

# Assessment of Early marker of Renal Dysfunction in Patients with beta - Thalassemia Major with Repeated Blood Transfusions

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## ABSTRACT

A case-control study include 60 patients with beta-thalassemia major type with recurrent blood transfusion were visited Babylon center of hereditary blood disorder in Babylon teaching hospital for maternity and children and 60 individuals who seemed to be healthy. This study aimed to assess N-acetyl-beta-glucosaminidase and others renal function and to look for renal complications in those patients if any. These patients and control groups divided into two subgroups according to age, group I were less than 18 year and group II were equal or more than 18 years. The present study's findings showed that there was a significant difference in the levels of Albumin to creatinine ratio, Beta2-microglobulin, N-Acetyl beta-D-glucosaminidase, serum creatinine level, and blood urea between patients group and its healthy group ( $p < 0.05$ ). In conclusion, beta-thalassemia major patients frequently have renal hemosiderosis and asymptomatic renal impairment, which are not found in routine renal investigations, it needs regular checks for early detection of tubular and glomerular failure.

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## INTRODUCTION

The thalassemia syndromes are a group of inherited autosomal recessive conditions associated with hemolytic diseases that are brought on by defects in the erythropoiesis process and the generation of hemoglobin.<sup>1,2</sup> The Greek terms "Thalassa," which means "the sea," and "Haima," which means "blood," are the origin of the word "thalassemia."<sup>3</sup>

Dr. Cooley first described thalassemia in 1925.<sup>4</sup> There are two main types of thalassemia, alpha and beta. According to polypeptide chain defects of hemoglobin in red blood cells, alpha thalassemia is caused by a failure in the rate of synthesis of alpha chains, and beta thalassemia is caused by a defect in the rate of synthesis of beta chains.<sup>3</sup>

Ineffective erythropoiesis(IE) and RBCs hemolysis are the reason for the anemia, therefore blood transfusions process of packed RBC are a common treatment for most thalassaemic patients.<sup>1</sup> Because 200 to 250 mg of iron are present in each unit of blood, repeated transfusions generate excessive iron loading, which can lead to transfusional siderosis with accumulation of iron in different organs.<sup>5</sup>

The excess of  $\alpha$ -chain is unstable, and it ultimately precipitates and disintegrates, causing damage to the red blood cell membrane. Premature hemolysis of damaged RBCs in the bone marrow and spleen causes accelerated RBC turnover, inefficient erythropoiesis, and severe anemia. Iron overload, caused by inefficient erythropoiesis and early hemolysis in the plasma and major organs for example liver, heart, and endocrine glands, is among the principal consequences of this hereditary condition.<sup>6</sup> As a result, iron chelators such deferoxamine, deferiprone, and deferasirox are necessary to keep these individuals alive. The survival of BTM patients with repeated blood transfusions is reduced without proper and suitable chelation treatment.

The kidney's putative role in  $\beta$ -thalassemia patients has received little consideration. Recent studies, however, have revealed the presence of various tubular and glomerular abnormalities[7]. Renal failure can develop in BTM patients without clinical manifestations before the onset of any additional

## KEYWORDS:

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consequences caused by chronic hypoxia, anemia, severe iron overload (haemosiderosis), and excessive chelation use[8]. It is essential to look into ways to effectively assess renal function in order to detect kidney disease early.

Previously, serum creatinine and other traditional tests were employed as a measure since they are easy to quantify from a blood sample. However, despite a regular blood test indicating serum creatinine as normal, 50% of persons have indications that their kidneys are impaired; this has a weak correlation with the most accurate evaluation of renal function.<sup>9,10</sup> So, early prognostic indicators of renal impairment in Beta-thalassemia major are urgently needed, as early management can considerably improve prognosis.<sup>11</sup>

The glomerular filter keeps higher molecular weight proteins in the circulation, smaller molecular weight proteins are readily filtered, reabsorbed, and catabolized within tubular cells. As a result, the presence of significant levels of protein in the urine indicates renal dysfunction.<sup>12</sup>

The enzyme N-acetyl-beta glucosaminidase (NAG) is a lysosomal enzyme is found in the kidneys' proximal convoluted tubules and can serve as a good indicator of proximal tubular injury and nephrotoxicity. The NAG molecule has a relatively greater molecular weight of around 130000-140000 Da, which prevents it from passing through the kidney's glomerular basement membrane. Finally, the liver quickly eliminates NAG from circulation.<sup>13</sup> Urinary NAG is a simple, quick, and noninvasive approach for detecting and monitoring renal tubular function in a variety of situations.

