J Surg Med. 2019;3(12):825-828.
DOI: 10.28982/josam.653402

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN: 2602-2079

Investigation of SCUBE-1 levels in pediatric patients with beta-thalassemia

Beta-talasemi majörlü pediatrik hastalarda SCUBE-1 düzeylerinin araştırılması

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Ethics Committee Approval: This study is approved by the Ethics Committee of Kahramanmaraş Sütçü İmam University with the protocol number 163 on 11.10.2017.
Etik Kurul Onayı: Bu çalışma, Kahramanmaraş Sütçü imam Üniversitesi Etik Kurulu tarafından 11.10.2017 tarih ve 163 numaralı protokol ile onaylandı.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 12/09/2019
Yayın Tarihi: 09.12.2019

Abstract

Aim: A thrombosis and inflammation marker, Signal peptide-CUB (complement C1r/C1 s)-EGF (epidermal growth factor)-like domain-containing protein 1 (SCUBE-1) is expressed in endothelial cells and activated platelets. We aimed to investigate the levels of this marker in patients with Thalassemia major (TM), a disease which causes a tendency to thrombosis.

Methods: Twenty patients with TM and age and gender-matched twenty healthy controls were included in this prospective case-control study. Other thrombosis markers such as D-dimer and homocysteine were studied in addition to SCUBE-1 in both groups.

Results: The SCUBE1 and D-dimer levels were higher in the TM group compared to the control group (P=0.004, P=0.023, respectively). A significant positive correlation was detected between platelet count and SCUBE1 (r=0.342, P=0.031).

Conclusion: We detected elevated SCUBE1 levels in children with TM compared to the healthy controls, which correlated with platelet count. SCUBE1 may be used as a thrombosis marker in TM in the early stages of the disease.

Keywords: Beta-thalassemia major, Children, SCUBE-1, Thrombosis

Öz

Amaç: Sinyal peptidi-CUB (tamamlayıcı C1r / C1s) -EGF (epidermal büyüme faktörü) benzeri alan içeren protein 1 (SCUBE-1), endotel hücreleri ve aktive olmuş trombositlerde eksprese edilen tromboz ve inflamasyon belirteci olarak kullanılan bir proteindir. Tromboza eğilim yaratan bir hastalık olan talasemi majörde bu belirtecin düzeylerini incelemeyi amaçladık.

Yöntemler: Bu kesitsel vaka-kontrol çalışmasına SCUBE-1 düzeylerini belirleyebilmek için 20 talasemi majorlü hasta ve kontrol grubu olarak da benzer yaş ve cinsiyette 20 sağlıklı çocuk dahil edildi. D-dimer, homosistein gibi diğer tromboz belirteçleri SCUBE-1 ek olarak her iki grupta çalışıldı.

Bulgular: Gruplar arasında SCUBE-1 ve D-dimer düzeyleri açısından anlamlı fark vardı (P=0,004, P=0,023, sırasıyla). Trombosit sayısı ile SCUBE-1 arasında anlamlı pozitif korelasyon tespit edildi (r=0,342, P=0,031).

Sonuç: SCUBE-1 seviyelerinin talasemi majorlü çocuk hastalarda sağlıklılara nazaran yüksek olduğunu ve trombosit sayısı ile korele olduğunu tespit ettik. SCUBE-1 belki erken evrelerde tromboz belirteci olarak kullanılabilir.

Anahtar kelimeler: Beta-talasemi major, Çocuklar, SCUBE-1, Tromboz

How to cite / Atıf için: Acıpayam C, Tuncel DA, Güneş H, Akkececi NS, Tolun Fİ, İpek S, Duyuran Ö. Investigation of SCUBE-1 levels in pediatric patients with beta-thalassemia. J Surg Med. 2019;3(12):825-828.

Introduction

Thalassemia is a hemolytic disorder associated with increased thrombosis incidence [1]. The prevalence of thromboembolic events is reported as 3-3.95% in cases with thalassemia major (TM) [2,3]. The main mechanisms underlying thromboembolism are endothelial cell activation, abnormal erythrocyte cell surface, and platelet activation. It is reported that hypercoagulability detected in thalassemic cases starts in childhood, continues for a lifetime and usually leads to thromboembolic events at older ages. There is a higher risk in patients with splenectomy due to increased abnormal erythrocytes and thrombocytosis [4].

