Evaluation of Serum RANKL Level in Acute Coronary Syndrome

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ABSTRACT

Background: Acute coronary syndrome (ACS) is a common disease, and a major determinant of morbidity and mortality in all races. The pleiotropic effects of the receptor activator of nuclear factor-kappa B ligand (RANKL) such as modulation of cell survival, mineralization and inflammation, make it an interesting candidate mediator in the progression and destabilization of atherosclerotic lesions.

Objectives: This study was performed to investigate the role of RANKL in the pathogenesis of ACS.

Methods: The levels of RANKL were measured by ELISA method in sera of 60 ACS patients, 31 patients with unstable angina (UA) and 29 patients with myocardial infarction (MI) in comparison with 20 apparently healthy controls.

Results: Current data indicated that there was a decrease in the median serum level of RANKL but statistically not significant (p>0.05)in ACS patients as compared with a healthy control group.

Conclusion: Current study revealed a low RANKL serum level but statistically not significant in ACS patients in addition there were no significant differences in median serum levels of RANKL in ACS patients according to gender type, family history, smoking habit and troponin enzyme.

Key words: RANKL, Unstable Angina, Myocardial Infarction

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Acute coronary syndrome (ACS) is a broad term encompassing a spectrum of acute myocardial ischemia and injury ranging from unstable angina (UA) and non-ST-segment elevation myocardial infarction (NSTEMI) to ST-segment elevation myocardial infarction (STEMI). ACS accounts for approximately 1.2 million hospital admissions in the United States annually [1].

An excessive inflammatory response to various forms of injurious stimuli to the arterial wall is characteristic for the process of atherosclerosis and plaque destabilization [2, 3], involving various inflammatory mediators including adhesion molecules, chemokines, and cytokines [3, 4].

Increased vasoconstriction, activation of platelets, vessel wall interaction and invasion of monocytes into the subintima as well as vascular smooth muscle cell proliferation, play a fundamental role in the pathogenesis of CAD. The interaction between the vulnerable atherosclerotic plaque and thrombus formation, a process referred to as atherothrombosis, is the cornerstone of ACS [5].Brogan (2003) showed that atherothrombosis playing a pivotal role in the pathophysiology of ACS. Alone or incorporated into plaque, thrombin causes vessel occlusion resulting in ACS. Inhibiting thrombin can improve clinical outcomes of ACS [6].

Receptor Activator of Nuclear factor-kappa B ligand (RANKL) belongs to the TNF superfamily, is expressed in bone, lung, bone marrow and lymphoid tissues, and exists as 3 isoforms: RANKL 1, 2 and 3. These three isoforms of this type II homotrimeric transmembrane protein can differentially regulate osteoclastogenesis and exists as a soluble and a membranous form. The soluble form has low capacity to generate osteoclasts [7].RANKL is also expressed in activated T-lymphocytes, lymph nodes, thymus, mammary glands, lungs, spleen and bone marrow [8].

The RANKL has been shown to regulate bone remodeling [9], and was more recently found to be involved in carcinogenesis as well as central thermoregulation [10,11]. It has also been linked to the development of atherosclerosis and plaque destabilization [12]. The aim of this study is to provide insight into the potential role of RANKL in the pathogenesis of ACS.

Methods. Sixty patients with ACS were enrolled in this study, their ages range from 25 to 83 years, Thirty four (about 57%) of ACS patients were males, while only (N=26, 43%) were females, Males/females ratio was 1.3:1.Patients presented with chest pain or typical symptoms suggestive for ischemic heart disease (IHD) presented to the coronary care unit (CCU) in AL-Kadhmiya teaching hospital seeking for medical help regarding their recently developed symptoms. The study was conducted during the period from January 2013 until the midst of May 2013; Samples were taken during the morning.

Determination of sRANKL serum concentration: Serum levels of soluble RANKL were measured with an Enzyme-Linked Immunosorbent Assay (ELISA). Frequency distribution for selected variables was done first. The outcome quantitative variable RANKL was non-normally distributed. Such variable is described by median and interquartile range. The difference in the median of a quantitative non-normally distributed variable between 2 groups was assessed by a non-parametric test (Mann-Whitney test). Among the outcome, quantitative variables were normally distributed, and therefore conveniently described by mean, standard deviation (SD), standard error (SE) and tested for statistical significance by t-test.

