Relationship between serums Osteoprotegrin / sRANKL ratio rand head trauma intensity

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Abstract: Introduction: The main lesions of trauma occurred secondary to injury. Immediately after trauma, a series of chemical and biochemical reactions started and the final products of this reaction had destructive effects in tissue. The aim of this study was to evaluate the Osteoprotegrin / sRANKL in patients with severe traumatic brain injury. **Materials and Methods:** In a case-control study that performed in the Department of Neurosurgery, Tabriz University of Medical Sciences on patients with head trauma, and the Osteoprotegrin / sRANKL level evaluated with intensity of trauma in patients with brain injury. **Results and conclusions:** In this study, we studied 84 patients with head trauma and 40 patients without head trauma as in the control group. Mean age of patients in case group was 39.13 ± 5.77 years and the mean age of patients in the control group was 38.15 ± 5.77 years (P=0.377). Mean of RANKL levels in the case group 146.92 ± 52.23 and mean RANKL levels in the control group was 98.63 ± 29.85(P<0.001). Mean of Osteoprotegrin levels of case group was 199.81 ± 63.96and in the control group was 152.46 ± 41.80(P=0.001. Mean of RANKL and Osteoprotegrin levels were significantly higher in patients with head trauma. Mean of RANKL and Osteoprotegrin levels in patients with severe trauma was significantly higher than other traumatic patients. A significant positive linear correlation was found between levels of Osteoprotegrin and RANKL in the studied patient's. A significant indirect linear correlation was found between RANKL and Osteoprotegrin with GCS of patient's with brain injury.
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1. Introduction

In the central nervous system trauma, neuronal damage occurs in both primary and secondary forms. Primary damage occurs due to the injuries to the lining of nerve cells immediately after traumatic injuries due to tensile and compressive forces; and this occurs in a small percentage of neurons. The main lesions occur due to the secondary damages after trauma. Immediately after injury, a series of chemical and biochemical reactions get started at the site of injury, the final products of which have destructive effect on the tissue (Kaptanoglu, 2000; Fujimoto, 2000; Sarrafzadeh, 2000). These reactions begin with the proliferation of neutrophil the site of injury. Release of free radicals from neutrophil is associated with damage to other biomolecules, and especially leads to start of lipid per oxidation in the cell wall that leads to increased fatty acids and free radicals as the final products of this reaction (Fujimoto, 2000). Regarding the sum of the evidences obtained from different studies, it can be concluded that the assessment of novel biomarkers in evaluation of recovery-treatment development and the Trauma-induced secondary effects is inevitable.

One of the relatively new markers recently used in newer studies is the assessment of changes. TNF is one of the super families of Osteoprotegrin receptor. Osteoprotegrin (OPG) and RANKL are cytokines regulating osteoclastogenesis. Both agents are classified into the superfamily of TNF and TNF receptor. RANKL binds on receptors on the surface of preosteoclasts and stimulates their differentiation into active osteoclasts, thus resulting in osteoresorption. It probably acts synergically with TNF-alpha (TNF acts via TNF-1 receptor and leads to a massive osteoclastogenesis after RANKL effect)(Zhang, 2001).

The aim of this study was to evaluate the relationship of Osteoprotegrin/ sRANKL with the intensity of damage in brain traumatic patients and comparing it with non-traumatic patients.

2. Material and Methods

In a case-control study at the Department of Neurosurgery, Tabriz University of Medical Sciences on traumatic patients, the relationship between Osteoprotegrin/ sRANKL and the intensity of injury in patients with brain trauma was evaluated.

Case group included the patients referred to Imam Reza Hospital of Tabriz who were enrolled into the study after diagnosis of trauma and grading it. The cases with GCS≤8 were considered as severe, 8<GCS<13 as moderate and GCS≥13 as mild trauma.

All patients were treated with the common corticosteroids and anti-seizure drugs. Thus, none of the patients will lose the routine treatments. In order to prevent the interventional effects of age, all the patients in this study were selected within the age range of 20-50 years. The control group, including healthy people without any traumatic effects and symptoms, were selected and evaluated.

To avoid the effects of nutritional and medical diets, all patients in either groups of case and control in this study were to use a similar and conventional diet; and no sudden change was allowed in their diet and medication.

Patients with multiple trauma or pathologies such as disorders or a history of systemic hereditary diseases, previous renal, cardiovascular and hepatic surgery according to the patients' medical records or interview when necessary and routine tests if needed, were excluded from the study.

The antibody part of sRANKL and Osteoprotegrin were measured by ELISA method.

Regarding primary and secondary pilot studies, previously conducted studies, and according to the formula for calculating the sample volume, the number of patients in the case group was estimated to be 60. In our study, 28 patients were selected in each group of the traumatic patients and were included in the study. 40 people were also selected as the control group with normal GCS and enrolled into the study.

