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Assessment of B2-Microglobulin Analysis for Renal Insufficiency in Iraqi β-Thalassemia Major Patients

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ABSTRACT: ***Background:*** *Beta-thalassemia is the most prevalent genetic hemoglobin apathy in the world. It is caused by a reduction or absence of beta-globin chain production, which is typically a portion of adult hemoglobin (HbA, which is α2β2). This genetic anomaly will lead to a rapid erythrocyte turnover, severe anemia, and compensatory ineffective erythropoiesis. The purpose of the current research is to reveal diagnostic, and predictive biomarkers that can be performed to detect the decline in renal function in β-thalassemia major patients with early stage renal impairment with high sensitivity and specificity, ascertain changes in B2-microglobulin as a biomarker in β-thalassemia patient.* ***Objective:*** *The samples were collected from Karama Hospital-Genetic Hematology Center/Baghdad during the period from the 1st of September 2022 until the 1st of January 2023.* ***Methods:*** *The patient group consisted of 45 patients with β-thalassemia major with repeated blood transfusion and the control group consisted of 45 individuals who seemed to be healthy. Age ranged between (18-35) years (for patients and control). The serum samples were used to measure biochemical parameters, S.Creatinine, blood urea, and Urine B2-microglobulin were measured from urine samples. B2-microglobulin level in Urine was estimated by Sandwich-enzyme-linked immunosorbent assay (ELISA) technique, while serum Blood Urea and Serum Creatinine concentration were calculated by Colorimetric Kit by Spectrophotometer method, and Ferritin level in blood was estimated by BioMrieux Mini Vidas.* ***Results:*** *The statistical examination was carried out using SPSS software. B2-microglobulin biomarkers in the thalassemia Patient group were significantly higher than those in the control group ($p<0.001$). While serum ferritin, B.Urea and S.Cr increased in patient groups compared to the control group ($p<0.005$).* ***Conclusions:*** *All parameters included in this study are significantly higher in β-thalassemia patients than in healthy subjects. Renal hemosiderosis and asymptomatic renal dysfunction are prevalent among β-thalassemia major patients with repeated blood transfusions, which are not found in routine renal investigations.*

KEYWORDS: *β-thalassemia major; B2-microglobulin; Ferritin; B.Urea; Creatinine*

INTRODUCTION

The alpha- or beta-globin chain of hemoglobin is completely absent or only partially synthesized in people with thalassemia, a genetic condition. Even while thalassemia is typically asymptomatic or only mildly related to anemia, several patients need lifelong blood transfusions to survive [1]. Although patient survival rates have greatly increased in recent years, this disease's consequences in several organs can still hurt patients' quality of life [2]. Therefore, β-thalassemia causes a decrease in the production of hemoglobin and an accumulation of α-globins that result in the formation of insoluble heme chromes, measurement of hemoglobin level for patients is required to determine the severity of anemia [3]. Oxidative stress and lipid peroxidation are caused by hypoxia and chronic anemia, which decrease tubular cell function [4]. Additionally, in thalassemic individuals, iron excess plays a significant role in the etiology of kidney damage [5]. Additionally, glomerular dysfunction

