https://doi.org/10.48047/AFJBS.6.16.2024.1100-1108

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Serum Amyloid A as A Biomarker of Iron Overload and Ineffective Erythropoiesis in Adult Egyptian Patients with β -Thalassemia

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Volume 6, Issue 16, Dec 2024
Received: 15 Oct 2024; Accepted: 05 Nov 2024; Published: 09 Dec 2024
doi: 10.48047/AFJBS.6.16.2024.1100-1108

Abstract

β-thalassemia (BT) is one of the most frequent hereditary hematologic diseases. The major complication of BT patients is iron overload that could lead to many organs damage and elevated mortality. The purpose of this study was to evaluate the role of serum amyloid A (SAA) in differentiate BT patients from other types of anemia and healthy controls. It was also to evaluate its association with iron overload and ineffective erythropoiesis in BT. Elevated SAA was related (P<0.0001) to BT (33.3 (31.2-48.5) ng/mL) compared to patients with other types of anaemia and healthy controls (14.7 (10.9-20.2) and 3.0 (1.0-6.5) ng/mL, respectively). It had a great power (AUC=1.00, P<0.0001) to discriminate BT patients. Its levels was increased (P=0.0399) in patients with ferritin>1000 ng/mL compared to those with <1000 ng/mL. It had a good ability to differentiate cases with iron overload (AUC=0.85, P=0.0129). Elevated SAA levels were significantly (P<0.05) correlated to elevated levels of ferritin (r=0.768), bilirubin (r=0.438) and ALT (r=0.675) and AST (r=0.678) activities, reduced hemoglobin (r=-0.691) and ineffective erythropoiesis [RBCs count (r=-0.256), MCV (r=-0.397) and PCV (r=-0.571)]. In conclusion, high level of SAA as new biomarker in patients with major BT is related to iron overload, mild/severe anemia and ineffective erythropoiesis.

Key words: β-thalassemia; Serum amyloid A; Biomarker; Iron overload; Ineffective erythropoiesis

1. Introduction

Beta (β)-thalassemia (BT) syndrome is the commonest autosomal recessive, monogenic hematologic disease. Worldwide with its high incidence and prevalence, it is becoming a serious major health problem [1, 2]. It is caused by β-globin gene mutation that is found in a locus located on chromosome 11 and β-thalassemia is characterized by absent or decreased beta globin chain production, causing declined hemoglobin (Hb) and red blood cells (RBCs) production and anemia [1, 3]. Each year, about 60000 newborns globally are born with the BT major, with the majority living in developing countries [4]. Carriers of BT account for about 3% of the world population. The Pacific islands, Melanesia, Southeast Asia, Indian subcontinent, Middle East, Mediterranean basin and the Africa are among the most affected areas [1, 5].

Inadequately untreated thalassemia patients, as a result of bone marrow expansion, commonly experience skeletal abnormalities, extramedullary hematopoiesis, leg ulcers, hepatosplenomegaly, weak musculature, jaundice, pallor, and growth retardation [2]. On the other hand, blood transfusions on a regular basis might cause blood iron excess. This iron accumulates in the liver, thyroid, pituitary, pancreas and heart and may lead to serious complication includes liver cirrhosis, hypothyroidism, hypogonadism, diabetes, heart failure and cardiomyopathy [6].

In β-thalassemia, the mention hemosiderosis leads to the production of free radicles particularly reactive oxygen species (ROS) and resulting in detrimental effects via Fenton reaction [7]. This is a vicious cycle of inflammation and oxidative stress. Into the circulation, a major effect of the released ROS and haeme is to promote vascular inflammation by activating NF-κB, which can then elevate adhesion molecules and pro-inflammatory cytokines expression on the endothelium [8]. One of the acute phase proteins (APPs) is serum Amyloid A (SAA) that bound to high-density lipoproteins (HDL) [9]. During phases of acute inflammation and because of its stimulation via pro-inflammatory cytokines, SAA levels can elevate 1000-fold [10]. In many studies as compared to C-reactive protein (CRP), SAA proved to be a more sensitive marker to measure active inflammation [10]. In BT patients, SAA levels before transplantation were markedly greater in cases who subsequently rejected the bone marrow transplant. Its levels increased after transplantation in acute graft versus host disease and rejection [11].

