Relationship between Serum Visfatin and Vascular Inflammation Markers Level in Beta Thalassemia Major Patients

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Original Article

ABSTRACT

Background: Understanding the possible role of visfatin in the pathogenesis of beta-thalassemia major (BTM) and its relationship with markers of endothelial function could help us to provide more effective therapeutic approaches for treatment of patients with BTM and its related complications. The aim of current study was to compare serum level of visfatin between patients with BTM and control group and determine its correlation with markers of endothelial function, intracellular adhesion molecule (ICAM) and vascular adhesion molecule (VCAM).

Methods: In this case-control study, patients with BTM receiving regular blood transfusion aged 10-20 years and a group of healthy subjects were enrolled. Selected subjects examined clinically and venous blood samples obtained for visfatin, ICAM, VCAM, cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol and ferritin measurements. Mean (standard deviation) of studied laboratory measurements compared in two studied groups and the relation between visfatin and ICAM, VCAM, ferrittin, body mass index determined.

Results: In this study 31 patients with BTM and 30 healthy controls studied. Mean of visfatin was significantly higher in patients with BTM than control group (133.9 ± 60.1 vs. 43.3 ± 27.9, $P < 0.001$).

Conclusions: The higher level of visfatin among patients with BTM indicated the possible inflammatory role of this adipocytokine in BTM. It seems that for understanding the underlying mechanisms and its relation with vascular inflammatory markers and endothelial function further studies with larger sample size is needed.

Keywords: Beta-thalassemia major, endothelial function, intracellular adhesion molecule, vascular adhesion molecule, vascular inflammatory markers, visfatin

INTRODUCTION

Beta-thalassemia major (BTM) considered as one of the most prevalent hemoglobinopathies in Mediterranean region including Iran.[1] BTM characterized by failure of the haemoglobin

synthesis leading to excess beta-globin chains, haemolysis and impair erythropoiesis.[2]

Though higher standards of care in BTM patients including blood transfusions combined with adequate chelation therapy have led to enhance years of survival but stimulantly the rate of its related complications such as cardiovascular disease (CVD) and arterial and venous thromboembolic events have increased also.[3,4]

The underlying mechanisms of BTM related complications were not determined clearly. Evidences indicated that inflammation and endothelial dysfunction have an important role in the pathophysiology of BTM and its related complications.[5-7]

Endothelium has a crucial role in the modulation of vascular tone. Furthermore endothelial dysfunction could be representative of vascular inflammation.[8] It is suggested that endothelial dysfunction through factors such as endothelial adhesion of thalassemic erythrocytes in microvessels, chronic hypercoagulable state and increased erythrocyte aggregation have role in this regard.[9-11]

It is believed that a chronic inflammatory state with increased level of pro-inflammatory cytokines is present in patients with BTM.[5] The most frequently studied adipocytokines are leptin and adiponectin.[12,13] Visfatin is a novel pro-inflammatory adipocytokine which predominantly expressed in visceral adipose tissue.[14] Several experimental studies indicated its role in inflammatory processes and chronic inflammatory diseases.[15,16] The potential properties of visfatin makes it as a target for novel therapeutic strategies in different inflammatory and metabolic diseases.[17] The role of visfatin in BTM has not studied yet.

It seems that understanding its possible role in the pathogenesis of BTM and its relationship with markers of endothelial function could help us to provide more effective therapeutic approaches for treatment of patients with BTM and its related complications. The aim of current study was to compare serum level of visfatin between patients with BTM and control group and determine its correlation with markers of endothelial function, intracellular adhesion molecule (ICAM) and vascular adhesion molecule (VCAM).

METHODS

In this case-control study patients with BTM receiving regular blood transfusion aged 10-20 years and a group of age, sex, body mass index and pubertal stages matched healthy subjects were enrolled.

Patients with BTM were selected from the Imam Ali clinic of endocrinology and hematology, affiliated to Shahrekord University of Medical Sciences from. Diagnosis of BTM was based on clinical and hematological (complete blood count and hemoglobin electrophoresis) evaluation. Control groups selected from healthy subjects referred to Imam Ali pediatrics clinic for regular annual follow-up.

