

Role of Serum Beta2 Microglobulin as Early Biomarker to Find Hidden Renal Dysfunction in Children with Beta Thalassemia Major

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Abstract

Background: For beta thalassemia major, there is advanced treatment and chelation therapy. But the unexpected complications like renal dysfunction is emerging nowadays.

Aim: To study the significant role of serum beta2 microglobulin as the early biomarker to find the hidden renal dysfunction in children with beta thalassemia major (β -TM).

Subjects and Methods: The study was performed on 30 children with β -TM and 30 apparently healthy children matched for age and sex as a control group. For all the enrolled children, a comprehensive medical history was obtained, and complete physical examination was performed, blood urea, serum creatinine, estimated glomerular filtration rate (eGFR) by original Schwartz formula and protein creatinine ratio in urine, serum beta2 microglobulin level were measured.

Results: A total of 60 children (30 cases and 30 controls) were included in the study. Children with β -thalassemia major had significantly higher serum β 2-microglobulin (B2MG) levels than controls 4.58 (3.91–15.6) vs. 1.35 (1.27–1.48), $p < 0.001$. Significant differences were also observed in serum creatinine, eGFR, urine protein, PCR, and hemoglobin levels (all $p < 0.001$). ROC curve analysis demonstrated that serum B2MG showed excellent diagnostic performance for the early detection of renal dysfunction, with an area under the curve (AUC) of 0.967, 96.7% sensitivity, and 100% specificity at a cut-off value of 1.79 mg/L. PCR also demonstrated excellent diagnostic accuracy (AUC = 0.962) followed by serum creatinine (AUC = 0.902) eGFR (AUC = 0.862) urine protein (AUC = 0.86) and urine creatinine (AUC = 0.647).

Conclusion: Beta2 microglobulin can be used as specific and sensitive early biomarker to monitor glomerular and tubular dysfunction in children with β -TM.

Keywords: Beta Thalassemia, Creatinine, Sensitivity, Specificity.

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Introduction

Beta thalassemia is a hereditary anaemia of autosomal recessive origin which is due to either reduced or absent production of beta globin chain of hemoglobin molecule. Beta thalassemia major or homozygous beta thalassemia is the disease with complete absence of beta globin chain synthesis. These patients need blood transfusion in the first year of life usually, as the fetal hemoglobin is replaced by the adult hemoglobin during the period of 6 months to 1 year of age [1]. Even though there is advanced treatment and chelation therapy for beta thalassemia major, the unrecognized complications like renal impairment are emerging nowadays [2].

Several factors contribute to the renal functional abnormalities in beta thalassemia major children. They are reduced life span of RBCs, increased iron

turnover, tissue deposition of excess iron and nephrotoxic iron chelators. Thalassemia major and iron overload are known to affect many organs like heart, lungs, liver, kidneys and endocrine glands [3] Thalassemia produces renal abnormalities like increased renal blood flow, decreased urine concentrating ability and renal tubular acidosis [6]. Many studies on patients with beta thalassemia major are demonstrating that the anaemia and iron toxicity are the causes for renal dysfunction. Iron chelation therapy also affects renal function in these patients. The newer oral iron chelator Deferasirox causes increase in serum creatinine, proteinuria and renal failure [7].

Renal dysfunction is prevalent among beta thalassemia major patients which has been demonstrated by many studies which reveals

proximal tubular dysfunction, proteinuria, aminoaciduria and low urine osmolarity [8,9].

Subjects and Methods

Subjects: This study was conducted on the patients attending the hematology outpatient clinic of the Institute of Child Health and Hospital for Children, Egmore, Chennai during the period from January 2019 to January 2020. Two groups of children were enrolled as Group 1 and Group 2.

The group 1 includes 30 children diagnosed as beta thalassemia major on regular transfusion therapy and chelation therapy from the age 3-12 years. Their diagnosis was based on the standard criteria including high performance liquid chromatography [HPLC]. Regular transfusion with packed RBCs and receiving chelation therapy regularly. The group 2 includes 30 healthy children from the age group of 3-12 years. The inclusion criteria for the cases included the patients with beta thalassemia major regularly transfused with packed RBCs and receiving chelation therapy in regular manner from the age group of 3-12 years and the parents or care givers willing to give informed written consent. The exclusion criteria for group 1 (cases) included patients with the history of recurrent urinary tract infections or systemic diseases which affect kidneys and history of intake of known nephrotoxic drugs and family history of renal diseases which are hereditary. The exclusion criteria for group 2 (controls) are the children with systemic diseases like anaemia, skeletal deformities, cardiovascular, liver and renal abnormalities.