Lipid profiles of patients and control subjects were determined and patients with very low HDL-C level and very high levels of cholesterol and triglycerides were excluded.

The first sampling was performed immediately after admission to EMS and before any medical intervention to assess the levels of sRANKL and Osteoprotegrin.

Subsequent samplings were obtained six days and two months after the trauma after 12 hours of fasting in the morning. The taken blood samples were centrifuged and separated to obtain the serum. The experiments were attempted to be performed immediately after C -70 sampling. Otherwise, the samples were stored in a freezer until experimentation.

3. Results

In this study, examining 84 traumatic patients, measuring their RANKL and Osteoprotegrin levels and evaluating their relationship with the intensity of trauma, the following results were obtained:

Mean age of the patients was 39.13±5.74 in the case group and 38.15±5.77 in the control group. There was no significant difference in the mean age of the two groups (p=0.377). Mean RANKL level was 146.92±52.23 in the patients of the case group and 98.63±29.85 in the patients of control group.

Mean Osteoprotegrin level was 199.81±63.96 in the patients of case group and 152.46±41.80 in the patients of control group. Mean Osteoprotegrin and RANKL levels in the patients of case group were significantly higher than in the control group (p=0.001). Mean GCS value was 10.47±2.93 in the traumatic patients (case group) and all patients in the control group had a GCS value of 15.

There was no linear relationship between the patients' age and RANKL levels (p=0.198 and R=0.116) and their Osteoprotegrin levels (p=0.327 and R=0.089) in the case group patients.

Table 1. Lipid profile of patients between groups

	Groups				P
	Mild	Moderate	Sever	Control	
TG	122.36 ± 41.69	131.75 ± 44.06	141.32 ± 45.64	130.90 ± 43.28	0.451
LDL	128.43 ± 13.61	131.39 ± 15.45	132.00 ± 14.62	131.18 ± 14.37	0.798
Choll	111.05 ± 31.68	112.23 ± 31.62	109.46 ± 22.06	112.44 ± 29.37	0.977
HDL	43.19 ± 8.73	41.74 ± 8.59	41.86 ± 9.05	41.93 ± 8.17	0.913

There was a significant positive linear relationship between patient's RANKL level and their Osteoprotegrin level in the patients under study (p<0.001 and R=0.318).

There was a significant inverse linear relationship between patient's RANKL level and their GCS value in the patients under study (p<0.001 and R=0.478).

There was a significant inverse linear relationship between patient's Osteoprotegrin level and their GCS value in the patients under study (p<0.001 and R=0.401).

4. Discussions

Nowadays, so much attention is paid to Osteoprotegrin, its ligand and their roles in bone metabolism. Bone growth and remodeling is a

dynamic process which occurs as a result of the balance created by multicore osteoclast cells between bone matrix synthesis by osteoblasts and their reabsorption (Kong, 1999; Takahashi, 1999).

Besides its crucial role in development and activation of osteoclasts, nowadays, OPGL has been identified as an important costimulation molecule involved in T cell–dendritic cell communication and in dendritic cell survival (Anderson, 1997; Bachmann, 1999).

RANK is the downstream signaling receptor for OPGL. Osteoprotegrin is a soluble decoy receptor for OPGL, neutralizing its ability to bind with RANK and induce a signal. Due to its expression on activated T cells and its role in inflammation and bone loss, OPGL has generated much interest in autoimmune disease research, in particular in inflammatory arthritis.

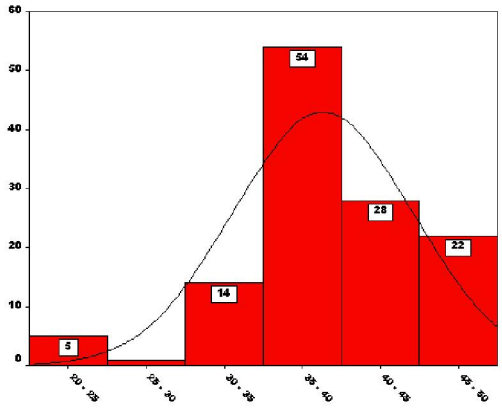

Chart 1. Age distribution of patients

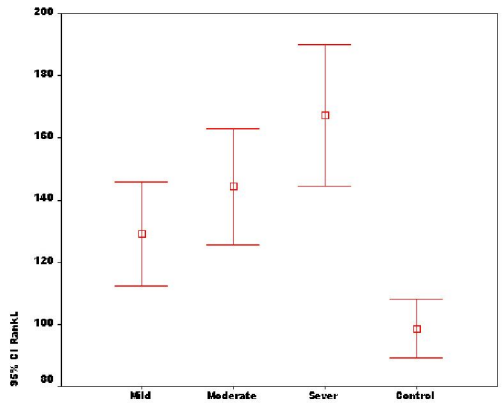

Chart 2. Distribution of RANKL level of patients with head trauma and control group

In patients with inflammatory arthritis, OPGL has been also emerged to be an important factor in the regulation of bone dissolution. Synovial tissue in patients with rheumatoid arthritis and osteoarthritis has been shown to have a high level of OPGL exposition. Similarly, patients with spondyloarthropathies have been also found to have a high level of OPGL exposition in damaged synovial (Haynes, 2003).