The aim of this study was to explore the possible utility of SAA for assessment of ineffective erythropoiesis and iron overload in adult Egyptian cases with β-thalassemia. Also, we aimed to evaluate the relationship of SAA with serum ferritin, as a routine assessment test, and RBC indexes in β-thalassemia patients.

2. Material and methods

2.1. The study population

This cohort study included 50 β-thalassemia adult cases, 30 with other types of anemia (20 with iron deficiency, 8 with hemolytic and 2 with hemorrhagic anemia) and 30 healthy individuals as controls. All patients were received blood transfusions regularly (thalassemia major), to keep hemoglobin level >9 g/dL, at the Hematology Unit, Mansoura Oncology Center, Mansoura University, Egypt. All the selected subjects were aged ≥18 years and BT was diagnosed with microcapillary method or high-performance liquid chromatography (HPLC). Patients' data and history such as blood transfusion frequency and family history of hematological disorder were collected. Cases who experienced malignancy, chronic infection or inflammation, acute inflammation (temperature>38°C), acute medical conditions (liver or heart failure) and pregnant females were excluded. Informed consents were obtained from all participants and the study protocol was approved by the Medical Research Ethics Committee, Institutional Review Board, Faculty of Medicine, Mansoura University (Code: R.23.02.2067) in accordance

with the ethical guidelines of the "Helsinki Declaration".

2.2. Laboratory measurments

Venous blood (5 mL) samples were collected from all participants and one part of the blood (treated with anticoagulant K-EDTA3) was subjected to complete blood count (CBC) estimation using completely automated hematology analyzer (Cell-Dyn emerald 1700, Abbott). Another untreated blood part was used for serum separation by centrifugation (4000 rpm, 15 minutes). Fresh cases' sera were screened for serum iron and ferritin using the chemistry auto-analyzer (Roche Cobas Integra-800, Roche Diagnostics, Switzerland). Also, liver and kidney function tests were determined using the same assay. The remaining of serum samples was directly frozen (-80°C) and stored for measurement of quantified SAA using the ELISA human kit provided from (Bioneovan, Beijing, China) based on the manufacturer's instructions.

2.3. Data analysis

The analyzed and collected data were processed using GraphPad version 6.0 and SPSS version 20 for Windows. Variables were expressed as mean±SD, absolute numbers or median (interquartile range), appropriately. Data analysis was conducted using the t-test, ANOVA and Kruskal-Wallis and Pearson correlation test, depending on the data distribution. The ability of SAA to differentiate BT patients was evaluated using area under the receiver operating characteristic (ROC) curve.

3. Results

3.1. Patients' characteristics

Healthy and patients with other types of anemia were sex- (P=0.129) and age-matched (P=0.441) to BT cases. The clinical and hematological data are shown in Table 1. Patients with BT were indicated mild to severe anemia included reduced hemoglobin, RBCs count and RBCs indices (Table 1). Levels of hemoglobin, mean cell volume (MCV), packed cell volume (PCV) and mean cell hemoglobin (MCH) were significantly (P<0.05) decreased compared to healthy controls. Moreover, as a result of regular blood transfusion for a long time in BT patients, there were high iron and ferritin levels (P=0.0001) (Table 1). Other iron overload-related consequences were associated with BT cases such as liver enzymes impairment (P=0.0001) and elevated bilirubin (P=0.0001) and WBCs count (P=0.001) (Table 1).