Methods:

Enrolled children are subjected to the following

1. Thorough history taking which includes the stress on the age at first transfusion, type of chelator on use, duration of chelation therapy and the patient's compliance for chelation therapy and the history of splenectomy undergone.
2. Clinical examination which includes the anthropometric measures height, general examination and systemic examination including the clinical evidence of iron overload complication and hemosiderosis of the skin.
3. Laboratory investigations.

Sampling Methods

Blood Sample: Venous blood 5ml was drawn from the intravenous line when it is fixed in the beta thalassemia major patients in the pretransfused stage for the purpose of blood transfusion. In the controls, the same amount of venous blood was drawn from the antecubital vein under strict aseptic precautions and distributed as follows:

1. Whole blood 1ml was drawn in an EDTA tube and mixed gently. This EDTA sample was used to measure the complete blood count.
2. Another 4ml of blood was drawn in the red capped tube with clot activator and allowed to separate the serum then centrifuged for 15 minutes at 1000×g. The serum was removed, aliquoted and stored at -20 degree Celsius.

The separated serum was used for

1. Serum urea and creatinine are measured using cobas 6000, C 501 from Roche Diagnostics by appropriate methods of chemical principles.
2. Serum beta2 microglobulin was measured using ELISA method.

Urine Sample: The second morning midstream urine sample of 5ml was obtained from the patients and controls and used for spot protein creatinine ratio analysis. In the anthropometric measures, the height of the cases and controls are measured.

Statistical Methods: Statistical analysis was performed using Jamovi version 2.7.7. The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables were expressed as median and interquartile range (IQR) because the data were not normally distributed. Comparisons between the case and control groups were performed using the Mann–Whitney U test. Categorical variables were presented as frequency and percentage and compared using the Chi-square test.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of serum beta2 microglobulin and other renal biomarkers. The area under the curve (AUC), 95% confidence interval (CI), optimal cut-off value, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. A p-value < 0.05 was considered statistically significant.

Results

The study was conducted among 60 children aged 3–12 years. The study population consisted of two groups: Group I included 30 children with β -thalassemia major, while Group II included 30 healthy controls. Among the cases, 17 (56.7%) were females and 13 (43.3%) were males, whereas the control group included 16 (53.3%) females and 14 (46.7%) males.

There was no statistically significant difference in gender distribution between the two groups ($p = 0.790$). Similarly, no statistically significant differences were observed in age and height between cases and controls. The median age was 8 (5.4–10) years in the case group and 8 (6.3–11) years in the control group ($p = 0.330$). The median

height was 121 (105–128) cm among cases and 120 (107–136) cm among controls ($p = 0.217$) (Table1).

Table 1: Socio-demographic characteristics of studied groups

Baseline Characteristics		Case (n=30)	Control (n=30)	Test Value	p-value
Gender	Female	17 (56.7%)	16 (53.3%)		0.79
	Male	13 (43.3%)	14 (46.7%)		
Age		8 (5.4–10)	8 (6.3–11)	384	0.33
Height		121 (105–128)	120 (107–136)	366	0.217

Clinical manifestations of the studied patients were as follows: the most presenting symptoms were moderate to severe degree of pallor and hepatomegaly in all patients 100%, jaundice in 95.5% and splenomegaly in 75.3% with splenectomy performed in 13.5% cases, the incidence of mongoloid features was 80.2%. The

onset of first blood transfusion presented early in life (mean 0.72 ± 0.42 years), age onset of iron chelation therapy was mean 2.72 ± 0.42 years and it was as follows: 56% of cases were treated by deferoxamine subcutaneous infusion, 36.5% were treated by deferasirox and 7.5% by Hydra.

Table 2: Comparison between the studied groups regarding laboratory investigations indicating renal function tests

Variable	Case (n=30) Median (IQR)	Control (n=30) Median (IQR)	Mann Whitney U Test	p-value
Urea	22.5 (16.1–27.8)	23 (22–24.8)	367.5	0.224
Creatinine	0.30 (0.26–0.40)	0.50 (0.4–0.50)	88	<0.001*
eGFR	203 (170–263)	145 (121–154)	124.5	<0.001*
Urine Protein	19.1 (10.3–26.9)	7 (6–9)	126	<0.001*
Urine Creatinine	41.5 (36.5–50.8)	48 (42–51.5)	317.5	0.051
PCR	0.469 (0.280–0.662)	0.156 (0.12–0.175)	34	<0.001*
B2MG	4.58 (3.91–15.6)	1.35 (1.27–1.48)	30	<0.001*
Hemoglobin	6.5 (5.5–7.1)	12 (11.6–12.3)	0	<0.001*