Signal peptide-CUB (complement C1r/ C1s, U egf, and Bmp1)-EGF (epidermal growth factor)-like domain-containing protein-1 (SCUBE-1) is a newly defined marker of platelet activation detected in thrombocyte and endothelial cells. Vascular endothelial cells play a significant role in pathophysiological processes such as cancer metastasis, angiogenesis inflammation, and vascular diseases [5,6]. It is shown that SCUBE-1 is stored in platelet α granules and to move to the cell surface during platelet activation [7,8]. Plasma SCUBE-1 protein is related to platelet and endothelial interactions and may be characteristic of platelet activation during acute ischemic circumstances. It is thought that this interaction between platelet and endothelial cells might have potential as a nonspecific marker of acute ischemia [9].

However, the studies that investigate SCUBE-1 functions are limited [10]. To date, the levels of SCUBE-1 are not studied in β-Thalassemia patients. We aimed to investigate the levels of SCUBE-1 in TM.

Materials and methods

For this cross-sectional and case-control study, we included 20 children with TM and 20 age- and gender-matched healthy children as the control group. TM patients were diagnosed by a pediatric hematologist based on hemoglobin electrophoresis and mutation results. Patients received blood transfusions and chelation therapy, as necessary. Those who had additional chronic, thrombotic, or neoplastic diseases, a major surgical intervention in the last three months or major trauma, those receiving oral or intravenous anticoagulants, those with chronic hepatitis and who were splenectomized were excluded. The control group consisted of healthy children cases who were referred to the outpatient clinic of our hospital, with no previously known chronic diseases, anemia or hemoglobinopathies. All patients' laboratory and demographic data were obtained from the hospital registry. Complete blood counts and routine biochemical parameters were recorded.

All blood samples were obtained under aseptic conditions, centrifuged for 20 minutes at 3500 rpm without any delay to have their plasma was separated and stored at -80 degrees for examination of SCUBE1 levels. Human SCUBE-1 serum levels were determined with ELISA (enzyme-linked immunosorbent assay) method by using the ELISA commercial kit (Shanghai Sunred Biological Technology Co. Ltd.), automatic Elisa reader (Thermo Scientific, Finland) and a software (Skanlt for Multiscan FC 2.5.1). The sensitivity and assay range were 0.852 ng/mL and 1 ng/mL-300 ng/mL, respectively.

Informed written consent was obtained from all patients and their parents; ethical approval was obtained from the local ethics committee before the study.

Statistical analysis

Data management and analysis were performed using SPSS program v.14 (SPSS Inc., Chicago, IL) and a two-sided *P*-value ≤0.05 was considered statistically significant. Baseline and skewness values as well as the mean and standard deviation of variables were calculated. Shao (2002) stated that the distortion and kurtosis values should be within the range of ±3 for the distribution of the data used in the research to be normal [11]. Continuous data were expressed as mean (standard deviation) or median and categorical data were expressed in percentages. The means were compared with an independent sample t-test, and in the case of non-normal distribution, Mann–Whitney U test was used. Chi-square test was utilized for the comparison of categorical data. Correlation analysis was performed with the Pearson correlation test for normally distributed variables and Spearmen correlation test was used for non-normally distributed variables.

Power analysis was used to determine the sample size of the study. Taking into account the statistical parameters of SCUBE1 in the reference studies (group 1: 1.40 (0.25), group 2: 1.22 (0.09)), alpha, beta errors and the power were set at 0.05, 0.20 and 0.80, respectively, to find that twenty individuals per group sufficed [12].

Results

The basic demographic and laboratory findings of the groups are presented in Table 1. There was no statistically significant difference between the groups in terms of age, gender, and body mass index. The SCUBE-1 and D-dimer levels of TM group were significantly higher than the control group (*P*=0.004 and *P*=0.023, respectively) (Figure 1). No difference was found between the homocysteine levels (*P*=0.179). None of the patients had cardiac or endocrinologic involvement.