The association between 2 categorical variables was assessed by Chi-square test. The statistical significance, direction and strength of linear correlation between 2 quantitative variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient. P value less than the 0.05 level of significance was considered statistically significant.

Results. This study showed that there is no statistical significant differences (p>0.05) in the median serum level of RANKL between patients groups (30.40, 19.10 and 42.20) and healthy controls (69.25 pg/ml). In addition, the comparison among three groups of patients also showed no significant differences (p>0.05) in median serum level of

Table 1: Descriptive statistics of serum RANKL level in ACS patients and healthy controls.

Studied groups	RANKL (pg/ml)			Statistical significance (p value)
	Median	Percentile 25	Percentile 75	
Control (N=20)	69.25	18.65	109.30	0.149 NS*
Patients (N=60)	31.10	15.90	68.30	

* NS=non significant (p>0.05).

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

Studied groups	RANKL (pg/ml)		
	Median	Percentile 25	Percentile 75
Control (N=20)	69.25	18.65	109.30
NSTEMI (N=11)	30.40[aNS]	15.90	65.50
STEMI (N=18)	19.10[aNS,bNS]	13.80	59.30
UA (N=31)	2.20[aNS,bNS,cNS]	17.30	73.70

a. Comparison with control.
b. Comparison with NSTEMI.
c. Comparison with STEMI.
NS=not significant (p>0.05).

Table 3: Descriptive statistics of RANKL in ACS according to gender type.

		Gender type		p value
		Female N=26	Male N=34	
RANKL (pg/ml)	Median	32.85	29.70	0.8 NS
	Percentile 25	14.50	16.50	
	Percentile 75	73.70	63.70	

NS=Non Significant (p>0.05).

Table 4: Descriptive statistics of RANKL in ACS according to family history.

		Family history		p value
		No N=38 (63%)	Yes N=22 (37%)	
RANKL (pg/ml)	Median	39.65	26.50	0.326 NS
	Percentile 25	14.50	17.30	
	Percentile 75	101.30	59.30	

NS=non significant (p>0.05).

Table 5: Descriptive statistics of RANKL in ACS according to smoking habit.

		Smoking		p value
		Yes N=22 (37%)	No N=38 (63%)	
RANKL (pg/ml)	Median	32.85	29.70	.963 NS
	Percentile 25	14.50	16.50	
	Percentile 75	73.70	53.40	

NS=non significant (p>0.05).

Table 6: Descriptive statistics of RANKL in ACS according to the seropositivity of troponin

		Troponin		p value
		Negative N=31 (52%)	Positive N=29 (48%)	
RANKL (pg/ml)	Median	38.40	27.70	0.398 NS
	Percentile 25	16.60	14.85	
	Percentile 75	84.50	52.20	

NS=non significant (p>0.05).

RANKL as shown in tables 1 and 2. Statistical analysis indicated that there were no significant differences (p>0.05) in median serum levels of RANKL in ACS patients regarding gender type, family history and the smoking habit, as illustrated in tables 3,4 and 5.

Regarding the differences in median serum levels of RANKL in ACS patients according to troponin enzymes , they current study showed that there were no significant differences (p> 0.005) in median serum level of RANKL in ACS patients positive for troponin and those patients were negative to troponin , as shown in table 6.

Discussion. The cause of an ACS is the rupture of an atherosclerotic plaque with subsequent platelet adherence, activation, aggregation, and activation of the clotting cascade[13]. RANKL/RANK/OPG system has an important role in several aspects of the processes leading to calcification. RANKL binds to its membrane receptor RANK and produces several intracellular signals that regulate the fusion, development, function, and survival of the osteoclasts[14].

The findings of the present study indicate that the serum RANKL levels in sera of ACS patients were decreased compared to controls. These results are consistent with other studies [15, 16].

The present study showed that there were no significant differences in median serum levels of RANKLin ACS patients regarding gender type, family history and the smoking. Correspondingly, Schett and associates observed that RANKL levels were not related to age, sex and smoking[17].

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