3.2. Reduced male serum TEST was associated with CRC

Increased SAA was related to BT, as BT cases (33.3 (31.2-48.5) ng/mL) were associated (P<0.0001) with greater SAA levels compared to patients with other types of anemia (14.7 (10.9-20.2) ng/mL) and healthy (3.0 (1.0-6.5) ng/mL) individuals (Figure 1). SAA had great ability (AUC=1.00; Figure 2) to differentiate BT patients from all non-BT (anemia patients and healthy combined).

3.3. Elevated SAA was associated with iron overload, ineffective erythropoiesis and liver impairments in BT patients

Among BT cases, SAA levels (ng/mL) was significantly (P<0.05) related to iron overload and its related consequences and implications. SAA was significantly (P=0.0399) increased in patients with ferritin>1000 ng/mL compared to those with ferritin<1000 ng/mL (Figure 3). It had a good ability to differentiate cases with iron overload (AUC=0.85; Figure 4). As shown in Table 2 and Figure 5, elevated SAA levels were significantly correlated to elevated levels of ferritin (r=0.768; P=0.0001), bilirubin (r=0.438; P=0.0007) and ALT (r=0.675; P=0.0001) and AST (r=0.678; P=0.0001) activities. In addition, it was associated with ineffective erythropoiesis including reduced hemoglobin (r=-0.691; P=0.0001), RBCs count (r=-0.256; P=0.0481), MCV (r=-0.397; P=0.019) and PCV (r=-0.571; P=0.0001).

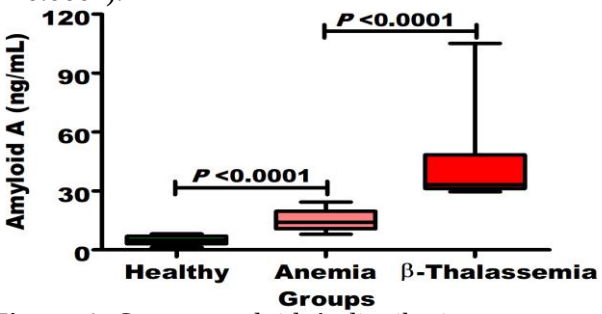

Figure 1. Serum amyloid A distribution among BT patients and controls.

Table 1. Clinical characteristics of patients and controls

Variables	Healthy	Anemia	β-Thalassemia	*P* value
Number	30	30	50	—
Sex (male/female)	16/14	15/15	29/21	0.129
Age (Years)	41.5±12.1	43.1±14.1	40.01±12.9	0.441
Hemoglobin (g/dL)	11.9±1.0	10.5±0.5	7.7±0.9	0.0001
RBCs ($\times10^{12}$/L)	4.4±0.6	4.1±0.8	3.2±0.8	0.045
MCV (fL)	73.6±11.6	67.9±3.3	64.1±6.1	0.018
MCH (pg)	26.9±5.3	23.5±5.4	22.3±4.4	0.015
PCV (%)	33.62±5.6	31.35±5.9	23.8±2.5	0.0001
WBCs ($\times10^{9}$/L)	4.7 (3.9-6.5)	4.6 (3.6-6)	9.5 (5.5-20.4)	0.001
Platelet count ($\times10^{9}$/L)	230 (200-370)	225.7 (137-363)	381 (145-560)	0.019
ALT (U/L)	13.1 (12-17.5)	14 (12-18)	32.5 (15.8-61.8)	0.0001
AST(U/L)	15.2 (12-19)	18.5 (15.9-23)	44 (27.5-68.3)	0.0001
Bilirubin (mg/dL)	0.59 (0.35-0.75)	0.6 (0.4-0.86)	1.72 (0.65-3.3)	0.0001
Albumin (g/dL)	4.15±0.38	3.87±0.66	3.88±0.66	0.220
Creatinine (mg/dL)	0.84±0.2	0.89±0.2	0.85±0.26	0.812
Ferritin (ng/mL)	12.6 (7-66.5)	13.5 (6.8-69)	908 (325-2455)	0.0001
Iron (ng/mL)	40.5 (30-72)	19.1 (10-49)	169.1 (130-201)	0.0001
Amyloid A (ng/mL)	3 (1-6.5)	14.7 (10.9-20.2)	33.3 (31.2-48.5)	0.0001
***Transfusion frequency**	10±4	—	—	—

