

Short Communication

Serum Vascular Endothelial Growth Factor (VEGF) levels in patients with alpha thalassemia

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Abstract

Background: Alpha-thalassemia syndrome includes a group of hereditary anemia in which expression of alpha globin chains is decreased or absent. Impaired RBC in patients with thalassemia causes vessel involvement and endothelial cell vessel disturbance. Vascular Endothelial Growth Factor (VEGF) is the most important regulator for endothelial cell proliferation. So, the aim of this study is to compare the serum VEGF levels in patients with alpha thalassemia and normal control group.

Materials and Methods: This case-control study was conducted on 17 patients with alpha thalassemia and 40 healthy people. Serum VEGF levels were measured by enzyme-linked immune sorbent assay (ELISA) kit. Then statistical analysis of results were performed using SPSS 16, value of $P < 0.05$ was considered statistically significant.

Results: Mean serum VEGF levels in case and control groups were 2294.19 ± 1552.39 and 598.09 ± 988.17 pg/ml, respectively. Serum VEGF levels were higher in patients with alpha thalassemia ($P < 0.01$). There was no significant correlation between serum VEGF levels and Hemoglobin. ($P = 0.73$).

Conclusion: Our study revealed that patients with alpha thalassemia have elevated levels of serum VEGF than normal control group. Further studies with larger sample size are recommended to confirm these observations.

Keywords: Alpha Thalassemia, Vascular Endothelial Growth Factor (VEGF), Angiogenesis

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Introduction

Thalassemia is the most common monogenic disorder in the Worldwide (1). Alpha-thalassemia syndrome includes a group of hereditary anemia in which expression of alpha globin chains are decreased or absent (2). Alpha thalassemia is heterogeneous in the molecular levels which is the result of point mutations, small base insertions or deletions that

affect the region encoding $\alpha 1$ and $\alpha 2$ genes on chromosome 16 (3). All four α -globin alleles are deleted or inactivated in Hemoglobin Bart syndrome. Deletion or dysfunction of three alleles results in HbH disease. Minor or trait alpha-thalassemia results from mutation in two alleles, and $\alpha+$ -thalassemia occurs when one allele has been mutated (4).

Impaired RBCs in patients with thalassemia result in vessel involvement and endothelial cell

vessel disturbance (5). In some cultural studies, low growth and disturbance of endothelial cell vessels in exposed to thalassemia serum were seen (6).

Angiogenesis is an important process in development and growth of new blood vessels. In this process, endothelial cell proliferation forms vascular networks for reestablishment of blood flow in injured tissues (7). Vascular endothelial growth factor (VEGF) is the most important regulator for this process (8). VEGF is a critical regulator of hemangioblastic differentiation and blood vessels formation (6). Tissue hypoxia is a main stimulus for the up-regulation of VEGF, so patients with anemia have elevated serum levels of VEGF. It seems that anemia might impact on the progression of angiogenesis (9).

The role of angiogenesis in several types of anemia was determined, but studies about its character in alpha thalassemia were too low. The aim of this study, is to compare the serum levels of VEGF as a marker of angiogenesis in patients with alpha thalassemia with normal control group.

Methods

This case-control study was carried out in Zafar Thalassemia Clinic, Tehran, during 2015-2016. Study population was consisted of 17 patients with alpha thalassemia and 40 healthy controls. Case and control groups were matched regarding the gender and age. Patients with other hemoglobinopathies, malignancies or other causes of anemia were excluded from the study. This study was approved in Ethical committee at Tehran University of Medical Sciences (TUMS) and

All of our participants in both groups had completed consent form.

A questionnaire consisted of demographic characterization such as gender and age was filled for every patients. Results of some laboratory and molecular tests were extracted from their clinical records.

Diagnosis of alpha thalassemia was based on laboratory test, peripheral blood smear, molecular test and clinical manifestations. Blood samples were collected with EDTA anticoagulant and Cell Blood

Count (CBC) test was done with Sysmex machine, KX21N (Sysmex Corporation Kobe, Japan). The samples were centrifuged for 10 minutes then sera were separated and stored at -70°C. Eventually, serum VEGF levels were measured by enzyme-linked immunosorbent assay (ELISA kit) Booster Biological Technology Co, Ltd). The detection limit of the serum VEGF assay was 9 pg/ml, the intra-assay precision was $\leq 6\%$ and the inter-assay precision was $\leq 10\%$. To adjust the serum VEGF level with platelet count and exclude the effect of the platelet count, serum VEGF (pg/ml) / platelet count ($\times 10^3/\mu\text{L}$) was calculated.

