SERUM LEVELS OF SOLUBLE RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA-B LIGAND (S-RANKL) IN PATIENTS WITH DIFUSE IDIOPATHIC SKELETAL HYPEROSTOSIS AND ANKILOSING SPONDILITIS

Mariela Geneva-Popova
Medical University, Plovdiv, University Clinic of Rheumatology, UMHAT “Sveti Georgi”, Plovdiv, Bulgaria, genevapopova@yahoo.com

Stanislava Popova
Medical University, Plovdiv, University Clinic of Rheumatology, UMHAT “Kaspela”, Plovdiv, Bulgaria, spopova92@abv.com

Abstract: Background: S-RANKL (Receptor activator of nuclear factor Kappa-B ligand) is a member of the TNF/TNF receptor superfamily. RANKL has been identified to control bone regeneration and remodeling. S-RANKL is secreted by osteoblasts and binds to the RANK receptor on osteoclast precursor and mature osteoclast cells. Variation in concentration levels of S-RANKL throughout several organs reconfirms the importance of RANKL in bone growth. S-RANKL is able to stimulate osteoclast formation and activity.

Methods: S-RANKL is estimated on 55 patients with Diffuse Idiopathic Skeletal Hyperostosis (DISH), 25 patients with Ankylosing Spondilitis (AS), 50 patients with spondylosis and 15 particularly healthy people aged 55-65. All patients were treated and monitored in University Clinic of Rheumatology, UMHAT “Sveti Georgi”, Plovdiv, Bulgaria. The measuring of the S-RANKL is done by ELISA-method with reader (Sitio-Microplate reader, Seac, Italy), 450 nm, with a kits of eBioscience, Austria. The statistic processing is done with SPSS 23 programme (p<0.01).

Results: The average measurements of S-RANKL of patients with DISH were 197.00±35.90 pg/ml, in patients with AS 190.86±18.54 pg/ml. S-RANKL in patients with spondylosis were 20,60±66,16 pg/ml, and in old control group were 23,33±15,07 pg/ml. The average measurements of s-RANKL in patients with DISH and AS were significant higher in comparison with the results of patients with spondylodyis and healthy people, regardless of their age (p<0.05).

Conclusion: S-RANKL is significantly increased in patients with DISH and spondylosis in comparison with the results of patients with AS and healthy old people. Hypothetically, this might be a result as a sequence of events, on-going osteoporosis and compresion fractures in patients with DISH and spondylitis. The endurance of the vertebral is decreased and the body increases the production of proteins from the osteoblasts to form compensatory more bone substance. The overproduction of S-RANKL is implicated in new bones formation in patients with DISH. Blocked up at S-RANK might have an important role in the suppression of pathologial processes in this diseases. The results indicate a possible common pathogenic connection between inflammatory joint disease (AS) and degenerative joint disease(DISH).

Keywords: level of S-RANKL, rheumatic diseases, bone growth

INTRODUCTION
S-RANKL (Receptor activator of nuclear factor kappa-B ligand) is a member of the TNF/TNF receptor superfamily. S-RANKL is identified as a factor capable to control bone regeneration and remodeling. (1, 2, 7). S-RANKL is secreted by the osteoblasts and connects through receptors to RANK on osteoclastic precursors and adult osteoclastic cells. Variations in the serum levels of RANKL from different organs and structures confirms the importance of RANKL in the process of bone substance increase (3, 4). S-RANKL has the ability to stimulate the formation and activity of the osteoclasts. (8). Understanding of osteoclast formation and activation has advanced considerably since the discovery of the RANKL/RANK/OPG system in the mid 1990s. Osteoblasts and stromal stem cells express receptor activator of NF-κB ligand (RANKL), which binds to its receptor, RANK, on the surface of osteoclasts and their precursors. This regulates the differentiation of precursors into multinucleated osteoclasts and osteoclast activation and survival both normally and in most pathologic conditions associated with increased bone resorption (1, 5, 6). The balance RANK-Ligand/OPG proved to be defining for the osteoclast differentiation, activity and survival rate. It has key role in the pathogenesis of Osteoporosis and other bone diseases, accompanied by increased bone resorption. (2, 6, 9)

Despite the fact that Osteoprotogerin/RANKL/RANK are studied in a variety of bone diseases (osteoporosis, multiple myeloma, metastases in bones etc.), in patients with Diffuse Idiopathic Skeletal Hyperostosis (DISH) and patients with (AS) they are not yet explored (2, 6).
PATIENTS AND METHODS

S-RANKL is studied in 55 patients, diagnosed with Diffuse Idiopathic Skeletal Hyperostosis (DISH), 25 patients with Ankylosing spondylitis (AS), 50 patients with Spondylosis and 15 practically healthy elderly individuals, similar in age and gender. All patients were treated and followed-up in Clinic of Rheumatology, University Hospital “Sveti. George”.

S-RANKL and Serum osteocalcin testing were performed with ELISA-technique with Sitio-Microplate reader, Seac, Italy, wave lenght λ 450 nm. Kits are produced by eBioscience, Austria. Biochemical tests performed were-serum calcium, ionised cacium, alkaline phosphatasis, uric acid, creatinine. Statistical analysis was performed with SPSS 23, confidence interval (p<0.001) (6).

RESULTS

Mean values of s-RANKL were significantly higher in patients with DISH and Spondylosis, in comparison to those with AS and the healthy elderly individuals used as controls (p<0.001). The highest individual value of S-RANKL was seen in one patient with Spondylosis - 3200 pg/ml. This result was significantly higher than the mean values of the rest of the results.