Normally and non-normally distributed data were expressed as mean± SD and median (interquartile range), respectively. RBC: red blood cell; MCV: mean cell volume; MCH: mean cell hemoglobin; PCV: packed cell volume; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Significant differences were determined using *ANOVA* and *Kruskal-Wallis* test, appropriately. $P<0.05$ was significant. * Frequency in a year; # Types of anemia other than β-Thalassemia.

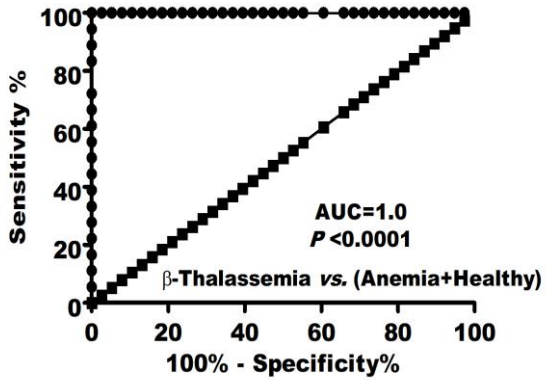

Figure 2. ROC curve analysis revealed the high diagnostic power of SAA for differentiating BT major patients.

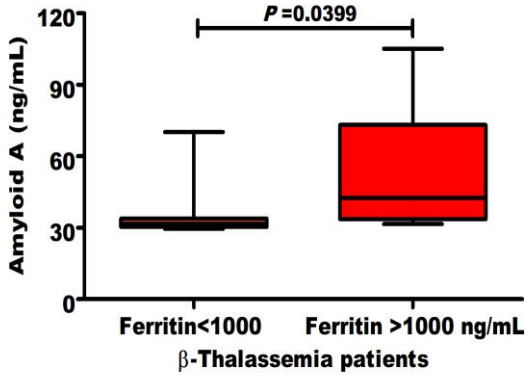

Figure 3. Serum amyloid A and iron overload. Patients with high ferritin levels (>1000 ng/mL) were associated with high SAA levels.

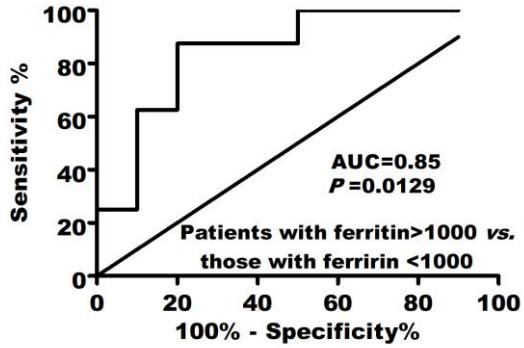

Figure 4. Serum amyloid A significantly differentiates patients with iron overload among BT patients.

Table 2. Correlation between SAA and other parameters related to β-Thalassemia

Factor correlated with SAA	Correlation coefficient (*r*)	*P* value
Ferritin (ng/mL)	0.768	**0.0001**
ALT (U/L)	0.675	**0.0001**
AST(U/L)	0.678	**0.0001**
Bilirubin (mg/dL)	0.438	**0.0007**
Hemoglobin (g/dL)	-0.691	**0.0001**
RBCs ($\times 10^{12}$/L)	-0.256	**0.0481**
MCV (fL)	-0.397	**0.019**
PCV (%)	-0.571	**0.0001**
MCH (pg)	-0.044	0.745
WBCs ($\times 10^{9}$/L)	0.179	0.187
Platelet count ($\times 10^{9}$/L)	-0.018	0.895

Pearson correlation was used for variables with interval scales.