Notes: *significant

There were no statistically significant differences between the two groups with respect to serum urea and urine creatinine levels ($p > 0.05$). The median serum urea level was 22.5 (16.1–27.8) mg/dL in cases and 23 (22–24.8) mg/dL in controls ($p = 0.224$), while the median urine creatinine level was 41.5 (36.5–50.8) and 48 (42–51.5), respectively ($p = 0.051$). There were statistically significant differences observed between cases and controls for serum creatinine, eGFR, urine protein, protein-creatinine ratio (PCR), B2MG, and hemoglobin (all

$p < 0.001$). Compared with controls, children with β -thalassemia major had significantly lower median serum creatinine 0.30 (0.26–0.40) vs. 0.50 (0.40–0.50) mg/dL and hemoglobin 6.5 (5.5–7.1) vs. 12 (11.6–12.3) g/dL. Conversely, the median eGFR 203 (170–263) vs. 145 (121–154), urine protein 19.1 (10.3–26.9) vs. 7 (6–9), protein-creatinine ratio 0.469 (0.280–0.662) vs. 0.156 (0.120–0.175), and B2MG 4.58 (3.91–15.6) vs. 1.35 (1.27–1.48) were significantly higher in cases than in controls (all $p < 0.001$).

Table 3: Diagnostic performance of Serum Beta2 Microglobulin and other renal biomarkers for the early detection of renal dysfunction in children with β -thalassemia major

Parameter	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
B2MG	0.967 (0.901–1.00)	1.79	96.67	100	100	96.77	<0.001
SPOT PCR	0.962 (0.907–1.00)	0.198	93.3	100	100	93.8	<0.001
Creatinine	0.902 (0.827–0.978)	0.35	70	100	100	76.9	<0.001
eGFR	0.862 (0.761–0.962)	156	83.3	86.7	86.2	83.9	<0.001
Urine Protein	0.860 (0.757–0.963)	12.5	70	100	100	76.9	<0.001
Urine Creatinine	0.647 (0.503–0.792)	41.5	50	80	71.4	61.5	0.046

Abbreviations: PPV, positive predictive values; NPV, negative predictive values.

ROC curve analysis demonstrated that B2MG had excellent diagnostic performance with an AUC of 0.967 (95% CI: 0.901–1.00, $p < 0.001$) at a cut-off value of 1.79 mg/L, yielding 96.67% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 96.77% negative predictive value

(NPV). Among the biomarkers evaluated in this analysis, B2MG showed the highest diagnostic accuracy. PCR also showed excellent diagnostic accuracy with an AUC of 0.962 (95% CI: 0.907–1.00, $p < 0.001$) at a cut-off value of 0.198, with 93.3% sensitivity, 100% specificity, 100% PPV,

and 93.8% NPV. Creatinine demonstrated good diagnostic performance with an AUC of 0.902 (95% CI: 0.827–0.978, $p < 0.001$) at a cut-off value of 0.35, with 70% sensitivity and 100% specificity.

Similarly, eGFR showed good diagnostic accuracy with an AUC of 0.862 (95% CI: 0.761–0.962, $p < 0.001$) at a cut-off value of 156, yielding 83.3% sensitivity and 86.7% specificity. Urine protein also demonstrated good diagnostic performance with an AUC of 0.860 (95% CI: 0.757–0.963, $p <$

0.001) at a cut-off value of 12.5, with 70% sensitivity and 100% specificity.

Among all parameters, urine creatinine showed the lowest diagnostic accuracy, with an AUC of 0.647 (95% CI: 0.503–0.792, $p = 0.046$) at a cut-off value of 41.5, demonstrating 50% sensitivity and 80% specificity.

Combined ROC curves - of serum B2MG and other renal biomarkers for the early detection of renal dysfunction in children with β -thalassemia major.

