Survey of the Status of Iron and Sclerostin in Major Thalassemia Patients

Leila Moinzadeh¹M.Sc., Mohammad Reza Keramati¹M.D., Mohammad Taha Jalali²Ph.D., Bejan Keikhaei³M.D.,Ph.D., Najmaldin Saki⁴Ph.D., Amal Saki Malehi⁵Ph.D., Zahra Mohammadii¹M.Sc., Mohammad Mirdoraghi⁶M.Sc. Seyyede Fatemeh Shams¹M.Sc., Mohammad Hadi Sadeghian⁷M.D.

¹Department of Hematology and Blood Banking, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
²Department of Laboratory Sciences, Faculty of Para-medical Sciences, Jundishapur University of Medical Sciences, Ahvaz, Iran.
³Department of Pediatric Hematology and Oncology, Jondishapour University of Medical Sciences, Ahvaz, Iran.
⁴Health Research Institute, Research Center of Thalassemia & Hemoglobinopathy, Jundishapur University of Medical Sciences, Ahvaz, Iran.
⁵Department of Biostatistics and Epidemiology, Faculty of Public Health, Jundishapur University of Medical Sciences, Ahvaz, Iran.
⁶Department of Radiology, Faculty of Para-Medicine, Tehran University of Medical Sciences, Tehran, Iran.
⁷Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

ABSTRACT

Background and Aims: Thalassemia is one the most prevalent genetic anemia in the world; homozygote patients usually suffer from severe disturbances. Osteopenia and osteoporosis are of various types of thalassemia complications which increase in rate in patients with iron overload conditions. Sclerostin is a protein which enhances bone loss by inhibiting osteoblasts. The aim of this study was to measure sclerostin protein and its association with iron overload in major thalassemia patients.

Materials and Methods: Forty patients with major beta-Thalassemia and 40 healthy control individuals were included in the study; Sclerostin protein and ferritin were evaluated using enzyme-linked immunosorbent assay method.

Results: Mean sclerostin protein was 100.7 pg/ml, in the case group; it was 143.1 pg/ml in the control group. There was a significant differences between sclerostin protein in case and control groups (p= 0.01). The association of sclerostin and ferritin was not significant in the case group (p= 0.7), while it was meaningful in the control individuals (p= 0.04).

Conclusions: Our findings suggest that sclerostin protein can play an important role in the pathogenesis of osteoporosis.
Introduction

Thalassemia is the most common genetic disease in the world, especially in Iran [1]. The effects of iron overload can be seen on the scale of damage to the blood and bone [2]. Disease generates from a defect in the formation of the globin beta chain [3]. The main symptom of loss, defect or non-production of hemoglobin units is that it leads to impairment in the process of hematopoiesis which results to severe anemia [4]. Infact, it is the most common genetic defect has been reported in 60 countries including Cyprus, Italy and Iran [5]. Diagnosis is made before the age of two [6]. If blood hemoglobin concentration is more than 10 g/dl, it largely neutralizes the effect of anemia. Maintaining hemoglobin levels within the recommended range ensures survival and growth, but it increases the body iron. Over the years, it leads to a problem with iron overload and hemosiderosis in patients with major beta thalassemia. Ultimately, due to iron overload, heart and endocrine disorders cause death before the age of twenty-two [7]. Osteoporosis is a common complication in patients with major beta thalassemia, [8] as a result of osteoclastic activity increment due to endocrine dysfunction [9]. Some researchers report that Wnt route plays an important role in bone formation. This pathway contains a large protein complex that transmits receptors from the cell surface to the genome [10]. Other studies have shown that inhibitors of this pathway, including sclerostin and dkk1, contribute to inhibition of bone metabolism and even osteoporosis [11, 12]. Sclerostin is a protein encoded by the gene (SOST) and located in human chromosome 17 (17 q11.2). This protein is produced by osteocytes and has anti-anabolic effects on bone structure [13, 14]. Therefore the study was conducted to measure and compare sclerostin, serum iron, ferritin, and bone parameters related to osteoporosis in patients with major thalassemia in comparison to control group.

Materials and Methods

This case control study was performed in Shafa Hospital in Ahvaz, Iran. For each group of patients and control, 40 people were selected. The study was approved by the Ethics Committee of Jundishapur University of Medical Sciences, Ahvaz, Iran. Blood samples were taken from patients who had referred to the laboratory unit, and then transferred to the blood bank of the hospital. After serum separation, measurement was maintained at -70°C.

Selection criteria

Healthy people without thalassemia and osteoporosis, who are not at risk of osteoporosis, and who are more likely to be closer to the patient in terms of age and gender, were chosen as controls. Other pieces of information were extracted from the patients' medical records. Clinical variables including age, sex, presence or absence of osteoporosis, presence or absence of splenomegaly, blood leukocyte count, hemoglobin, and red blood cell count were recorded.
Laboratory tests

In this study, additional serum samples taken from patients were used for specific tests, therefore patients' costs will be reduced. Five ml whole blood was collected and the serum was separated and frozen until the test was conducted. The samples were stored at -70°C. Serum samples were used for sclerostin examination in vitro. Also the latest laboratory results of patients (up to one month before sampling for recent cross-match) such as ferritin, white blood cells, hemoglobin, hematocrit, height, and weight were recorded from the patients' medical records. People with osteoporosis were diagnosed with bone mineral density test. Enzyme-linked immunosorbent assay (ELISA) test was used for measuring the sclerostin. The ELISA kit of 155440 anti-Human SOST abcam from the company of the USA was used for this assay. Osteoporosis parameters that were evaluated in this study were Z and T score. The Z-score is the comparison of the age-matched normal and is generally applied in cases of severe osteoporosis. T score is the related scale when screening for osteoporosis. It is the bone mineral density (BMD) at the area when compared to the young normal reference average. It is a comparison of a patient's BMD to that of a healthy 30 years old [15].