4. Discussion

As major BT is intrinsic hemolytic disease and, up to the date, regular and repeated blood transfusion is the main therapy, major BT is the worst group of patients having the characteristic of severe anemia causing failure to thrive [12]. Owing to blood hemolysis, increased iron overload, and frequent blood transfusion, several iron overload-related organs injuries and health complications is the leading cause of morbidity and mortality in BT patients [13]. This study was conducted to study the possible utility of SAA in adult with BT major compared to patients with other types of anemia and healthy volunteers. Also, this study aimed to explore SAA for the evaluation of iron overload, mild to

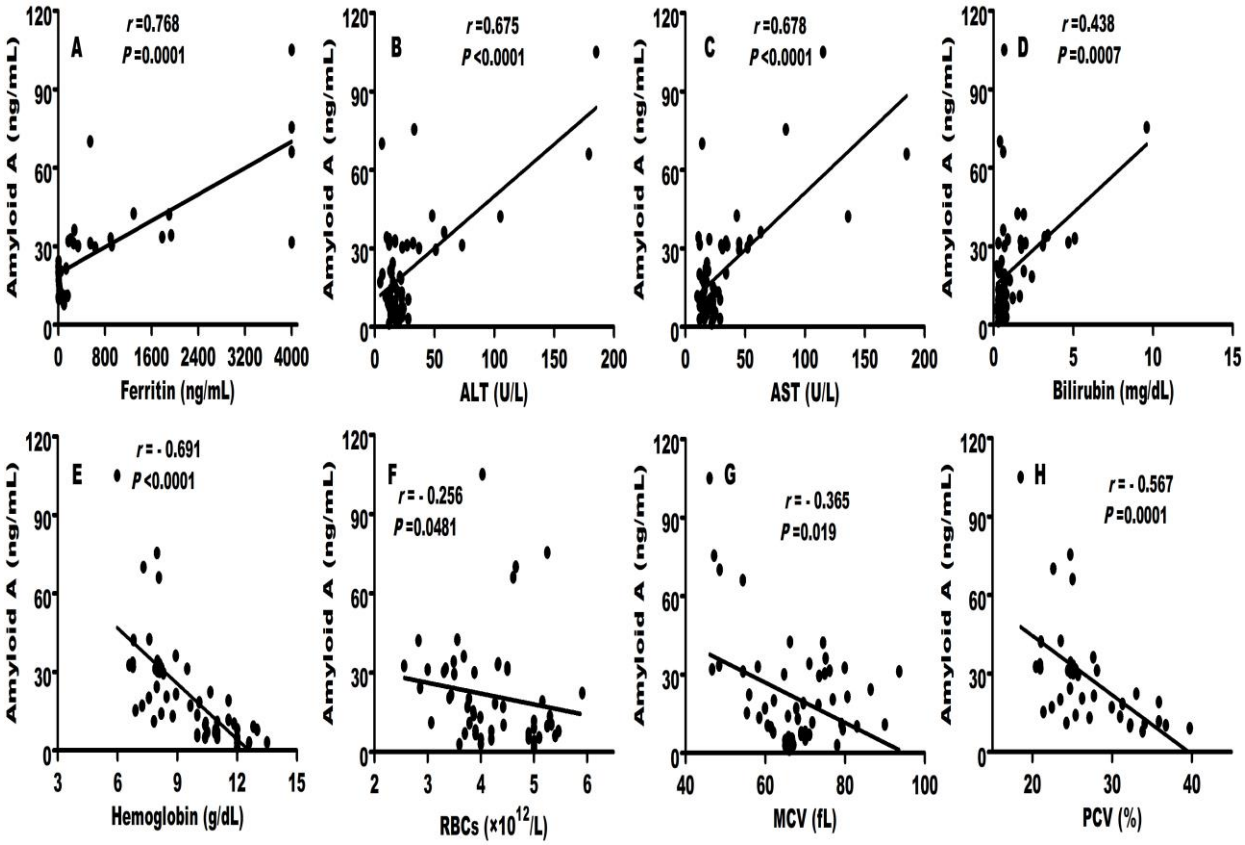

Figure 5. Correlation between SAA and iron overload and severe anemia related parameters in BT patients. Elevated SAA was significantly correlated with elevated levels of (A) ferritin, (B) ALT and (C) AST activities and (D) bilirubin. In addition, it was associated with ineffective erythropoiesis including reduced (E) hemoglobin, (F) RBCs count, (G) MCV and (H) PCV.

severe anemia and ineffective erythropoiesis in Egyptian BT major.

In this study, it was reported that elevated SAA was related (P<0.0001) to BT (33.3 (31.2-48.5) ng/mL) compared to patients with other types of anemia and healthy controls (14.7 (10.9-20.2) and 3.0 (1.0-6.5) ng/mL, respectively). It had a great power (AUC=1.00, P<0.0001) to discriminate BT patients. Interestingly, SAA levels (ng/mL) was significantly (P<0.05) related to iron overload and its related consequences and implications. Its levels was increased (P=0.0399) in patients with ferritin>1000 ng/mL compared to those with <1000 ng/mL. It had a good ability to differentiate cases with iron overload (AUC=0.85, P=0.0129). Elevated SAA levels were significantly (P<0.05) correlated to elevated levels of ferritin (r=0.768), bilirubin (r=0.438) and ALT (r=0.675) and AST (r=0.678) activities, reduced hemoglobin (r=-0.691) and ineffective erythropoiesis [RBCs count (r=-0.256), MCV (r=-0.397) and PCV (r=-0.571)].

In major BT patients, inflammation is known to have an important role in chronic inflammatory state and the pathogenesis of BT. Thus, pro-inflammatory cytokines such as IL-6 and APPs and other inflammation markers including CRP and SAA are may be increased in thalassemic cases [14, 15]. The findings of some former studies are in consistent with our results and may explain the association between elevated SAA and major BT and its related iron overload and ineffective erythropoiesis.