can be a side effect of iron chelator poisoning; in addition, renal impairment may be brought on by iron overload-induced hepatic and cardiac dysfunction [6]. Hemoglobin (Hb), a hemoprotein found in red blood cells, is principally responsible for carrying oxygen from the lungs to the tissues. Two other functions are the transfer of carbon dioxide (CO_2) and the buffering action. Heme is required for oxygen transport even though globin protects it from oxidation, makes it soluble, and allows for variation in oxygen affinities [7]. The structure of the HB is Heme groups and polypeptide chains are present in each of the four HB subunits. The iron protoporphyrin IX prosthetic heme group is connected to a polypeptide chain with residues of 141 (alpha) and 146 (beta) amino acids in all hemoglobins. A histidine's N is coupled to the heme's ferrous ion. Its polypeptide chain contains phenylalanine that forces the porphyrin ring into its pocket. The two types of polypeptide chains known as alpha and beta chains that make up adult hemoglobin are comparable in length but distinct in the sequence of their amino acids [8]. The main pathophysiological characteristic of β-thalassemia is the reduced production of β-globin chains. The inescapable imbalance in the α / β-globin ratio and accumulation of α-globin causes the erythroid lineage to experience oxidative stress, apoptosis, and ineffective erythropoiesis. Iron overload and increased intestinal iron absorption are caused by inadequate hepcidin production and compensatory hematopoietic development [9]. Later clinical signs include splenomegaly, extramedullary hemopoiesis, marrow enlargement, and bone marrow. In addition, excessive iron from blood transfusions and ineffective erythropoiesis build up in vital organs, causing neurological issues, diabetes, liver fibrosis, and heart failure [10]. Renal dysfunction in β - thalassemia major (BTM) advances in the management of patients (BTM) and the advent of effective chelators have led to the discovery of many renal complications. Mechanisms of renal impairment in BTM are still not fully investigated [11]. Chronic anemia and hypoxia may result in oxidative stress lipid peroxidation and finally impairment in tubular cell function. In addition, iron overload due to repeated blood transfusions is a critical factor in the pathogenesis of kidney injury in thalassemic patients [12]. β2-microglobulin is a single-chain polypeptide, with low molecular weight (11.8 kDa) released by all nucleated human cells [13]. The production of β2-microglobulin in normal subjects is quite constant, about 0.13 mg/h kg. The catabolism is almost exclusively through renal elimination [14]. The protein readily passes the glomerular membrane; subsequently, more than 99.9% of the filtered β2-microglobulin is reabsorbed and degraded in the proximal tubules, with only about 5 micrograms/h of the protein appearing in the final urine. Proximal tubular dysfunction leads to an increased urinary concentration shown in Figure 1 [15]. The amount of this marker is very low in healthy individuals, but its level increases in conditions such as neoplastic, inflammatory, and immunologic conditions [16]. Impaired uptake as a result of tubular injury results in increased β2-microglobulin urinary excretion, and thus B2M is considered a direct marker of tubular dysfunction. However, increased β2-microglobulin production or isolated glomerular disease may increase urinary excretion as well [17]. Furthermore, clinical studies have investigated β2-microglobulin as a useful biomarker for predicting poor outcomes in patients with kidney [18]. In CKD the serum β2- microglobulin had the best diagnostic value. Periodic renal assessment of renal patients is mandatory as they may be affected by hidden renal dysfunction [19].

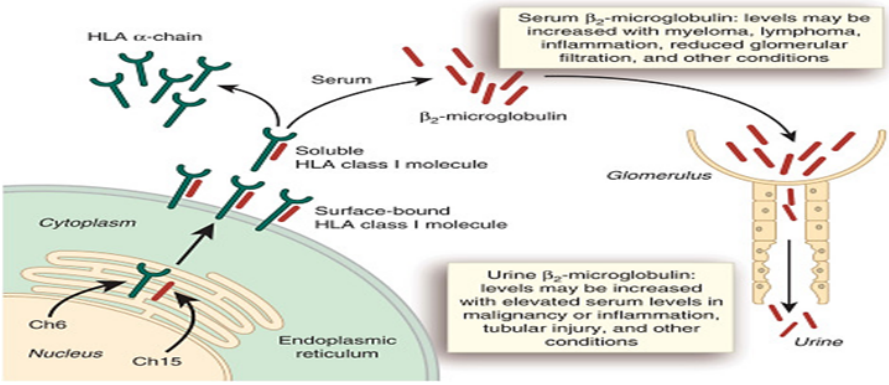

Figure 1. production, circulation, catabolism and excretion of B2 macroglobulin in health and disease [20]

MATERIALS AND METHODS

The samples were collected from Karama Hospital-Genetic Hematology Center/Baghdad during the period from the 1st of September until the 1st of January 2023. In this case-control study, there are

two groups: β-thalassemia major patients, and those who appear to be healthy as a control group.

Patients and Control

The patient group consisted of 45 patients with β-thalassemia major with repeated blood transfusion who had received 6 units of blood or more within 6 months. The control group consisted of 45 individuals who seemed to be healthy.