In a wide range of hematologic and non-hematologic disorders, serum beta2 microglobulin has now been recognized as a significant prognostic predictor. Despite normal plasma levels, urine beta-2-microglobulin levels are high in renal tubular diseases, reflecting a problem with proximal tubule reabsorption.<sup>14</sup>

## MATERIALS AND PROCEDURES

### Study Design

In this case control study, there are two groups: the first includes 60 beta thalassemia major patients, and the second includes 60 individuals those who appear to be healthy. This research was carried out at the laboratory of the College of Medicine / University of Babylon. The collecting of samples carried out from November 2021 to January 2022.

### Ethical Approval and Consent

All participants in this study were informed before to collecting samples, and verbal agreement was obtained from each of them.

### Criteria for Exclusion:

1. Any patient with nephropathy.
2. Any patient with diabetes mellitus.
3. Pregnant.
4. Any patient with chronic liver disease.
5. Patients with other hemoglobinopathies.

### Methodologies

Venous samples oh whole blood were obtained from control group and patients and the serum that obtained from the

blood were used to estimate serum ferritin by vidas technique, serum creatinine, and blood urea by spectrophotometer. Early morning urine sample collected from patients and control used to estimate urinary NAG by ELISA technique, B2M by vidas technique, and albumin to creatinine ratio by DCA Vantage device.

## RESULTS AND DISCUSSION

### General Characteristics

Sixty patients (27 females and 33 males) with B- thalassemia major and 60 healthy control (27 females and 33 males) were contributed in this study. The age of patients were between (6-35) years. These patients and control groups divided into subgroup according to age, group I were less than 18 year, , group II were equal or more than 18 years. The mean and other statistical parameters as displayed in Table 1.

### Biochemical Parameters

The comparison of some renal function markers between patients with BTM and healthy control showed that significant difference in all parameters in this study. The statistical analysis shown in Table 2.

Albumin to creatinine ratio between the patients and the control group in this study were found to be significantly different, as shown above. Three studies among many that have looked for urine albumin/creatinine ratio in those sufferers of beta-thalassemia reported that beta-thalassemia patients had a considerable increase in this parameter compared to healthy individuals, similar to our findings.<sup>15-17</sup>

BTM anemia lowers systemic vascular resistance, which leads to increase glomerular filtration rate and renal blood flow. These modifications can contribute to glomerular capillary stretching and subsequent capillary damage, as well as macromolecule transudation into the mesangium, which is linked to glomerular dysfunction. Furthermore, apoptosis, cytokine release, tubulointerstitial damage, and glomerulosclerosis are

**Table 1:** Age distribution in study group, patients and control

Age group	N	Mean ± SD		P-value
		Patient	Control	
Group I (< 18 years)	38	14.00 ± 2.75	12.76 ± 2.86	0.059
Group II (≥18 years)	22	23.13 ± 4.45	22.09 ± 4.22	0.429

**Table 2:** Comparison of some renal function markers between patients and control

Parameter	Mean ± SD		P- value
	Patients (N=60)	Control (N=60)	
ACR (mg/g)	51.2 ± 23.14	15.5 ± 11.31	0.005
NAG (ng/ml)	131.5 ± 55.2	77.0 ± 25.1	< 0.001
B2M (mg/l)	0.12 ± 0.048	0.06 ± 0.035	0.003
Blood Urea (mg/dl)	31.7 ± 5.8	20.6 ± 5.13	<0.01
Creatinine (mg/dl)	0.57 ± 0.1	0.51 ± 0.1	0.04

ACR, Albumin creatinine ratio; B2M, Beta2-microglobulin; NAG, N-acetyl-beta-D-glucosaminidase

all caused by persistent hypoxia and heavy iron overload in tubular cells.<sup>18</sup>