Subjects who were smoker or had a history of diabetes mellitus, hypertension, hypothyroidism, hyperlipidemia, valvular heart disease, heart failure and renal or hepatic disease were excluded from the study.

The protocol of study was approved by Pediatrics Review Board and Regional Bioethics Committee of Shahrekord University of Medical Sciences. Written informed consent was obtained from all selected patients or their parents.

Selected patients were recalled and demographic characteristics and details related to the history of disease in thalassemic patients were recorded by a trained nurse using a questionnaire.

Selected subjects examined clinically by a pediatrician (anthropometrics measurements, vital signs and presence of any complications) and venous blood samples obtained for visfatin, ICAM, VCAM, cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and ferritin measurements. Blood samples obtained after 12 h fasting at 7:30-9:30 am. In thalassemic patients blood samples obtained before blood transfusion.

Mean (standard deviation [SD]) of studied laboratory measurements compared in two studied groups.

Laboratory measurements

Visfatin level was measured by ELISA method using Human Visfatin Kit (ADIPOGEN Inc., South Korea) with the sensitivity of 30 pg/ml.

ICAM and VCAM measured by ELISA method using Bender Med system, Human sVCAM-1-BM232-Austria and Bender Med System, Human sICAM-1-BM 201-Austria.

Normal range of visfatin, ICAM and VCAM was 14-50 ng/ml, 347-629 ng/ml and 675-1693 ng/ml, respectively.

Ferritin level was measured by ELISA method using human from Human company kit (Germany).

Cholesterol, triglyceride, HDL-C and LDL-C were measured using Pars Azmoon Kits (Tehran, Iran).

Statistical analysis

Obtained data analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, U.S.A.) software. Quantitive variables were presented as mean (SD). Mean of the study variables between groups were compared using *t*-test. Frequencies of higher level of visfatin, ICAM and VCAM between groups were compared using Chi-square test. $P < 0.05$ were considered significant.

RESULTS

In this study 31 patients with BTM and 30 healthy controls studied. Demographic and laboratory findings of studied population are presented in Table 1. High level of visfatin was reported in 25 (80.64%) and 9 (30.00%) of patients in BTM and control groups, respectively ($P < 0.001$). There was not any cases with higher rate of ICAM and VCAM.

Table 1: Mean±SD of demographic and laboratory variables in patients with beta-thalassemia major and healthy controls

Variables	Patients with beta-thalassemia major *n*=31	Control group *n*=30	*P* value
Female/male	12/19	11/19	0.54
Age (years)	14.45±4.82	14.56±4.29	0.92
BMI (kg/m^2)	18.37±2.72	19.55±2.72	0.07
Ferritin (ng/ml)	926.43±216.40	139.6±80.13	<0.001
ICAM (ng/ml)	15.25±6.44	17.72±8.12	0.215
VCAM (ng/ml)	57.64±15.89	81.98±14.72	0.025
Visfatin (ng/ml)	133.99±60.10	43.35±27.93	<0.001
Cholesterol (mg/dl)	116.58±21.12	116.26±20.44	0.95
Triglyceride (mg/dl)	120.09±36.73	81.53±22.59	0.06
LDL-C (mg/dl)	58.96±18.02	61.43±19.16	0.6
HDL-C (mg/dl)	36.41±7.4	38.4±4.37	0.21

SD=Standard deviation, BMI=Body mass index, ICAM=Intracellular adhesion molecule, VCAM=Vascular adhesion molecule, LDL-C=Low density lipoprotein cholesterol, HDL-C=High-density lipoprotein cholesterol

DISCUSSION

In this study we investigated the serum concentration of visfatin as well as ICAM and VCAM, the vascular inflammatory markers in patients with BTM and a group of healthy subjects to determine their possible role in the pathophysiology of BTM. Our findings indicated that visfatin was significantly higher in patients with BTM but there was not any relationship between visfatin and ICAM, VCAM and ferritin in two studied groups.