Statistical analysis

SPSS v. 19 was used for analyzing the data. P value less than 0.05 was considered significant.

Results

In our study, the number of case and control groups were equal. The case and control groups comprised of 10 males (25%) and 30 females (75%). Gender distribution was not different significantly in the two groups on the basis of the chi-square test (p= 0.23). The average, minimum and maximum age were 22, 15 and 34 years in the case group respectively. T-test did not show any significant differences between the groups (p= 0.06). The factors associated with osteoporosis in the case group are shown in Table 1. Serum sclerostin level in case and control groups were 100.07 and 143.1 pg/ml, respectively. The minimum, maximum, and mean±SD sclerostin levels in the case group were 13.4, 258.1 and 100.07±66.08, respectively; these values amounted to 27.4, 212.2 and 143.1±54.7 pg/ml in the control group, respectively (Table 2).

According to T-test, there was a significant difference between the patients and the control group in the amount of serum sclerostin (p=0.01). Sclerostin and ferritin showed association in the control group (p=0.04), but that was not seen in the case group (p=0.7). Figure 1 discloses two parameters correlations. Also, the relationship between sclerostin and hematocrit (p=0.05) as well as sclerostin and hemoglobin (p=0.03) was significant. There was no significant relationships between sclerostin and White blood cells count (p=0.5) and bone parameters such as T score of spine (p=0.45), Z score of spine (p=0.3), T score of femur (p=0.7) and Z score of femur (p=0.9). However, evaluating the statistical results revealed a significant difference between ferritin and bone parameters such as T score of spine (p=0.05), Z score of spine (p=0.02), T score of femur (p=0.04) and Z score of femur (p=0.03).
Table 1. Osteoporosis parameters in case group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean SD</th>
<th>Max SD</th>
<th>Min SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine</td>
<td>-2.40 -2.32</td>
<td>1.06 1.05</td>
<td>-0.51 -0.38</td>
</tr>
<tr>
<td>Femoral</td>
<td>-1.59 -1.65</td>
<td>0.86 0.86</td>
<td>0.30 0.30</td>
</tr>
</tbody>
</table>

T= T score; Z= Z score

Table 2. Laboratory findings in patients with thalassemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean SD</th>
<th>Max SD</th>
<th>Min SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heamoglobin(g/dl)</td>
<td>8.05 1.03</td>
<td>10.0 5.0</td>
<td></td>
</tr>
<tr>
<td>Heamatocrit (%)</td>
<td>23.4 3.22</td>
<td>30.1 14.2</td>
<td></td>
</tr>
<tr>
<td>Ferritin (mg/ml)</td>
<td>2770.33 2135.86</td>
<td>10000 430</td>
<td></td>
</tr>
<tr>
<td>White blood cell x10³ (µL)</td>
<td>9005.00 6297.53</td>
<td>26000 1200</td>
<td></td>
</tr>
<tr>
<td>Sclerostin(pg/ml)</td>
<td>100.07 66.08</td>
<td>258.1 13.4</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Sclerostin is a glycoprotein that is made up of osteocytes, and has anti-anabolic effects. It can inhibit WNT signaling pathway by binding to LRP5/6. The inhibition of the WNT pathway can inhibit bone formation [16]. Sclerostin is a protein encoded by the gene...
(SOST), this protein is produced by osteocytes [13, 14]. Bone formation rate and superficial levels of serum alkaline phosphatase enzyme and osteocalcin increment have been reported in SOST mutation carriers; the accumulation of excessive bone is likely due to increase in osteoblast activity, as well as loss and reduction of SOST expression [17]. Some studies suggest that the ability of SOST to decrease bone osteoblast activity may be explained by anti basic metabolic panel I [18].

SOST causes apoptosis in human osteoblast cells. It has basic metabolic panel antagonist activities such as noggin and chordin [19]. In this study, there was a significant difference between the patients and control in the mean sclerostin serum (p=0.015). This suggests that sclerostin protein may play an important role in the pathogenesis of osteoporosis, but these results are not in line with the findings of studies that indicate protein expression enhancement in patients with major thalassemia [13, 16]. In our study, the relationship between sclerostin levels and ferritin was not significant in the patients, but in the controls it showed a significant relationship (p=0.04). In another study, iron overload along with its effects on osteoporosis was examined. Those who had blood transfusion, but did not consume iron chelator, experienced reduction in the osteoblasts activity and synthesis of new bones; it resulted in osteoporosis and osteopenia [20].

Conclusion

Based on the results, there is no significant relationship between sclerostin, ferritin and osteoporosis parameters. It suggests that the sclerostin protein may play an important role in the pathogenesis of osteoporosis. For better results, it is suggested to examine a larger community.

Conflict of Interest

None to declare.

Acknowledgment

This research project was supported by Mashhad University of Medical Sciences (MUMS). We thank the staff of Ahvaz Jundishapur university of medical sciences for their technical supports.

References

[7]. Sarker NR, Ghosh AK, Saha SK, Shahriar A. Recent advances in the management of