Chemicals and Methods

1. The sandwich-ELISA kit as the method was used to determine urine β2-microglobulin. In this kit, an antibody specific to β2-microglobulin was pre-coated to the micro-ELISA plate (Bioassay Technology Laboratory, ELISA kit).
2. Serum Blood Urea, and Serum Creatinine concentration was calculated by Colorimetric Kit (France-Biolabo) by Spectrophotometer method, and Ferritin levels in blood were estimated by BioMrieux Mini Vidas (France) is an enzyme-linked fluorescent immunoassay (ELFA) performed in an automated instrument.

RESULTS AND DISCUSSION

Demographic Characteristics in Patients and Control

1 Age Distribution in Patients and Control

The distribution of patients with β- thalassemia major according to age is shown in Table 1. A total of patients with thalassemia was (45) included in this study whose ages ranged (18-35) years. The control group (45).

Table 1. Age distribution in patients and control

Parameters	Mean ± SD		P-value
	patients group (n=45)	Control group (n=45)	
Age (18-35)	26.82 ± 5.97	26.06 ± 6.56	0.904

SD= standard deviation; p 0.05: significant

2 Gender Distribution in Patients and Control

Among 45 patients with β-thalassemia and the healthy group who contributed to this study, there were 18(40%) males and 27(60%) females.

Beta2-microglobulin (β2M)

Assessment of β2-microglobulin in Thalassemia patients showed that the Mean ± SD for the patient was (7.1 ±1.6) and for the healthy control was (1.4 ± 0.95) respectively. The results revealed that there was a significant difference in the level of β2M between the patients and their control group (p-value<0.05), the results are listed in Table 2 and in Figure 2.

Table 2. Comparison of β2M level in patients and control

	group	N	Mean ± SD	P-value
B2-microglobulin mg/ml	patients	45	7.1 ±1.6	0.001
	control	45	1.4 ± 0.95	

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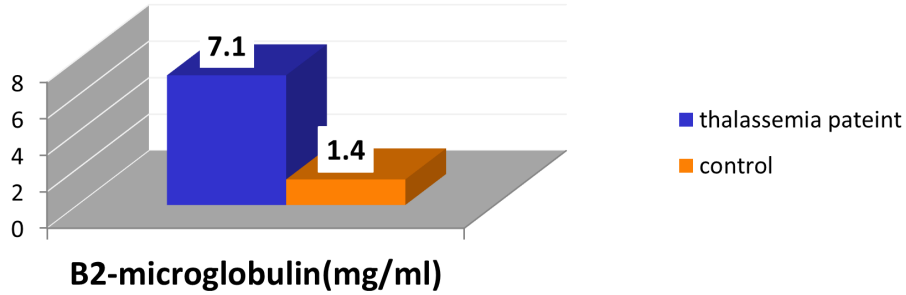

Figure 2. Comparison of β2-microglobulin level in patients and control groups

A low-molecular-weight protein called urinary β2 microglobulin (u β2MG) is freely filtered by glomeruli, reabsorbed by renal tubules, and then eliminated. Due to its continual production, is thought to be a more reliable endogenous measure of early glomerular filtration rate (GFR) affection than creatinine. For monitoring glomerular and tubular dysfunction in β -TM, β2MG is a sensitive early biomarker [21]. The study documented that the mechanism leading to the damage is not clear, but that it may be related to the increase in oxidative stress secondary to the accumulation of iron in the tissues [22]. Furthermore, the current study revealed that there was a significant difference in urine β2M between the patient groups and the healthy group. β2M as well as serum ferritin and liver iron deposition were found to be significantly positively correlated, according to Kacar *et al.* [16]. Serum ferritin levels were discovered to be associated with β2M levels by Uzun *et al.* [23]. We found that serum ferritin is correlated with the level of β2 M. The risk of glomerular and tubular dysfunction may increase with iron buildup in the body.

Serum Creatinine and Blood Urea

The results revealed that there was a significant difference in the level of serum Creatinine and Urea between the patients and the control group (p-value<0.05), the data are listed in Table 3.