As mentioned the quality of care and life expectancy of patients with BTM have improved significantly due to proper treatment strategies but in the other hand the rate of BTM related CVD, endocrine and metabolic complication have increased also.[18] Though the responsible factors have not determined clearly but inflammation and endothelial dysfunction considered as potential factors in this field.[5,19]

The role of some adipocytokines and inflammatory factors in the pathogenesis of BTM and its complication have been studied in previous studies.[20] In this study we investigated the role of visfatin in this field. To the best of our knowledge it is the first study which evaluated the role of visfatin in BTM.

The role of visfatin in the pathogenesis of other diseases such as diabetes mellitus, insulin resistance, atopic dermatitis, chronic kidney disease and rheumatic disease have been reported.[21-24]

Kim *et al.* in Korea have indicated that visfatin could increases expression of ICAM and VCAM through reactive oxygen species-dependent NF-κB activation in endothelial cells.[25]

Evidences demonstrated that ICAM and VCAM considered as early endothelial dysfunction markers.[26] Thus in this study we examined the relation between visfatin with mentioned endothelial factors. It is supposed that in the presence of relationship between visfatin and vascular inflammatory factors we could identify high risk population by measuring the marker and consequently we could provide preventative strategies.

In this study though serum visfatin was higher in patients than control group but there was not significant relationship between visfatin and ICAM and VCAM. The level of ICAM was not different significantly in control and patients with BTM. Regarding VCAM, though it was significantly higher in control group than patients with BTM but it was in normal range of VCAM (normal range = 675-1693 ng/ml).

There was not any similar study in this field. But there were some similar studies which examined the role of other adipocytokines in this field.

Aggeli *et al*. in Greece evaluated serum levels of inflammatory mediators including interleukin-6, sVCAM-1 and sICAM-1 in 67 patients with BTM and 71 healthy controls. They showed that patients with BTM had impaired endothelial function and increased level of studied markers. They concluded that inflammation and endothelial dysfunction may have potential role in occurrence of BTM related complications.[19]

In another recent study in Greece, Chaliasos *et al.* have examined the serum levels of leptin and adiponectin and their correlation with vascular inflammation markers including endothelin (ET)-1, ET-3, VCAM-1, ICAM-1, L-selectin and E-selectin in 28 patients with BTM and a group of healthy controls. Their results indicated that patients with BTM have significantly lower level of leptin and higher level of adiponectin than control group. Patients had higher level of ET-1, VCAM-1 and E-selectin than control group.[27]

They did not find any correlations between leptin and vascular inflammation markers. There was positive correlation between adiponectin and ET-1. They concluded that from studied adipocytokines, adiponectin have a possible role in endothelial damage and CVD complication in patients with BTM.

In current study similarly we did not find any significant correlation between visfatin and ICAM and VCAM. Obtained data could be explained as follows considering the low mean age of our studied patients, possibly the process of endothelial dysfunction has not initiated yet. Also, it may be due to that it was the first stage of endothelial dysfunction when even we have not seen any increasing in vascular inflammation markers levels.

A confirmatory data was that lipids level both in patients and control groups were similar. Mean age of our studied population was 14.5 years whereas in the study of Aggeli *et al*. and Chaliasos *et al*. mean age of studied population was 24.6 and 18 years respectively.

In addition mean of ferritin was also lower than the mentioned studies. It may be due to lower duration of the disease. Lower duration of the disease could also explain the lack of relationship between mentioned factors with ferritin. Another explanation was small sample size of patients.

The limitation of current study was that endothelial function was not evaluated by intima-media thickness or flow-mediated dilatation. However they considered as reliable and noninvasive method for this purpose.

CONCLUSIONS

The higher level of visfatin among patients with BTM indicated the possible inflammatory role of this adipocytokine in BTM. It seems that for understanding the underlying mechanisms and its relation with vascular inflammatory markers and endothelial function further studies with larger sample size is needed.

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