ROC Analysis

Combined ROC Curves

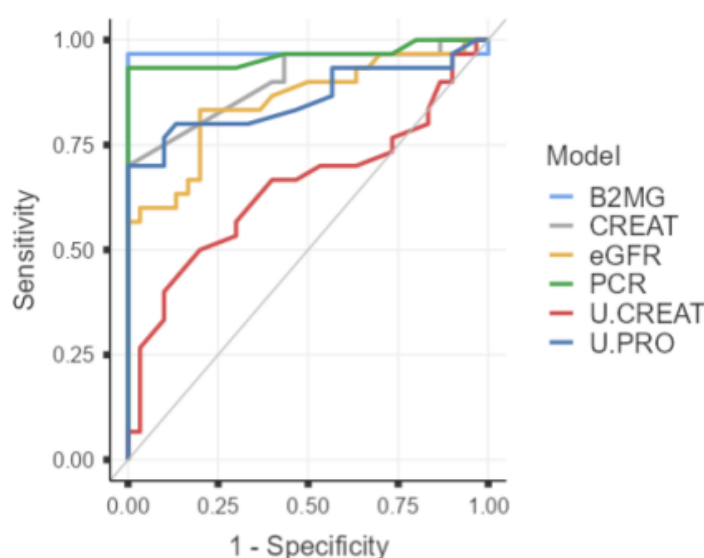


Figure 1: shows the combined ROC curves of serum B2MG and other renal biomarkers.

B2MG demonstrated the highest diagnostic performance among the evaluated biomarkers, with its ROC curve closest to the upper left corner (AUC = 0.967), followed by PCR (AUC = 0.962), whereas urine creatinine showed the lowest diagnostic accuracy (AUC = 0.647).

Discussion

Chronic anaemia and iron overload are the main factors responsible for tubular damage, interstitial fibrosis, glomerulosclerosis in patients with beta thalassemia major [20]. Kacar et al found that- B-2 microglobulin and urea levels were increased in the thalassemic patients group in comparison with control group, while no statistically significant differences were observed between thalassemic patients group and control group with regard to serum cystatin-C, serum creatinine and creatinine clearance, which supports the evidence that chronic hypoxia occurs due to anemia-disrupted kidney function.[29] In the present study, there were

statistically significant positive correlations between beta2 microglobulin and protein/creatinine ratio, duration of chelation therapy and frequency of blood transfusion/year Voskaridou et al who stated that serum β -2 microglobulin was found to have inverse correlations between Hb and β -2 microglobulin.[37]

Herrero-Morin et al also found that serum β -2 microglobulin reflects the decrease in the renal function and negative correlation between serum β -2 microglobulin, and Hb. They documented that impaired renal function and hyperproduction of Beta2 microglobulin are both associated with increased serum levels. So, it is suggested that β -2 microglobulin concentration in the blood is largely affected by the changes in the GFR. [38]

The underlying mechanisms for tubulopathy and glomerulopathy in thalassemia major patients include.

1. Long standing anaemia
2. Chronic hypoxia
3. Iron overload
4. Iron chelating drugs [6]

In the present study, beta thalassemia major patients showed higher levels of serum beta2microglobulin and urine protein creatinine ratio, eGFR, and lower levels of serum creatinine and blood hemoglobin than control group.

In the study of Ali and Mahmoud et al. they found that the higher levels of serum beta2microglobulin and urine protein creatinine ratio in the thalassemic patient group than the control group.[27]

Kacar et al found that chronic hypoxia occurs due to anemia disrupted kidney function.[29]

Koliakos et al found normal serum creatinine and creatinine clearance in thalassemia patients who received subcutaneous deferoxamine treatment.[13]

In the present study, there were statistically significant positive correlation between beta2 microglobulin and Urine protein, and spot PCR, duration of Chelation therapy and frequency of blood Transfusion / year and there were statistically significant negative correlation between beta2 microglobulin and Hb.

According to Ali and Mahmoud, they concluded that in thalassemia major patients, glomerular dysfunction is a Common complication. So beta2 microglobulin is an useful early biomarker for periodic renal assessment in those patients to detect the hidden renal dysfunction.[27]

Economou et al reported that 36%. Of their Beta thalassemia major patients had elevated Serum beta2microglobulin which suggests that beta2 microglobulin is the early and superior biomarker to serum creatinine for detection of impaired renal function in cross sectional studies.[23]

Voskaridou et al found inverse Correlation between Hb and beta2 microglobulin levels.[37] There were statistically significant differences observed between cases and controls for serum creatinine, eGFR, urine protein, protein-creatinine ratio (PCR), B2MG, and hemoglobin (all $p < 0.001$).