There was a positive correlation between SCUBE-1 levels and platelet count (r=0.342, *P*=0.031), and a negative correlation between the SCUBE-1 levels and hemoglobin (r=-0.414, *P*=0.008), hematocrit (r=-0.412, *P*=0.008), RBC (r=-0.366, *P*=0.02), and ALT (r=-0.414, *P*=0.008) levels (Table 2). We did not detect any correlation between SCUBE-1, homocysteine and D-dimer values (r=-0.068, *P*=0.677 and r=-0.002, *P*=0.988, respectively).

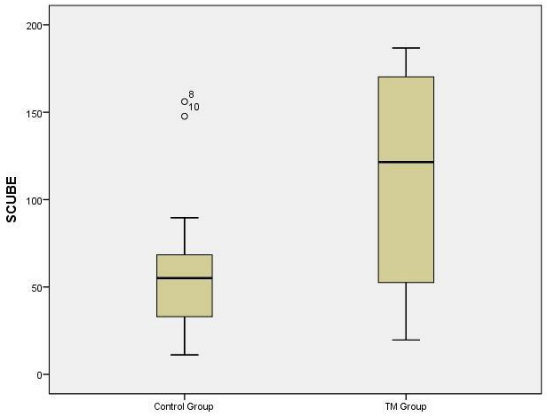

Figure 1: SCUBE-1 levels in patient and control groups

Table 1: Demographic and laboratory data of the study population

	TM group (n=20)	Control group (n=20)	*P*-value
Age, median (IQR), years	11.5(10-13.7)	11.5(10-13.7)	0.900
Gender, male/female	11/9	12/8	0.749*
Body mass index, median (IQR), kg/m^2	16.5(15.2-19.1)	17.1(16.2-22.0)	0.212
Laboratory Findings			
WBC, median (IQR),10^9/L	8.1(6.4-11.6)	8.3(6.6-10.1)	0.752
RBC, mean (SD),10^9/L	3.0 (0.4)	4.09 (0.45)	<0.001
Hb, mean (SD), g/dL	8.2 (1.1)	13.4 (1.20)	<0.001
Hematocrit, mean (SD), %	24.6 (3.6)	39.4 (3.40)	<0.001
MCV, mean (SD), fl	79.8 (4.8)	79.6 (4.70)	0.917
MPV, mean (SD), fl	10.5 (0.7)	9.7 (0.8)	0.002
Platelet Count, median (IQR), 10^9/L	334 (282-467)	327 (313-419)	0.752
Fasting blood glucose, mean (SD), mg/dL	102.9 (13.62)	88.6 (12.8)	0.001
Urea, mean (SD), mg/dL	15.6 (3.57)	12.1(2.7)	0.001
Creatinine, median (IQR), mg/dL	0.4 (0.3-0.4)	0.4 (0.4-0.5)	0.004
AST, mean (SD), U/L	29.9 (11.8)	31.0 (15.9)	0.685
ALT, median (IQR), U/L	16.5 (10.7-26.7)	18 (14-29)	0.752
Ferritin, mean (SD), µg/L	1339.9 (984.7)	-	-
SCUBE-1, median (IQR), ng/mL	121.4 (51.2-173.9)	55.0 (32.5-71.8)	0.004
D-dimer, median (IQR),mg/L	0.2 (0.2-0.3)	0.1 (0.1-0.2)	0.023
Homocysteine, median (IQR), µmol/L	10.3 (7.67-12.0)	9.4 (8.8-10.2)	0.179

*Chi-Square test, ALT: Alanine transaminase, AST: Aspartate transaminase, Hb: Hemoglobin, MCV: Mean corpuscular volume, MPV: Mean platelet volume, RBC: Red blood cell, WBC: White blood cell

Table 2: Correlation coefficients for SCUBE-1

Variables	r	*P*-value
Hemoglobin	-0.414	0.008
Hematocrit	-0.412	0.008
Platelet	0.342	0.031
RBC	-0.366	0.020
ALT	-0.414	0.020

ALT: Alanine aminotransferase, RBC: Red blood cell

Discussion

To the best of our knowledge, this was the first study investigating SCUBE-1 levels in children with TM, and we found that the median SCUBE-1 levels of TM patients were higher than the healthy control group. Furthermore, we demonstrated that there was a positive correlation between SCUBE-1 levels and platelet count.