In some disorders that associated with iron overload, such as Alzheimer's disease, iron overload can cause elevated production of β-amyloid by affecting the processing and expression of amyloid precursor protein [16]. In a chronic renal failure patient, an interesting study found that amyloidosis in the bone marrow causes resistance to erythropoietin and consequently patient's anemia did not improve (after 6 months of erythropoietin treatment) [17]. Taking into account knowledge, this patient did not have any other erythropoietin resistance etiology such as hematological disorders, chronic inflammatory diseases, hyperparathyroidism, aluminum toxicity and iron deficiency [17].

As BT patients suffer from a chronic inflammatory condition [18], iron overload may has an important role in producing pro-inflammatory cytokines such as TNF-α and IL-6. Increased serum levels of these cytokines is relevant to BT pathophysiology, and this increased cytokines production may be due to macrophages overstimulation as a result of

repeated transfusion treatment and associates with iron metabolism abnormalities [19, 20]. Also in major BT, the increase in non-transferrin bound iron has related to ROS increase and its activation resulting in such pro-inflammatory cytokines production [21, 22]. These pro-inflammatory cytokines induce the expression of several APPs, including SAA, CRP and plasminogen activator inhibitor-1 [23-26].

Similar to our results, before transplantation and compared to patients without complications, it was reported that SAA levels were significantly greater in patients who subsequently rejected the transplant in BT patients under bone marrow transplantation [11].

5. Conclusions

This study confirmed that elevated levels of SAA are found to be associated with patients with major BT, but not with other types of anemia like iron deficiency, hemolytic or hemorrhagic anemia. Elevated SAA levels in BT cases could be used as a biomarker of iron overload, severe anemia and ineffective erythropoiesis.

Disclosure statement

None

Funding

None

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