Statistical analysis of results was performed using SPSS software version 16. Quantitative variables were expressed as mean and standard deviation (mean \pm SD). Qualitative variables were expressed as count and percentage. 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 17 patients with alpha thalassemia of which 6 (35.3%) were male and 11 (67.4 %) were female. Frequency of the different types of alpha thalassemia syndrome in our patients as follows: 2(11.7%) silent carrier, 10 (58.82%) alpha thalassemia trait and 5(29.41%) Hb H disease. Their mean age was 37.06 ± 3.1 . A total of 40 healthy peoples as control group were enrolled in the study, 18(40.91 %) of them were male and 26 (50.09%) were female. The mean age of the control group was 25.73 ± 5.04 .

Serum VEGF levels were higher in the case group.

Because of the low number of patients, Shapiro-Wilk test was used to assess the normality of the data which showed non-normal distribution. Comparison of serum VEGF levels between case and control groups by Mann-Whitney U test revealed significant differences between two groups.

Table 1: It demonstrates the amounts of serum VEGF levels in case and control groups.

	Case(mean \pm SD)	Control(mean \pm SD)	P value
VEGF (pg/ml)	2294.19 \pm 1552.39	598.09 \pm 488.17	<0.01

The mean range of Hemoglobin in patients with alpha- thalassemia was 11.41 ± 2.2 and there was not any statistically significant correlation between serum VEGF levels with Hemoglobin. (P= 0.73)

There wasn't correlation between age and gender with serum VEGF levels (P=0.86, P=0.43).

Statistical analysis showed no significant association between serum VEGF levels and number of deletion mutation. (P = 0.63)

Discussion

Vascular endothelial growth factor (VEGF) is a key regulator of physiological angiogenesis and plays an important role during development of the vascular system, wound healing, menstrual cycle, etc. It has also been implicated in pathological angiogenesis associated with tumors and other conditions (10) Inhibition of angiogenesis in these situations can alleviate symptoms of disease (11). VEGF is produced in response to tissue hypoxia in anemia and some tumors (12). Continuance of hypoxic state leads to high expression of Hypoxia Induced Factor (HIF) which is the main gene to balance oxygen by some pathways like angiogenesis (13).

Enhancement of VEGF level has been investigated in different kinds of anemia. However, its role as a marker of angiogenesis has not been enough assayed in alpha thalassemia. Our study showed that serum VEGF levels were significantly higher in patients with alpha thalassemia than control group that it was similar to Butthep and et al study. They found that serum VEGF level increase in patients with alpha and beta thalassemia (5).

Also, we were not observed significant correlation between serum VEGF levels and hemoglobin ,that it seems probably due to most patients were mild alpha thalassemia, this finding

similar to Olgar and et al study ,in which they also showed no significant correlation between VEGF levels and Hemoglobin (14). We found no significant correlation between serum VEGF levels and patients' age that was contrast to shitrit and et al study (6). One of our big limitation in this study, was low sample size, so further studies with larger population is recommended to confirm these observations.

Conclusions

Our study showed that patients with alpha thalassemia have elevated levels of serum VEGF than normal control group. Further studies with larger sample size are recommended to confirm these observations.

Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

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References

1. Galanello R, Origa R.,Beta-thalassemia. Orphanet journal of rare diseases. 2010;5(1):1172-5.
2. Vichinsky EP, Clinical manifestations of α -thalassemia. Cold Spring Harbor perspectives in medicine. 2013;3(5):011742.
3. Zarbakhsh B, Eghbalpour F, Farshadi E, Fallah M. S, Karimipour M, Kaeini Moghadam, Z, et al. Frequency of alpha thalassemia carriers detected in Tehran premarriage screening using molecular techniques. Scientific Journal of Iranian Blood Transfusion Organization. 2013;9(4):414-21.
4. Origa R, Moi P, Galanello R, Cao A. Alpha-thalassemia. 2013. 21.

