Original article

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

Farzane Farokhi[1], Javad Razaviyan[2, 3], Mehrnoush kosaryan[4], Mostafa Roudbari[5], Samira Esmaeili Reykande[3, 6], Aily Aliasghariyan[7], Maryam Dehghani[8]

1-.Dep. of Biology, Azad University of Sari Branch, Mazandaran, Iran.

2-MSc Student of Clinical Biochemistry, Medical School, Tehran University of Medical Sciences, Tehran, Iran.

3-Student of Scientific Research Center, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran.

4-Professor of pediatrics, Hemoglobinopathy Institute, Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

5-Dep. of Laboratory Medicine, Faculty of Allied Medicine, Mazandaran University of Medical Sciences, Mazandaran, Iran.

6-Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran.

7-Msc student of medical microbiology, Thalassemia Research center, Mazandaran university of medical sciences, Sari, Iran

8-Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Corresponding author: Dr Farzane Farokhi. Email: farzane.farokhi@gmail.com

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*

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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 was 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 ($\times 10^3/\mu L$) 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%)

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males with mean age of 26.7 ± 5.90 years were in the control group.

Table1: Demographic and hematologic features of case group

Parameter	Value(mean±SD)
Male, number (%)	19(52.8%)
Age (years)	26.77 ± 4.94
Age at starting iron chelators (years)	5.21 ± 4.12
Age at diagnosis (years)	1.91 ± 1.89
Platelets ($\times 10^9$/l)	387 ± 186
WBC (/mm^3)	11.7 ± 7.6
Hb (g/dl)	8.6 ± 0.9
HCT (%)	25.6 ± 2.9
Ferritin (ng /ml)	3687 ± 3012

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

	Case (mean±SD)	Control (mean±SD)	p-value
Ferritin (ng/ml)	3687.12 ± 3012.64	91.00 ± 70.08	<0.05
VEGF (pg/ml)	153.82 ± 77.57	120.2 ± 45.40	0.037

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.

Table3: correlation of serum VEGF level and laboratory findings

Laboratory Findings	p- value
Hemoglobin (g/dl)	0.481
Hematocrit (%)	0.815
WBC (/mm^3)	0.545
PLT count ($\times 10^3$/µL)	0.619
Serum ferritin level (ng/ml)	0.251

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 Deferrioxamine (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, hemangiomas, 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 erythropoiesis (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 sever 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 duo 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 Fahmy 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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