Hereunto, several clinical studies have been conducted and the results obtained are somehow incoherent. These differences may be due to the use of different methods for measuring the Osteoprotegrin probands. Moreover, Osteoprotegrin /RANKL ratio is determining for clinical interpretations. Yet, there is no unique method and instrument available for measuring RANKL. Bone remodeling parameters indicate a specific relationship with Osteoprotegrin. For example, Osteoprotegrin and osteocalcin, deoxipiridonolin and Osteoprotegrin, and Osteoprotegrin and C terminal collagen I propeptide (Seidel, 2001).

On the other side, other studies show no further relationship between Osteoprotegrin and bone markers (Szulc, 2001). Some authors have shown a shortly negative relationship between Osteoprotegrin and calcium density and a shortly positive relationship between Osteoprotegrin and PTH (Browner, 2001). Osteoprotegrin density seems to have a relationship with age in human which increases as age goes up. Also, a subtle relationship has been observed between Testosterone and Estradiols. Probably, Osteoprotegrin has increased in above mentioned cases; however, Osteoprotegrin /RANKL ratio has initially reduced, and then increased (Szulc, 2001).

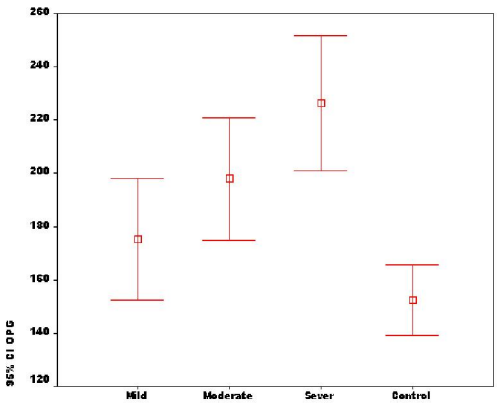

Chart 3. Distribution of Osteoprotegrin level of patients with head trauma and control group

Only some studies have evaluated and approved the relationship between Osteoprotegrin and bone density in human; the other studies have not approved this relationship (Szulc, 2001; Browner, 2001; Yano, 2001). No clear study has approved the relationship between Osteoprotegrin and incidence of osteoporosis (only one study by Ad Hoe has investigated the relationship between the rate bone fractures and Osteoprotegrin in women.) (Browner, 2001).

Osteoprotegrin is a protein that attaches to the ligand which activates the RANK nuclear receptor available on osteoclasts and prevents RANKL from attaching to the corresponding receptor and causes a change in cellular activities of osteoclasts and consequently, prevents them from destructing bones (Duarte, 2007).

Generally, normal metabolic activity of bone and stability of bone mass depends on the balance between RANKL and Osteoprotegrin. RANKL and Osteoprotegrin have complicated signals which require several factors to work interactively (Trouvin, 2010).

Catabolic activity of RANKL is controlled by Osteoprotegrin; so that Osteoprotegrin, binding to the receptor of RANKL, i.e. RANK, prevents the activation of RANKL and thereby, causes increased bone mass (Cheng, 2004).

In our study, the mean levels of RANKL and Osteoprotegrin were significantly higher in patients with brain trauma. Mean levels of RANKL and Osteoprotegrin in patients with severe trauma were significantly higher than in other traumatic patients. There was a significant positive linear relationship between the level of RANKL and the level of Osteoprotegrin in patients under study. There was a significant inverse linear relationship between the levels of RANKL and Osteoprotegrin of patients with brain trauma and their GCS rate.

In the patients of the control group instead, there was no significant linear relationship between the level of RANKL and the level of Osteoprotegrin in patients under study; which reflects the role of these two markers in patients with brain trauma.

Conclusions

Mean levels of RANKL and Osteoprotegrin were significantly higher in patients with brain trauma. Mean levels of RANKL and Osteoprotegrin in patients with severe trauma were significantly higher than in other traumatic patients. There was a significant positive linear relationship between the level of RANKL and the level of Osteoprotegrin in patients under study. There was a significant inverse linear relationship between the levels of RANKL and Osteoprotegrin of patients with brain trauma and their GCS rate.

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