Frequency of Glomerular Dysfunction in Children with Beta Thalassaemia Major

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ABSTRACT: Objectives: This study investigated the frequency of glomerular dysfunction in children with beta thalassaemia major (β-TM) by using different markers and correlating them with serum ferritin and iron chelation therapy. Methods: The study, carried out between August 2011 and May 2012, included 100 patients with β-TM, in two groups. Group Ia (n = 62) received chelation therapy (deferoxamine). Group Ib (n = 38) received follow-up care at the Pediatric Hematology Outpatient Clinic, Minia University Children's Hospital, Egypt. Group II included 50 apparently healthy controls, age- and sex-matched to Group I. All patients underwent a thorough history-taking, clinical examination and laboratory investigations. Results: Compared to Group II, Group Ia and Ib had significantly higher levels of cystatin C, serum creatinine and serum ferritin, and a higher albumin/creatinine ratio in their urine, and a significantly lower estimated glomerular filtration rate (eGFR) and creatinine clearance (P <0.05). Moreover, Group Ia had a significantly lower eGFR and creatinine clearance than Group Ib. Cystatin C had a highly significant strong negative correlation with eGFR and creatinine clearance and a significantly strong positive correlation with serum ferritin, and a higher sensitivity and specificity than serum creatinine and creatinine clearance for small changes in GFR. Conclusion: β-TM patients had a high frequency of glomerular dysfunction—possibly attributable to chronic anaemia, iron overload or chelation therapy. Periodic renal assessment is mandatory to detect renal complications. Cystatin C is a promising marker to monitor glomerular dysfunction, having a higher sensitivity and specificity than serum creatinine and creatinine clearance for small changes in GFR.

Keywords: Abnormalities, glomerular; beta-Thalassemia; Cystatin C; Chelation Therapy; Egypt.

The objectives of this study were to investigate the frequency of glomerular dysfunction in β-TM patients and to correlate this with serum ferritin and iron chelation therapy. A total of 100 patients with β-TM were included, divided into two groups: Group Ia (n = 62) received chelation therapy (deferoxamine) and Group Ib (n = 38) received follow-up care. Group II included 50 apparently healthy controls, age- and sex-matched to Group I. All patients underwent a thorough history-taking, clinical examination and laboratory investigations. Compared to Group II, Group Ia and Ib had significantly higher levels of cystatin C, serum creatinine and serum ferritin, and a lower albumin/creatinine ratio. Cystatin C had a strong negative correlation with eGFR and creatinine clearance and a strong positive correlation with serum ferritin. Cystatin C is a promising marker to monitor glomerular dysfunction.

Advances in Knowledge
- Glomerular dysfunction in children with beta major thalassaemia (β-TM) may be subclinical and, as this dysfunction may not be detected by routine testing, the use of early markers is recommended.

Application to Patient Care
- In β-TM patients, a close follow-up of glomerular dysfunction is necessary as it is not a rare complication.

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Beta thalassaemia (β-TM) is an inherited haemoglobin disorder characterised by the failure of the patient to produce beta haemoglobin chains, usually resulting in severe anaemia in its homozygous and compound heterozygous forms. The use of regular and frequent blood transfusions in patients with β-TM major has improved patients’ life spans and quality of life, but can lead to chronic iron overload. Unlike other organs, it is unclear whether kidney damage results solely from intravascular haemolysis, chronic transfusion or as a complication of iron chelation therapy.

Patients with β-TM are known to have severe cardiopulmonary and reticuloendothelial dysfunction, but renal involvement has received little attention.

Cystatin C is a 122-amino acid non-glycosylated protein of low molecular weight (13 kilodaltons [kDa]) that inhibits cysteine proteases. It is filtered by glomeruli and followed by tubular reabsorption and degradation, resulting in the excretion of a minute amount of cystatin C in the urine. It is not secreted by the renal tubules or reabsorbed back into the serum. Therefore, its serum levels serve as an endogenous parameter of glomerular filtration rate (GFR). Due to its small size, cystatin C is freely filtered by the glomerulus, and is not secreted but is fully reabsorbed and broken down by the renal tubules. This means that the primary determinate of blood cystatin C levels is the rate at which it is filtered at the glomerulus, making it an excellent marker of GFR. A recent meta-analysis demonstrated that serum cystatin C is a better marker for GFR than serum creatinine.