The result in Table (2) show comparison of the level of NAG in urine between the patient group and the healthy control group that revealed a high significant increase in the patient group ( $p < 0.05$ ). Increased NAG level in the urine indicate the presence of an injury to the renal tubes, as previous studies have shown, Mohkam et al.2008 and Koliakos et al.2003, they discovered that increasing urine NAG in individuals with  $\beta$ -thalassemia major, which they identified as the primary cause of this dysfunction as iron excess.<sup>8, 18</sup>

The increase level of urinary NAG is attributed to tubular impairment and considering that NAG is not of plasmatic source and is not filtered by the glomeruli, it is regarded as a sensitive and dependable indicator of proximal tubule damage and a probable prediction of proteinuria.<sup>19</sup>

The results in present study show significant difference of B2M level between patients and control at ( $p$ -value 0.003), which similar to Ong-ajyooth et al.1998, one of the first studies that searched renal tube abnormalities in patients with beta-thal/ Hb E disease. The authors explained that it is unclear how the

harm occurs, however they think that it may be associated to the increase in oxidative stress brought on by the accumulation of iron in the tissues, increased serum and urine malondi-aldehyde (MDA) levels serve as an indication of this condition.<sup>20</sup>

In our study, there statistically significant differences of B2M level were found between patients subgroup according to age, as shown in Table (3) this result similar to study was done by Jalaly et al. 2011, revealed that older age, more blood transfusions, and hypercalciuria can all increase the risk of renal impairment in thalassemia major patients.<sup>21</sup>

Although the level of Urea and creatinine are within the acceptable range, but significant difference when compered the patients and the control group. Moreover, there is a significant difference for creatinine between group I and group II of patients, and this is similar to the study conducted by Helin et al .1998, Ali et al. 2008.<sup>22,23</sup>

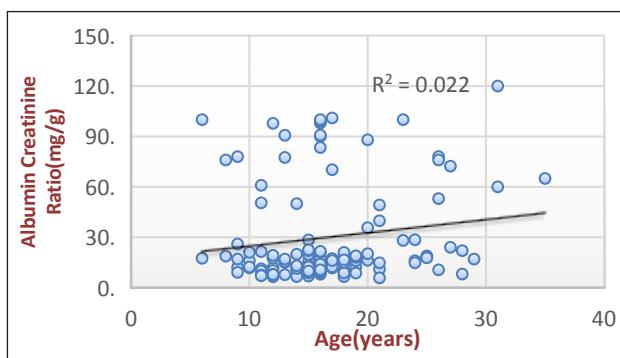
Excess iron and shortened red cell lifespan cause functional and physiological problems in numerous organ systems, with a significant frequency of renal tubular abnormalities in  $\beta$ -thalassemia patients.<sup>24</sup>

In this study, age has a positive correlation with ACR and 2M. P-value: 0.000, p-value: 0.003 respectively, as seen in figures 1 and 2. As well as between age with serum creatinine and urea. It is possible that the increase in the renal dysfunction markers with increasing the age of patient is a result of the increase duration of disease and therefore the number of blood transfusions. Significant positive correlation between ACR with B2M and significant positive correlation between NAG with B2M, as seen in figures 3 and 4.

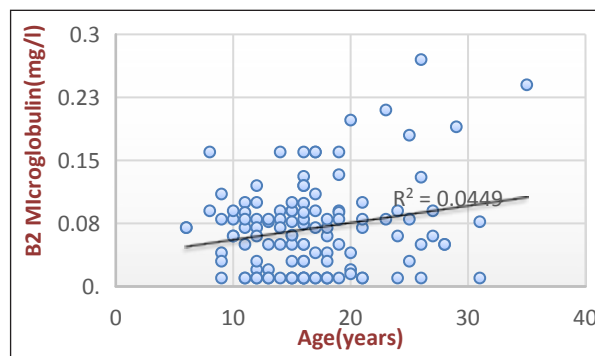
ROC curve for the sensitivity and specificity of NAG (ng/mL) and the diagnostic value of this marker to assess the renal dysfunction in beta thalassemia major, (Cut-off point was  $\geq$

