Original article

The relationship between serum vascular endothelial growth factor (SVEFG) and beta thalassemia major

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Abstract
Background: Beta thalassemia is an inherited disorder characterized by absent or reduced amounts of beta globin chains. Vascular Endothelial Growth Factor (VEGF) is a significant regulator of hemangioblast differentiation. This study was aimed to assess serum VEGF levels in patients with beta thalassemia major in comparison with control group.

Methods: This historical cohort study was conducted on 36 patients with β–thalassemia major who had received regular blood transfusion and 26 healthy people which were referred for checkup in a general hospital, Sari, north of Iran, during March to May 2015. Demographic characterization and laboratory tests such as Complete Blood Count (CBC), and evaluation of levels of serum ferritin, serum VEGF, hepatitis B virus antibody and hepatitis C virus antibody were carried out for our patients. The statistical analyses were performed by SPSS (16) software. The Pearson correlation coefficient test was used to test the significant correlations for quantitative parameters. A value of P<0.05 was considered statistically significant.

Results: Mean serum VEGF level in case and control groups was 153.8 ± 77.5 and 120.2± 45.4 pg/ml, respectively. Serum VEGF level was higher in beta thalassemia major (p= 0.037). Serum VEGF level was significantly higher in splenectomized patients (P=0.006). There was not any significant correlation between serum VEGF levels and Hemoglobin, WBC and platelet count and neither was with serum ferritin level (p>0.05).

Conclusion: Serum VEGF level was higher in thalassemic patients. Splenectomized patients had higher serum VEGF levels than others.

Keywords: Thalassemia major, Vascular Endothelial Growth Factor, Angiogenesis, Splenectomy
Introduction
Beta thalassemia is an inherited disorder in which expression of beta globin chains is decreased or absent (1, 2). The higher standards of care in β-thalassemia have led to significant increase in the life expectancy in the severely affected patients. Enhanced years of survival have led to the unmasking of management related complications, which were infrequently encountered (3, 4). Arterial and venous thromboembolic episodes in beta-thalassemia major patients especially splenectomized non transfusion dependent patients have been reported (5). Endothelial cell activation and impaired flow-mediated dilation in the brachial arteries of beta-thalassemia patients, as shown in previous in vivo studies, implicate endothelial dysfunction in the pathogenesis of vascular complications. Endothelial dysfunction generally leads to vascular remodeling and potential changes in mechanical properties (3, 6). Also impaired red blood cells in thalassemia patients cause vessels involvement and endothelial cell vessels disturbance in these patients (7). Evidences of culture showed low growth and endothelial cell vessels disturbance in presence of thalassemic serum (8).

Angiogenesis, growth of new blood vessels, is a significant process in development and growth, and it is essential for reestablishment of blood flow in injured tissues with formation of vascular network (7, 9). It is regulated with different cytokines and the most important regulator is Vascular Endothelial Growth Factor (VEGF). VEGF is an essential regulator of hemangioblast differentiation (10, 11) and without its regulatory function, formation of blood vessels are disturbed (8, 10).

The role of angiogenesis in different types of anemia such as malignancy related to anemia and sickle cell anemia was discovered, but its role on thalassemia patients was not appreciated enough (3, 11). Previous studies showed various correlation between serum VEGF levels and different factors in thalassemia patients (7, 10, 12).

Objectives
The aim of this study was to compare the serum VEGF in thalassemia major patients with normal controls, in a center located in the north of Iran.

Method
This historical cohort study was carried out in Thalassemia Research Center, Sari, north of Iran, during March to May 2015. Study population consisted of 36 patients with beta thalassemia major on regular blood transfusions and, 26 healthy people which were referred for checkup to hospital and they didn’t have any disease and didn’t receive any drugs and matched with case group regarding the gender and age. Patients with other hemoglobinopathies, malignancies, or other anemia were excluded from the study. All of our participants in both groups had completed consent form.

A questionnaire consisted of demographic characterization such as gender, age, date of last blood transfusion, age of onset of chelation therapy, the type of chelation therapy was filled for every patient. Result of some laboratory tests were extracted from clinical records. Blood sampling from thalassemic patients was at least 3 weeks after the last blood transfusion.

Diagnosis of beta thalassemia major was based on pre-transfusion CBC and hemoglobin electrophoresis according to texts and clinical manifestations. Blood samples were collected with EDTA anticoagulant and CBC test was done with Sysmex machine, KX21N (Sysmex Corporation Kobe, Japan). After centrifugation, plasma were collected in other labeled tube and saved in -70°C freezer. Serum VEGF levels were assessed with ELISA kit (Booster Biological Technology Co, Ltd). The detection limit of the VEGF assay was 9 pg/ml, the intra-assay precision was ≤ 6 % and the inter-assay precision was ≤10%. To adjust serum VEGF level with platelet count and exclude the effect of the platelet count, serum VEGF (pg/ml) / platelet count (x10^9/μL) was calculated.

Serum ferritin was measured with enzyme-linked immunosorbent assay (ELISA) method (Padtan Elm Co). HBV and HCV antibodies were also measured with enzyme-linked immunosorbent assay (ELISA) method (DiaPlus ELISA kit).

Statistical analysis were performed using Statistical Package for Social Science (SPSS) software version 16. Quantitative variables were expressed as mean and standard deviation (mean ± SD). Qualitative variables were expressed as count and percentage. Cross tabulation test was used for comparison between percentage values. Student t-test or non-parametric equivalent Mann-Whitney U test was used for comparison between means of two groups. The Pearson correlation coefficient test was used to test the significant correlations for quantitative parameters. A value of P<0.05 was considered statistically significant.

