation with CAC than either RANKL or OPG concentration alone (Gerber, 2009). An imbalance in the RANKL/ OPG molecular triad system has been suggested to be responsible for the calcification process of atherosclerotic plaques (Van Campenhout *et al*., 2009). In addition the present study showed no significant correlation between RANKL and OPG in ACS, this result disagree with study done by Giaginis *et al.,* who reported that RANKL concentrations showed a strong positive correlation with OPG (Giaginis *et al.,* 2012).

5. Conclusion

These findings indicated that there was high significant elevation in serum level of OPG in acute coronary syndrome patients. In addition the present data enforce the clinical utility of OPG in atherosclerosis and suggested that RANKL/OPG ratio could be a biomarker for acute coronary syndrome.

References

1) Torres, M. & Moayedi, S. (2007). Evaluation of the acutely dyspneic elderly patient. *Clin. Geriatr. Med.*; 23 (2): 307–25.
2) Dallas, T.X. (2002). American Heart Association. *Heart Disease and Stroke Statistics-2003 Update*.
3) British Heart Foundation. Coronary Heart Disease Statistics, (2004): London; British Heart Foundation.
4) Sandberg, W.J., Yndestad, A., Oie, E. *et al*. (2006). Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler Thromb Vasc Biol.;* 26:857–63.
5) Anand, D.V., Lahiri, A., Lim, E., Hopkins, D., & Corder, R. (2006). The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subject. *Journal of the American College of Cardiology*; 47 (9): 1850–1857.
6) Abedin, M., Tintut, Y., & Demer, L.L. (2004). Vascular calcification: mechanisms and clinical ramifications. *Arteriosclerosis, Thrombosis, and Vascular Biology*; 24(7): 1161–1170.
7) Simonet, W.S., Lacey, D.L., Dunstan, C.R. *et al*. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*; 89: 309 –19.
8) Boyle, W.J., Simonet, W.S., Lacey, D.L. (2003). Osteoclast differentiation and activation. *Nature*; 423:337–42.
9) Abedin, M., Omland, T., Ueland, T., Khera, A., Aukrust, P., Murphy, S.A., Jain, T., Gruntmanis, U., McGuire, D.K., & de Lemos, J.A. (2007). Relation of osteoprotegerin to coronary calcium and aortic plaque (from the Dallas Heart Study). *Am J Cardiol*; 99, 513-518.
10) Omland, T., Ueland, T., Jansson, A.M., Persson, A., Karlsson, T., Smith, C., Herlitz, J., Aukrust, P., Hartford, M., Caidahl, K. (2008). Circulating osteoprotegerin levels and long-term prognosis in patients with acute coronary syndromes. *J Am Coll Cardiol*; 51:627– 633.
11) Jono, S., Otsuki, S., Higashikuni, Y. *et al*. (2010). Serum osteoprotegerin levels and long-term prognosis in subjects with stable coronary artery disease. *J Thromb Haemost*; 8: 1170–75.
12) Venuraju, S.M., Yerramasu, A., Corder, R., & Lahiri, A. (2010). Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *Journal of the American College of Cardiology*;

55(19): 2049–2061.
13) Shamsara, J., Ramezani, M., Mohammadpour, A.H. (2009). The RANKL: osteoprotegerin (OPG) ratio as a new biomarker for coronary artery disease. *Iranian Journal of Medical Hypotheses and Ideas*; 3: 1.
14) Rackley, C.E., & Weissman, N.J. (2009). The role of plaque rupture in acute coronary syndromes. *Arteriosclerosis, Thrombosis, and Vascular Biology*; 54:743–759.
15) Giaginis, C.**,** Papadopouli, A., Zira, A.T., Katsargyris, A., Klonaris, C., Theocharis, S. (2012). Correlation of plasma osteoprotegerin (OPG) and receptor activator of the nuclear factor kB ligand (RANKL) levels with clinical risk factors in patients with advanced carotid atherosclerosis. *Med Sci Monit*; 18(10): 597-604.
16) Golledge, J., McCann, M., Mangan, S., Lam, A., Karan, M. (2004). Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. *Stroke*; 35:1636 –1641.
17) Ueland, T., Kjekshus, J., Froland, S.S., Omland, T., Squire, I.B., Gullestad, L., Dickstein, K., Aukrust, P. (2005). Plasma levels of soluble tumor necrosis factor receptor type 1 during the acute phase following complicated myocardial infarction predicts survival in high-risk patients. *J Am Coll Cardiol*; 46: 2018 –2021.
18) Jensen, J.K., Ueland, T., Atar, D., *et al*. (2010). Osteoprotegerin concentrations and prognosis in acute ischaemic stroke. *Journal of Internal Medicine*; 267(4): 410–417.
19) Semb, A.G., Ueland, T., Aukrust, P., Wareham, N.J., Luben, R., Gullestad, L., Kastelein, J.J., Khaw, K.T., Boekholdt, S.M. (2009). Osteoprotegerin and soluble receptor activator of nuclear factor-kappab ligand and risk for coronary events: a nested case-control approach in the prospective epic-norfolk population study 1993–2003. *Arterioscler Thromb Vasc Biol*; 29: 975–980.
20) Mohammadpour, A.H., Shamsara, J., Nazemi S., Ghadirzadeh, S., Shahsava S., & Ramezani, M. (2012): Evaluation of RANKL/OPG Serum Concentration Ratio as a New Biomarker for Coronary Artery Calcification: A Pilot Study. *Thrombosis*; 2012: 4.
21) Gerber, T. C. (2009). Diagnostic and prognostic implications of coronary artery calcification detected by computed tomography. *Calcified Tissue International*; 91: 123–137.
22) Van Campenhout, A., & Golledge, J. (2009). Osteoprotegerin, vascular calcification and atherosclerosis. *Atheroscler;* 204: 321–329.

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