Table 1. Results from the mean values of s-RANKL in patients with DISH, spondylosis, AS and healthy controls pg/ml.

<table>
<thead>
<tr>
<th>Disease</th>
<th>N</th>
<th>x ± S x</th>
<th>Results</th>
<th>Sx</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Minimal</td>
<td>Maximal</td>
<td></td>
</tr>
<tr>
<td>DISH</td>
<td>55</td>
<td>197.00±35.90</td>
<td>10.00</td>
<td>1430.00</td>
<td>266.27</td>
</tr>
<tr>
<td>AS</td>
<td>25</td>
<td>200.60±66.167</td>
<td>10.00</td>
<td>3200</td>
<td>467.85</td>
</tr>
<tr>
<td>Spondylosis</td>
<td>50</td>
<td>90.86±18.542</td>
<td>20.00</td>
<td>430.00</td>
<td>88.92</td>
</tr>
<tr>
<td>Elderly controls</td>
<td>15</td>
<td>83.33±15.076</td>
<td>30.00</td>
<td>250.00</td>
<td>58.39</td>
</tr>
</tbody>
</table>

P – difference in the results of s-RANKL in patients with DISH and AS in comparison to those with spondylosis and elderly controls.

Patients with DISH have increased mean value of S-RANKL in comparison to patients with spondylosis and healthy controls (p<0.001). This results do not differ significantly from those of the patients with AS. (p>0.05).

The individual values of S-RANKL in patients with DISH, spondylosis and AS increase gradually with age, while it decreases with age in the results of the healthy controls (p<0.05) (Figure 1). Significant distraction of the individual results is present in all patients groups.

A. Mean Values of s-RANKL in patients with DISH

B. Mean values of s-RANKL in patients with Spondylosis
C. Mean values of s-RANKL in patients with AS.

D. Mean Values of s-RANKL in healthy controls

Figure 1. Dynamics of the individual values of s-RANKL in patients with DISH, Spondylosis, AS and healthy controls divided according to their age.

Correlational dependence between the values of S-RANKL in patients with DISH and some biochemistry tests is shown on table 2. Confident dependency between s-RANKL and the serum level of calcium, ionised calcium, phosphorus, uric acid, creatinine and urea exists (p<0.01).

Table 2. Correlational dependencies between the levels of s-RANKL, biochemistry tests and bone metabolism test is shown (R_{x,y}) as well as their confidence interval.

<table>
<thead>
<tr>
<th>Test</th>
<th>R_{x,y}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium mmol/l</td>
<td>0.76</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ionised calcium mmol/l</td>
<td>0.81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum phosphorus mmol/l</td>
<td>0.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Alkaline phosphatasis</td>
<td>0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Uric acid mmol/l</td>
<td>0.78</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine mmol/l</td>
<td>0.62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urea mmol/l</td>
<td>0.67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum osteocalcin ng/ml</td>
<td>0.69</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

DISCUSSION
The present data for the importance of S-RANKL shows the role of this biomarker for the evaluation of bone metabolism. Despite this in patients with DISH - a typical disease with formation of new bone and ossification of soft tissues, this metabolism is not yet studied.

Osteoclasts stimulating factor- S-RANKL is proved to be with significantly higher mean values in patients with DISH and AS in comparison to those with Spondylosis and elderly controls. (p<0.01). The individual values of S-RANKL in patients with DISH, spondylosis and AS gradually increase with age, while it decrease in elderly controls.

In the current stage of development of the medical science it is impossible to answer why the values of s-RANKL are increased in patients with DISH. It is hypothetically possible that the increase in s-RANKL is result from the primary increased bone synthesis through metaplas of the monocyte precursors/macrophageal cells into polynuclear osteoclasts, through which the balance between the bone synthesis and bone destruction is restored. On the other hand it is possible that increase in the levels of s-osteoprotegerin occurs in order to control the increase of levels of s-RANKL thus preventing its superproduction. Whether those changes in the markers of bone destructions are enough to control the balance between osteosynthesis and osteodestruction is not yet clear.

DISH and AS are two totally different diseases, presenting with change due to pathologic process in axial skeleton and peripheral enthesias, that leads to bone expansion in the extraspinal enthesias. Although the onset and clinical
picture of those diseases is different, in their late stages, accompanied by acceleration of loss of spinal mobility and radiographic changes, it gets harder to distinguish between them. Indifferentiation of both of the diseases is obligatory for the proper treatment of the patients and thus avoiding non-effective, unnecessary and risky therapies. This is even more important nowadays due to the treatment with expensive blockers of TNF-a in patients with AS, that do not have effect in patients with DISH. Large number of patients, that did not have clinical and laboratory improvement during therapy with TNF-alpha, actually suffered from DISH that was not recognised. In conclusion DISH is accompanied by increase in the studied biomarker for bone metabolism, bone remodelling is active and processes of bone synthesis predominates. Those results in the study of bone metabolism in patients with DISH are the onset of new understanding of the osteogenesis and hyperostosis. This is only the beginning of multiple and vast studies. The bone metabolism process is multilateral, multilayered with participation of variety of factors (hormones, cytokines, vitamins, growth factors etc) and additional studies are needed that should approve or reject our results in order to clear the importance of this metabolism for the pathogenesis, diagnose and differential diagnose of the patients with DISH.

REFERENCES