TM causes a tendency to thromboembolic events such as recurrent or transient ischemic attacks, arterial or venous thrombosis, and stroke [13]. Numerous factors may play a role in the pathogenesis of thromboembolic events in TM. Thrombin formation is facilitated by the asymmetry in membrane phospholipids due to increased phosphatidylserine exposure in red blood cells (RBC), which in turn increases thrombosis [13]. The structural anomalies found in the RBCs of TM patients during the first months of their life increase the life-long risk of thromboembolic events. The other mechanism that causes a tendency to thrombosis is platelet activation. Menichelli et al. [14] demonstrated in vivo platelet activation in children with TM. In the study of Koçak et al. [15] high platelet count found in children with TM increased thrombosis risk. There are many other studies on this issue: Eldor et al. [16] showed increased urine metabolites of thromboxane A2 in TM patients. Similarly, Atichartakan et al. [17] found elevated β-thromboglobulin levels in TM patients. SCUBE-1 protein is stored in the alpha granules of the inactive platelets [18]. By the activation of thrombin, the protein migrates to the surface of platelets, inducing platelet activation, thus increasing the level of SCUBE-1 [19,20]. Dai et al. [21] demonstrated that plasma SCUBE1 levels in acute ischemic stroke patients were higher than the healthy control group. Türkmen et al. [22] showed that SCUBE-1 levels were statistically significantly higher in the 6th hour of mesenteric ischemia compared to the control group. Bolayır et al. [23,24] stated that SCUBE1 levels increased in the initial stages of ischemic stroke and demonstrated an increase in the levels of SCUBE-1 in myocardial infarct. Similarly, we found elevated median levels of SCUBE-1 in children with TM compared to healthy controls. This may be associated with increased thrombotic tendency in TM. We also detected a positive correlation between SCUBE-1 and platelet count, which is known risk factor of thrombosis. This may also contribute to the association of SCUBE-1 with thrombosis.

According to our results, D-dimer and MPV had significantly increased in the TM group. Elevated MPV is another indicator of platelet activation, leading to thrombosis [25,26]. Çıkrıkçıoğlu et al. [27] found higher MPV values in β Thalassemia intermedia. However, we have not detected any correlation between D-dimer and homocysteine levels. This may be related our sample size or the weakness of SCUBE-1 in detecting thrombosis.

Although no thrombotic events were seen in any of our cases during the study period, this does not change the fact that TM poses a risk for thrombosis [13].

The difference between hemoglobin, hematocrit, and RBC in groups was due to the already known TM.

Limitations

The first limitation of this study is our small sample size. The fact that we did this conduct this study among our clinic's regular patients reduced the number of individuals we included in our study. Another limitation is the lack of inclusion of thalassemia patients who experienced thrombotic events, which made it impossible to compare and provide a cut-off value for SCUBE-1. More randomized controlled trials with increased number of patients are needed to illuminate this issue.

Conclusion

Almost all our patients, who had not experienced any thrombotic events, had elevated levels of SCUBE-1, a new thrombosis marker, compared to healthy controls. TM patients should be carefully monitored for any thrombotic events and precautions should be taken.

Acknowledgments

The authors thank the nursing staff of the department for their help and care for the patients.

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This paper has been checked for language accuracy by JOSAM editors.
The National Library of Medicine (NLM) citation style guide has been used in this paper.

Suggested citation: Patrias K. Citing medicine: the NLM style guide for authors, editors, and publishers [Internet]. 2nd ed. Wendling DL, technical editor. Bethesda (MD): National Library of Medicine (US); 2007-[updated 2015 Oct 2; cited Year Month Day]. Available from: http://www.nlm.nih.gov/citingmedicine