Therefore, this study aimed to investigate the frequency of glomerular dysfunction in children with β-TM by using different markers and correlating them with serum ferritin and iron chelation therapy.

**Methods**

This cross-sectional study included an experimental group (Group I) of 100 patients, 72 males (65.5%) and 28 females (34.5%) aged 8–16 years and recruited from August 2011 to May 2012. Exclusion criteria included a patient history suggestive of recurrent urinary tract infections; the presence of systemic diseases affecting the kidney; the presence of rheumatic heart, thyroid, hepatic diseases, or diabetes mellitus; the intake of trimethoprim, corticosteroids or cephalosporin in the past seven days; a history of nephrotoxic drug use, and a family history of hereditary renal diseases. The control group included 50 apparently healthy volunteers who were age- and sex-matched to those in Group I.

The patients with β-TM were divided into two groups. Group Ia (n = 62) received regular chelation therapy consisting of deferoxamine (DFO) in doses of 20–40 mg/Kg per day via subcutaneous pump infusions over 8–12 hours/night for five days a week. Group Ib (n = 38) did not receive chelation therapy. Those in Group Ib underwent regular follow-up in the Pediatric Hematology Outpatient’s Clinic of the University Children’s Hospital of Minia University, Egypt. Written consent was received from the patients’ caregivers after the approval of the study by the University’s Ethical Committee.

Both groups underwent a through history-taking and clinical examination. Morning fasting blood samples and urine specimens were collected and tested for different biochemical function profiles, including simple urine analysis, an evaluation of the albumin/creatinine (A/C) ratio in the urine using the National Kidney Foundation’s level recommendations as a reference (female <3.5 mg/mmol, male <2.5 mg/mmol) and creatinine clearance.

The estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula for children, where constants were 0.44 for children <2 years and 0.55 for children ≥2 years:

\[
eGFR \ (\text{ml/min per } 1.73 \text{ m}^2) = \frac{\text{height (cm) \times constant}}{\text{serum creatinine (mg/dL)}}
\]

Renal dysfunction was defined as eGFR <90 ml/min per 1.73 m². A complete blood count (CBC) was done using a Sysmex Complete Blood Count Analyzer (Sysmex Corporation, Kobe, Hyogo, Japan) and serum ferritin (ug/dL) was done by enzyme-linked immunosorbent assay (ELISA)
Renal function tests include blood urea with a reference range of 3.0–6.0 mmol/L and serum creatinine with a reference value for females of 40–90 μmol/L and males of 50–100 μmol/L by a creatinine-Jaffe enzymatic assay using a spectrophotometer.\(^9\) Finally, serum cystatin C level was measured by a Quantikine\(^{®}\) human cystatin C immunosorbent assay (ELISA) kit (R&D Systems) with a reference value of 0.80–0.90 mg/L.\(^{10}\)

The data were coded and verified prior to data entry. The Statistical Package for the Social Sciences (SPSS), Version 13 (IBM, Corp., Chicago, Illinois, USA), was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was done using the Student’s t-test and Mann-Whitney U test for normal and non-parametric variables, respectively. The chi-squared test (χ\(^2\)) was used to compare the frequency of qualitative variables among the different groups. Pearson’s and Spearman’s correlation tests were used for correlating parametric and non-parametric variables, respectively. Multiple regression analysis was also performed to determine the effect of various factors on a dependent variable. A P value of >0.05 was determined as non-significant while a P value of <0.05 was significant. A Z-test was used to compare proportions and correlations.\(^{13}\)

A receiver operating characteristic (ROC) analysis was done to determine the accuracy of serum cystatin C versus serum creatinine and creatinine clearance by plotting the sensitivity and specificity of the experimental and control groups.