There were no statistically significant differences between the two groups with respect to serum urea and urine creatinine levels ($p > 0.05$). The median serum urea level was 22.5 (16.1–27.8) mg/dL in cases and 23 (22–24.8) mg/dL in controls ($p = 0.224$), while the median urine creatinine level was 41.5 (36.5–50.8) and 48 (42–51.5), respectively ($p = 0.051$). Compared with controls, children with β -thalassemia major had significantly lower median serum creatinine [0.30 (0.26–0.40) vs. 0.50 (0.40–0.50) mg/dL] and hemoglobin [6.5 (5.5–7.1) vs. 12 (11.6–12.3) g/dL]. Conversely, the median eGFR

[203 (170–263) vs. 145 (121–154)], urine protein [19.1 (10.3–26.9) vs. 7 (6–9)], Urine protein-creatinine ratio [0.469 (0.280–0.662) vs. 0.156 (0.120–0.175)], and B2MG [4.58 (3.91–15.6) vs. 1.35 (1.27–1.48)] were significantly higher in cases than in controls (all $p < 0.001$).

ROC curve analysis demonstrated that B2MG had excellent diagnostic performance with an AUC of 0.967 (95% CI: 0.901–1.00, $p < 0.001$) at a cut-off value of 1.79 mg/L, yielding 96.67% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 96.77% negative predictive value (NPV).

Among the biomarkers evaluated in this analysis, B2MG showed the highest diagnostic accuracy.

PCR also showed excellent diagnostic accuracy with an AUC of 0.962 (95% CI: 0.907–1.00, $p < 0.001$) at a cut-off value of 0.198, with 93.3% sensitivity, 100% specificity, 100% PPV, and 93.8% NPV.

Creatinine demonstrated good diagnostic performance with an AUC of 0.902 (95% CI: 0.827–0.978, $p < 0.001$) at a cut-off value of 0.35, with 70% sensitivity and 100% specificity.

Similarly, eGFR showed good diagnostic accuracy with an AUC of 0.862 (95% CI: 0.761–0.962, $p < 0.001$) at a cut-off value of 156, yielding 83.3% sensitivity and 86.7% specificity.

Urine protein also demonstrated good diagnostic performance with an AUC of 0.860 (95% CI: 0.757–0.963, $p < 0.001$) at a cut-off value of 12.5, with 70% sensitivity and 100% specificity.

Among all parameters, urine creatinine showed the lowest diagnostic accuracy, with an AUC of 0.647 (95% CI: 0.503–0.792, $p = 0.046$) at a cut-off value of 41.5, demonstrating 50% sensitivity and 80% specificity. B2MG demonstrated the highest diagnostic performance among the evaluated biomarkers, with its ROC curve closest to the upper left corner (AUC = 0.967), followed by PCR (AUC = 0.962), whereas urine creatinine showed the lowest diagnostic accuracy (AUC = 0.647).

Previous studies demonstrated the relationship between arterial stiffness and renal insufficiency, including vascular structure changes, increased atherogenic lipoprotein, decreased fibrinolysis and oxidative stress; these studies demonstrated that endothelial dysfunction is the main link between renal insufficiency and cardiovascular diseases.[33–36] Hyperfiltration is present in patients with thalassemia major which leads to overestimated GFR by schwartz formula [3,11,13,14].

The median eGFR₂₀₃(170-263)vs.145(121-154) is seen in the present study. Quinn et al. have found that eGFR calculated by the schwartz formula

overestimated the creatinine clearance measured in their study by 30ml/min per 1.73sq.m. Most of the patients had hyperfiltration especially those with lower serum creatinine levels [14]. Overestimation of GFR by the equations based on serum creatinine is due to low serum creatinine levels in thalassemia major patients in the present study.

Many factors contribute to low serum creatinine levels urinary creatinine excretion is significantly lower than expected value. In healthy individuals, urinary creatinine excretion is expected to equal creatinine generation.[28,29]

Lower creatinine excretion by thalassemia major patients indicates the decreased generation by the muscles. Reduced muscle mass is seen in thalassemia major patients. The hemoglobin level in 100% cases group shows the lower level compared to the controls. All controls are having normal hemoglobin level which is 100%. The comparison is done by the Mann Whitney U test which gives the p value of < 0.001 which is highly significant.

In beta thalassemia major, the alpha globin chains are in excess to beta globin chains which precipitate in red cell precursors and damaging the red cell membrane leading to hemolysis and anaemia [37]. The degree of tubular abnormalities are correlated with the severity of anaemia [6,11]. Anaemia can cause oxidative stress [3,21] which leads to lipid peroxidation and DNA damage [3] Hemolysate and hemoglobin ameliorate oxidation induced DNA damage.[24] The markers of oxidative damage to tubules favour the role of anaemia and iron overload in renal dysfunction in thalassemia major patients [11-13,20].

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