**Table 3:** Comparison of some renal function markers between age subgroup in patients

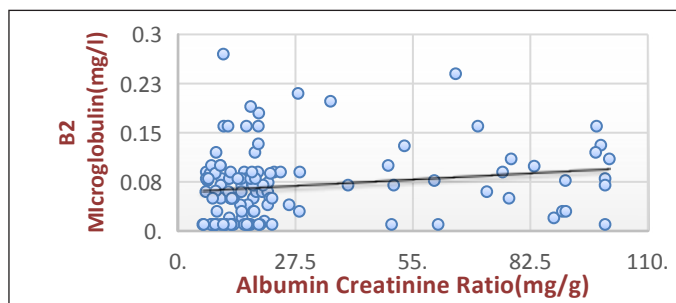
Parameter	Mean $\pm$ SD		P- value
	Group I (N=38)	Group II (N=22)	
ACR (mg/g)	36.4 $\pm$ 18.12	42.3 $\pm$ 26.75	0.515
NAG (ng/ml)	134.9 $\pm$ 53.8	125.8 $\pm$ 58.4	0.54
B2M (mg/l)	0.6 $\pm$ 0.03	0.11 $\pm$ 0.06	0.04
Blood Urea (mg/dl)	31.0 $\pm$ 4.6	32.9 $\pm$ 7.4	0.23
Creatinine (mg/dl)	0.54 $\pm$ 0.08	0.61 $\pm$ 0.11	0.01



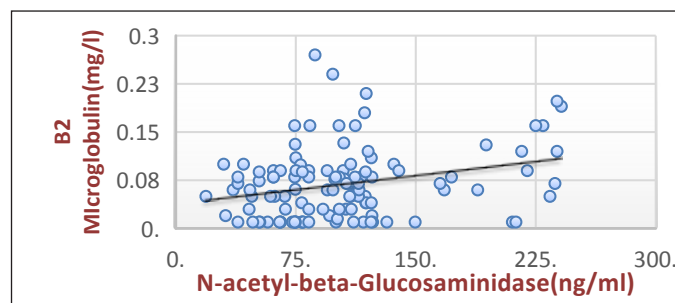
**Fig. 1:** The correlation between age and ACR



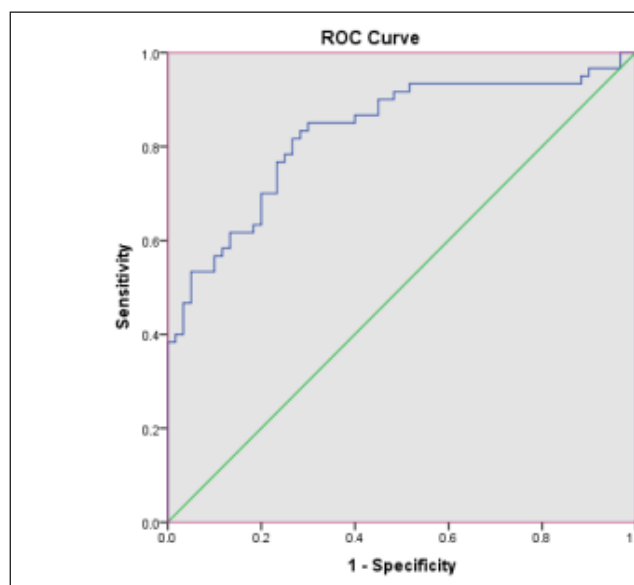
**Fig. 2:** The correlation between age and B2M



**Fig. 3:** The correlation between B2M and ACR



**Fig. 4:** The correlation between B2M and NAG



**Fig. 5:** ROC curve for urinary NAG level in-patient with BTM

93.3 (ng/mL)), AUC=0.83, P= <0.001\*, 95% CI (0.755-0.904), the sensitivity and the specificity was 83.3 %, 71.7 % respectively, positive predictive value (PPV) was 74.6%, negative predictive value (NPV) was 81.1%, as shown in figure (5)

## CONCLUSIONS

Compared to healthy participants, B-thalassemia patients had considerably higher levels of all study-related parameters.

In B-thalassemia major patients receiving frequent blood transfusions, renal hemosiderosis and asymptomatic renal impairment are common, which are not found in routine renal investigations. Urinary NAG are sensitive, specific, and highly predictive early indicators for acute renal injury in individuals with BTM when subclinical kidney damage or dysfunction is expected before serum creatinine increases.

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