Results
Case group was consisted of 36 beta thalassemia major patients of which 19 (52.8%) were male and 17(48%) were female. Table 1 shows demographic and hematologic features of case group. Twenty six healthy people of 17(65.4%) female and 9(34.6%)...
males with mean age of 26.7 ± 5.90 years were in
the control group.

Table 1: Demographic and hematologic features of
case group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value(mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, number (%)</td>
<td>19(52.8%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.77 ± 4.94</td>
</tr>
<tr>
<td>Age at starting iron chelators</td>
<td>5.21 ± 4.12</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>1.91 ± 1.89</td>
</tr>
<tr>
<td>Platelets (×10⁹/l)</td>
<td>387 ± 186</td>
</tr>
<tr>
<td>WBC (/mm³)</td>
<td>11.7 ± 7.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.6 ± 0.9</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>25.6 ± 2.9</td>
</tr>
<tr>
<td>Ferritin (ng /ml)</td>
<td>3687 ± 3012</td>
</tr>
</tbody>
</table>

Serum VEGF and ferritin levels were higher in
case group. Table 2 demonstrates amount of serum
VEGF and ferritin levels in case and control
groups.

Table 2: The characteristics of serum VEGF and
ferritin levels in case and control groups

<table>
<thead>
<tr>
<th></th>
<th>Case (mean±SD)</th>
<th>Control (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (ng/ml)</td>
<td>3687.12 ± 3012.64</td>
<td>91.00 ± 70.08</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>153.82 ± 77.57</td>
<td>120.2 ± 45.40</td>
<td>0.037</td>
</tr>
</tbody>
</table>

There wasn’t correlation between type of chelation
therapy and serum VEGF level (P=0.7).
There was not any statistically significant
correlation between serum VEGF levels and serum
ferritin, and neither was with Hb, WBC and platelet
count. Table 3 shows laboratory findings and
serum VEGF level in thalassemia major patients.

Table 3: correlation of serum VEGF level and
laboratory findings

<table>
<thead>
<tr>
<th>Laboratory Findings</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>0.481</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.815</td>
</tr>
<tr>
<td>WBC (/mm³)</td>
<td>0.545</td>
</tr>
<tr>
<td>PLT count (×10⁹/µL)</td>
<td>0.619</td>
</tr>
<tr>
<td>Serum ferritin level (ng/ml)</td>
<td>0.251</td>
</tr>
</tbody>
</table>

None of our patients had hepatitis B virus antibody
and only one of them had hepatitis C virus
antibody. All patients had received regular blood
transfusion with 3-4 week intervals. The patients
were following three iron chelating therapies as
follows: twenty one of them were using Deferoxamine (DFO). Thirteen (36.1%) patients
were using DFO in combination with Deferiprone
and 2 (5.6%) patients were using Deferasirox.

Discussion
Angiogenesis is classified into two forms as
physiological and pathophysiological phenomenon.
Physiological angiogenesis is a highly regulated
process and occurs in cases such as wound healing,
the menstrual cycle, placental growth and etc. While pathophysiological angiogenesis is a
condition with uncontrolled proliferation of
endothelial vessels in conditions such as diabetes,
hamangiomas, tumor growth and metastasis (13,
14). Inhibition of angiogenesis in these situations
can improve symptoms of disease (15). VEGF is
produced in response to hypoxic state in anemia
and also in some tumors (3, 16, 17). Chronic
hypoxia can lead to high expression of Hypoxia
Induced Factor (HIF) and it is the main gene to
balance oxygen by some pathways like angiogenesis and, strengthening of glycolysis and
erthropoiesis (18, 19).
Our study showed that VEGF was significantly
higher in thalassemia patients than controls. Some
studies suggest that high serum levels of VEGF in
thalassemia patients is due to hypoxia caused by
low hemoglobin, which is explained by hypoxic
state in these patients(10, 12). We could not find
significant correlation between VEGF levels with
hemoglobin in our study (p=0.481) that is probably
because the anemia was not severe enough to induce

tissue hypoxia, this finding is in concordance with
other studies.
In our study, within the patients, VEGF levels were
higher in splenectomized ones (p= 0.006) and this
finding was similar to some studies which express
that patients who underwent splenectomy, had
higher platelet count in their peripheral blood, and
platelets are source of VEGF and secrete it (3, 8,
11). Most of our patients were using desferrioxamine
and we couldn’t find correlation between type of
iron chelators and serum VEGF level, this finding
was similar to studies that were done by Olgar et al
and Fahmey et al (3, 11).
There wasn’t statistically significant correlation
between VEGF and ferritin levels. This is similar to
the study of Fahmey et al and is probably due to
taking regular iron chelators by thalassemia major
patients (3).
In our patients, the mean age of beginning iron
chelating therapy was 5.21 ± 4.12 years old and its
correlation with serum VEGF level wasn’t
significant (p = 0.737) which contrasts with similar
studies (3, 11). Duration of disease wasn’t significantly correlated
with serum VEGF level in the study of Fahmey et al,
and in our study there was not any correlation

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between duration of disease and serum VEGF level as well (p = 0.269) (11).
In this study, there was not a significant correlation between platelet count and serum VEGF level. This is in contrast to current explanation that platelets play a role as a reservoir for serum VEGF (10). Probably this is because of our low sample size and we need to research more to verify the source and potential pathological importance of serum VEGF in thalassemia major patients.

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References