### Results

In this study, the frequency of glomerular dysfunction in children with β-TM was investigated by using different markers and correlating them with serum ferritin and iron chelation therapy. A comparison of Groups Ia and Ib in regards to clinical findings showed that Group Ia had a significantly higher frequency of blood transfusions than group Ib (P = 0.02) [Table 1]. Concerning the laboratory parameters, Table 2 shows that Groups Ia and Ib had statistically significant higher levels of serum cystatin C, serum creatinine and serum ferritin, and a higher albumin/creatinine (A/C) ratio of the urine than Group II where P <0.05. On the other hand, they had a significantly lower eGFR as determined by Schwartz and a lower creatinine clearance than Group II.

A comparison between Groups Ia and Ib demonstrated that Group Ia had a significantly lower eGFR and creatinine clearance than Group Ib (P = 0.004 for each). In regards to different correlations, there were statistically significant strong negative correlations between serum cystatin C with eGFR and creatinine clearance where r = -0.91, P = 0.001 and r = -0.80, P = 0.005, respectively. Moreover, there was an insignificant negative weak correlation with the frequency of blood transfusions (r = -0.14, P = 0.3) and a significant positive correlation with duration of chelation therapy, serum ferritin and A/C ratio in the urine (r = 0.29, P = 0.04; r = 0.9, P = 0.001; r = 0.82, P = 0.0001, respectively) [Table 3].

A ROC test was done for serum cystatin C and serum creatinine in β-TM patients and control groups, and it was found that the area under the curve (AUC) for serum cystatin C was significantly higher than that for serum creatinine [Figure 1]. Moreover, serum cystatin C had higher sensitivity and specificity than serum creatinine (65% versus 27% and 91% versus 79%) [Table 4]. Table 5 and Figure 2 show that the AUC for serum cystatin C was significantly higher than that for creatinine clearance and serum cystatin C had higher sensitivity and specificity than creatinine clearance.

### Table 1: Comparison between beta thalassaemia major patient subgroups by clinical characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient subgroups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, range (mean ± SD)</td>
<td>Group Ia with chelation (n = 62)</td>
<td>Group Ib without chelation (n = 38)</td>
</tr>
<tr>
<td></td>
<td>10–16</td>
<td>8–14.5</td>
</tr>
<tr>
<td></td>
<td>(9.6 ± 1.1)</td>
<td>(9.8 ± 1.7)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td>Male</td>
<td>40 (64.5)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22 (35.5)</td>
</tr>
<tr>
<td>Age of onset of transfusion in months, mean ± SD</td>
<td>7.8 ± 3.2</td>
<td>8.7 ± 2.6</td>
</tr>
<tr>
<td>Frequency of blood transfusion per year, mean ± SD</td>
<td>11.3 ± 1.9</td>
<td>9.4 ± 4.03</td>
</tr>
<tr>
<td>Splenectomy, n (%)</td>
<td>Positive</td>
<td>6 (9.7)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>56 (90.3)</td>
</tr>
</tbody>
</table>

SD = standard deviation; *Significant.
(67% versus 63% and 93% versus 66%, respectively).

**Discussion**

In this study, it should be noted that less attention was paid to renal complications. Koliakos et al. and others have conducted investigations of renal involvement in adult β-TM patients.14,15 The current study was designed to investigate glomerular dysfunction in children with β-TM by using different markers, including cystatin C, and to correlate those findings with serum ferritin and iron chelation therapy. The current study found that Group Ia had statistically significant higher frequencies of blood transfusion than Group Ib (P = 0.02) [Table 1], this was perhaps due to the potential ignorance of the Group Ib parents and their failure to comply with treatment.

Concerning laboratory data, the current study found that β-TM patients, whether receiving chelation therapy or not, had statistically significant higher levels of serum cystatin C, serum creatinine and serum ferritin than those in the control group. On the other hand, they had a significantly lower eGFR and creatinine clearance than the control group. This could be explained by the possibility that renal dysfunction in patients with β-TM was due to long-standing anaemia, chronic hypoxia and iron overload.14 In addition, the shortened life span of red blood cells with rapid iron turnover and DFO therapy may have proven nephrotoxic.15 These results were in agreement with the results obtained