5. Butthep P. Increased circulating activated endothelial cells, vascular endothelial growth factor, and tumor necrosis factor in thalassemia. *American journal of hematology*. 2002;70(2):100-6.
 6. Shitrit D, Tamary H, Koren A, Levin C, Bargil-Shitrit A, Sulkes J, et al. Correlation of vascular endothelial growth factor with the severity of thalassemia intermedia. *Blood Coagulation & Fibrinolysis*. 2008;19(7):611-4.
 7. Kofler NM, Simons M. Angiogenesis versus arteriogenesis: neuropilin 1 modulation of VEGF signaling. *F1000 prime reports*. 2015;3(7).
 8. Dunst J. Anemia and elevated systemic levels of vascular endothelial growth factor (VEGF). *Strahlentherapie und Onkologie*. 2002;178(8):436-41.
 9. Fahmey SS, Naguib HF, Abdelshafy SS, Alashry RE. Vascular endothelial growth factor in children with thalassemia major. *Med J hematology and Infectious Dis*. 2013;5(1).
 10. Ferrara N, Gerber HP, LeCouter J, The biology of VEGF and its receptors. *Nature Med*. 2003;9(6):669-76.
 11. Risau W. Mechanisms of angiogenesis. *Nature*. 1997;386(6626):671-4.
 12. Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, et al. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. *The Journal of neuroscience*. 1995;15(7):4738-47.
 13. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. *Journal of Neuroimmune Pharmacology*. 2014;9(2):142-60.
 14. Olgar S, Kara A, Hicyilmaz H, Balta N, Canatan D. Evaluation of angiogenesis with vascular endothelial growth factor in patients with thalassemia major. *Pediatrics International*. 2010;52(2):247-51.
1. Galanello R, Origa R. Beta-thalassemia. *Orphanet journal of rare diseases*. 2010;5(1):1.
 2. Vichinsky EP. Clinical manifestations of α -thalassemia. *Cold Spring Harbor perspectives in medicine*. 2013;3(5):a011742.
 3. Zarbakhsh B, Eghbalpour F, Farshadi E, Fallah M, Karimipour M, Zeinali S. Frequency of alpha thalassemia carriers detected in Tehran premarriage screening using molecular techniques. *Scientific Journal of Iranian Blood Transfusion Organization*. 2013;9(4).
 4. Origa R, Moi P, Galanello R, Cao A. Alpha-thalassemia. 2013.
 5. Butthep P, Rummavas S, Wisedpanichkij R, Jindadamrongwech S, Fucharoen S, Bunyaratvej A. Increased circulating activated endothelial cells, vascular endothelial growth factor, and tumor necrosis factor in thalassemia. *American journal of hematology*. 2002;70(2):100-6.
 6. Shitrit D, Tamary H, Koren A, Levin C, Bargil-Shitrit A, Sulkes J, et al. Correlation of vascular endothelial growth factor with the severity of thalassemia intermedia. *Blood Coagulation & Fibrinolysis*. 2008;19(7):611-4.
 7. Kofler NM, Simons M. Angiogenesis versus arteriogenesis: neuropilin 1 modulation of VEGF signaling. *F1000prime reports*. 2015;7.
 8. Dunst J, Becker A, Lautenschläger C, Markau S, Becker H, Fischer K, et al. Anemia and elevated systemic levels of vascular endothelial growth factor (VEGF). *Strahlentherapie und Onkologie*. 2002;178(8):436-41.
 9. Fahmey SS, Naguib HF, Abdelshafy SS, Alashry RE. Vascular endothelial growth factor in children with thalassemia major. *Mediterranean journal of hematology and infectious diseases*. 2013;5(1).
 10. Ferrara N, Gerber H-P, LeCouter J. The biology of VEGF and its receptors. *Nature medicine*. 2003;9(6):669-76.
 11. Risau W. Mechanisms of angiogenesis. *Nature*. 1997;386(6626):671-4.
 12. Stone J, Itin A, Alon T, Pe'Er J, Gnessin H, Chan-Ling T, et al. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. *The Journal of neuroscience*. 1995;15(7):4738-47.
 13. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. *Journal of Neuroimmune Pharmacology*. 2014;9(2):142-60.
 14. Olgar S, Kara A, Hicyilmaz H, Balta N, Canatan D. Evaluation of angiogenesis with vascular endothelial growth factor in patients with thalassemia major. *Pediatrics International*. 2010;52(2):247-51.