Table 3. Comparison of S-Cr,and Urea levels in patients and control groups

	Group	N	Mean± SD	P-value
B.Urea mg/dl	Patients	45	30.4 ± 8.97	0.004
	Control	45	19.2 ± 7.47	
Creatinine mg/dl	Patients	45	0.60 ± 0.10	0.003
	Control	45	0.45 ± 0.23	

Although the levels of urea and creatinine are within the normal limit, there is a notable difference between the patients and the control group, and this is similar to the study conducted by M. Economou *et al.* 2010 [24].

Serum urea, creatinine, and urine protein excretion levels are monitored in deferasirox users to manage renal function. Previous research, however, has indicated that the main renal damage stages are not effectively predicted by serum creatinine and proteinuria levels [25]. The fact that muscle mass, protein intake, inflammatory conditions, and liver disorders can all have an impact on serum creatinine and urea levels [26].

Assessment of Ferritin in Thalassemia patients showed that the Mean ±SD for the patient was (3701 ±1974) and for the healthy control was (135 ± 88). The findings demonstrated a significant difference in Ferritin levels between patients and their healthy group (P<0.01), as illustrated in Table 4.

Table 4. Differences between patients and control groups for Ferritin level

	Group	N	Mean ± SD	P-value
Ferritin ng/ml	patients	45	3701 ±1974	0.001
	control	45	135 ± 88	

Ferritin levels provide for predicting variables to predict a range of clinical outcomes in β-thalassemia patients. β-thalassemia patients had a significantly higher frequency of renal Iron overload [27].

Iron deposits in organs such as the heart, kidney, endocrine glands, liver, and others as a result of repeat blood transfusions harm the tissue and lead to organ malfunction or failure. Multi-organ failure caused by hemosiderosis is still widespread, although chelating treatment significantly increases survival in patients receiving continuous transfusions [28]. On the other hand, the measurement of ferritin is even now a repeatable and low-cost method to assess iron overload and the performance of iron chelation therapy [29].

Serum ferritin levels and renal disorder significantly positively correlated (P<0.001). In conclusion, serum ferritin levels may be used as a prognostic marker for predicting renal impairment in patients [30]. This result similar to the study was done by (Mohamed R. El-Shanshory *et al.* 2021) [31].

ROC Curve of β 2-microglobulin (B2M)

ROC curve for the sensitivity and specificity of B2M (mg/l) for diagnosis of renal dysfunction in BTM, (Cut-off point was $\geq$ 3.8 (mg/l)), AUC=0.96, P=<0.001, the sensitivity and the specificity was 95.6 %, 91.2 % respectively, as shown in Figure 3.

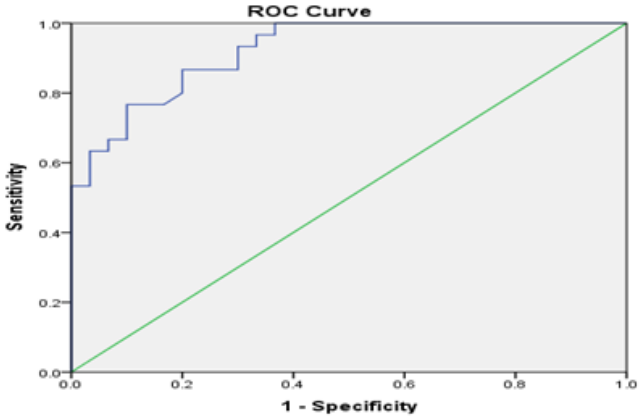

Figure 3. ROC Curve of (B2-MG)

CONCLUSION

All parameters included in this study are significantly higher in β-thalassemia patients than in healthy subjects. Renal hemosiderosis and asymptomatic renal dysfunction are prevalent among β-thalassemia major patients with repeated blood transfusion s, which are not found in routine renal investigations. B2-MG is a sensitive, specific, and highly predictive early indicator for acute renal injury in individuals with BTM when subclinical kidney damage or dysfunction is expected before serum Creatinine increases.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Estabraq Salman Alwan: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing original draft preparation, and writing review. Thana Mohammed Juda and Liqaa M. Majed: Editing, visualization, supervision, and project administration.

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None.

DATA AVAILABILITY STATEMENT

Data is available in the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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