| Parameter                                      | Group Ia with chelation (n = 31) | Group Ib without chelation (n = 19) | Group II control (n = 35) | P value  
|-----------------------------------------------|----------------------------------|------------------------------------|--------------------------|--------
| Cystatin C in mg/dL, range (mean ± SD)        | 0.7–2 (1.9 ± 0.2)                | 0.5–1.8 (1.6 ± 0.3)                | 0.3–0.6 (0.6 ± 0.1)      | 0.01*  
| Serum creatinine in mg/dL, range (mean ± SD)  | 0.7–1.5 (0.9 ± 0.1)              | 0.5–1.2 (0.7 ± 0.2)                | 0.5–0.8 (0.4 ± 0.08)     | 0.01*  
| eGFR by Schwartz formula in ml/min per 1.73 m², range (mean ± SD) | 44–88.1 (77.4 ± 30.4) | 56–99 (83.2 ± 36.4) | 58.9–154 (102.9 ± 23.4) | 0.01*  
| Creatinine clearance in ml/min, range (mean ± SD) | 33–134 (34.2 ± 5.9)              | 40.3–140 (46.3 ± 6.05)             | 88–133 (89.7 ± 2.1)      | 0.001*  
| Serum ferritin in ng/ml, range (mean ± SD)    | 760–2500 (1120 ± 45.1)           | 98–1001 (995.09 ± 35.5)            | 12–76 (15.3 ± 2.5)       | 0.001*  
| Albumin/creatinine ratio in urine in mg/mmol, range (mean ± SD) | 1.1–112.8 (75.5 ± 24.4)         | 0.6–101.4 (62.2 ± 30.1)            | 0.4–2.2 (1.3 ± 0.2)      | 0.001*  

SD = standard deviation; eGFR = estimated glomerular filtration rate; *Significant.

**Figure 1:** Receiver operating characteristic curve for serum cystatin C and creatinine among beta thalassaemia major patients and the control group.

**Figure 2:** Receiver operating characteristic curve for serum cystatin C and creatinine clearance among beta thalassaemia major patients and the control group.
by Grundy et al. sixteen and Hamed and ElMelegy seventeen who reported higher serum levels of serum cystatin C and serum creatinine and lower levels creatinine clearance. In contrast to the current study’s findings, Koliakos et al. found normal serum creatinine and creatinine clearance in β-TM patients who received subcutaneous DFO treatment.

Concerning the urinary findings, the current study found that Groups Ia and Ib had statistically significant higher ratios of A/C in the urine than the control group. This albuminurea was attributed mainly to the destruction of the glomerular filtration membrane which may be due to massive iron deposition in the tissues, resulting in an increase of free radical production via the Fenton reaction, leading to cell death by binding cell proteins and disturbing their production.

In addition, albuminurea could result from prolonged hyperfiltration, prostaglandin secretion and chronic anaemia.

A comparison between Groups Ia and Ib in regard to some laboratory data showed that Group Ia had a significantly lower eGFR and creatinine clearance than Group Ib. This might be due to the shortened red blood cell life span with rapid iron turnover and tissue deposition of excess iron and the deferoxamine (DFO) therapy that may have proven nephrotoxic.

In addition, Group Ia had a higher frequency of blood transfusion than Group Ib, where each 1 ml of packed red cells increases the body’s iron load by 1 mg, resulting in iron overload. Concerning different correlations, the current study found that serum cystatin C had a statistically higher, significantly strong negative correlation with eGFR and creatinine clearance where r = -0.91, P = 0.001 and r = -0.80, P = 0.005, respectively [Table 3]. Because its biological variation is low, serum cystatin C gives a good assessment of GFR changes during follow-up. Furthermore, cystatin C had a statistically higher, significantly strong positive correlation with ferritin where r = 0.90 and P = 0.001. This may be due to increased iron deposition coming from multiple life-long transfusions and enhanced iron absorption, resulting in secondary haemosiderosis with secondary renal dysfunction.22

Serum cystatin C had a statistically significant, fair positive correlation with A/C ratio in the urine where r = 0.82 and P = 0.001. This could be explained by the possibility that proteinuria and microalbuminuria could be related to prolonged glomerular hyperfiltration and glomerulosclerosis. Also, serum cystatin C had a highly significant positive correlation with the duration of chelation therapy, where r = 0.29 and P = 0.04.

In the present study, serum cystatin C and creatinine values were measured as markers of GFR in β-TM patients and a control group and therefore a ROC test was done for serum cystatin C versus serum creatinine by plotting the claimed sensitivity and specificity of the β-TM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of chelation in months</td>
<td>0.29</td>
<td>0.04*</td>
</tr>
<tr>
<td>Frequency of transfusion per year</td>
<td>-0.14</td>
<td>0.3</td>
</tr>
<tr>
<td>eGFR in ml/min per 1.73 m²</td>
<td>-0.91</td>
<td>0.001*</td>
</tr>
<tr>
<td>Creatinine clearance in ml/min</td>
<td>-0.80</td>
<td>0.005*</td>
</tr>
<tr>
<td>Serum ferritin in ng/ml</td>
<td>0.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>Albumin/creatinine ratio</td>
<td>0.82</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*eGFR = estimated glomerular filtration rate.
*Significant; †Grades of r: 0.00–0.24 (weak or no association); 0.25–0.49 (fair association); 0.50–0.74 (moderate association); >0.75 (strong association).

Table 3: Correlations between cystatin C and different parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Threshold value</th>
<th>AUC, mean ± SD</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C in mg/dl.</td>
<td>1.03</td>
<td>0.84 ± 0.03</td>
<td>65</td>
<td>91</td>
<td>0.75–0.89</td>
</tr>
<tr>
<td>Serum creatinine in mg/dl.</td>
<td>0.6</td>
<td>0.71 ± 0.04</td>
<td>27</td>
<td>79</td>
<td>0.60–0.82</td>
</tr>
</tbody>
</table>

AUC = area under the curve; SD = standard deviation; CI = confidence interval.

Table 4: Diagnostic accuracy of reduced glomerular filtration rate from serum cystatin C and serum creatinine among beta thalassaemia major patients and the control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Threshold value</th>
<th>AUC, mean ± SD</th>
<th>Sensitivity %</th>
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<tbody>
<tr>
<td>Cystatin C in mg/dl.</td>
<td>1.03</td>
<td>0.82 ± 0.02</td>
<td>67</td>
<td>93</td>
<td>0.75–0.92</td>
</tr>
<tr>
<td>Creatinine clearance in ml/min</td>
<td>112</td>
<td>0.36 ± 0.04</td>
<td>63</td>
<td>66</td>
<td>0.25–0.46</td>
</tr>
</tbody>
</table>

Table 5: Diagnostic accuracy of reduced glomerular filtration rate from serum cystatin C and creatinine clearance among beta thalassaemia major patients and the control group
and control patients. This test strongly suggested that serum cystatin C was indeed superior to serum creatinine for the detection of GFR as the AUC was 0.85 ± 0.04 for cystatin C versus 0.73 ± 0.05 for serum creatinine with higher sensitivity and specificity (66% and 92% versus 64% and 75%) [Table 4 and Figure 1]. This finding was in agreement with the results obtained by Larsson et al., who stated that plasma cystatin C provided a better indication of changes of GFR than serum creatinine.24

Table 5 and Figure 2 show that the AUC for serum cystatin C was significantly higher than that for creatinine clearance (0.85 ± 0.04 versus 0.35 ±0.05) when comparing β-TM patients with the control group. Also, the sensitivity and specificity of serum cystatin C were higher than creatinine clearance (66% and 92% versus 26% and 75%, respectively). This finding was in agreement with the findings obtained by Finney et al., who demonstrated that, in contrast to creatinine clearance, serum cystatin C concentration was effectively constant by the first year of life, and remained constant throughout adulthood up to 50 years of age.25 They also suggested that serum cystatin C might offer a considerable advantage to paediatric nephrologists in the diagnosis of GFR due to its sensitivity in the detection and reduction of GFR.

Conclusion

This study has shown that β-TM patients have a high frequency of glomerular dysfunction. This can be attributed to chronic anaemia, iron overload and DFO toxicity. Periodic renal assessment of those patients is mandatory as they may be affected by hidden renal dysfunction. Cystatin C is a promising marker to assist in monitoring glomerular dysfunction for small changes in GFR with higher sensitivity and specificity than serum creatinine and creatinine clearance.

References


