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Role of Cystatin C as Early Predictor of Renal Dysfunction in Iron Overloaded Beta Thalassemia Major Patients

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ABSTRACT

Background:
Beta thalassemia major patients are at risk to developing renal dysfunction over a period of time, secondary to chronic anemia, iron overload and chelation therapy. Early observation of renal insufficiency is necessary for intervention.

Objectives:
The objective of this study was to assess the value of cystatin c as an early marker of renal dysfunction in pediatric patients with iron overload Beta thalassemia major and investigates the related clinical factors.

Patients and Methods:
This was a cross sectional study which was conducted in Babylon center for hereditary blood disorders in Babylon governorate, 74 patients with beta thalassemia major whose parents accepted to participate in this study. Data collection was done by direct interview with parents. It was done by using performed questionnaire and tested for epidemiological and clinical factors. The concentration of cystatin C, creatinine, ferritin and haemoglobin were determined. Apparent correlations with cystatin C were analyzed for several factors, including age, age at presentation, duration of the disease, age at first blood transfusion and compliance with therapy and then take blood sample and examine the patient height.

Results:
A total of 74 patients with beta thalassemia major were enrolled in this study. Cystatin C adjusted concentrations showed a trend to increase with the later age at diagnosis ($P < 0.0001$); patients with diagnosis by 3-5 years were found the highest (1.42 ± 0.230 mg/L) compared to those diagnosed earlier at < 1 year (1.08 ± 0.248 mg/L) or between 1-3 years (1.11 ± 0.201 mg/L). Age at the first blood transfusion ($P = 0.001$) and therapy adherence ($P = 0.032$) were significantly associated with cystatin C levels and were higher in the non adherence group. Correlation of cystatin C was statistically in significant with serum creatinine ($P = 0.047$) while no correlation with age ($P = 0.476$) and duration of disease ($P = 0.7$) was observed unlike creatinine. There was significant correlation between cystatin C and serum ferritin.There was cut-off point of 1.34 mg/L serves as a critical warning sign, prompting further investigation and potentially earlier initiation of nephroprotective measures.

Conclusion:
Cystatin C proved to be a more sensitive biomarker than serum creatinine for the early detection of renal dysfunction in iron-overloaded β-thalassemia major patients. The

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study demonstrated that Cystatin C levels were significantly elevated in these patients, even before conventional markers such as serum creatinine showed abnormalities. This indicates that Cystatin C can play an essential role in the early prediction and monitoring of renal impairment, allowing for timely interventions to prevent progression, Additionally, demographic factors such as age and duration of blood transfusion therapy were found to influence renal function deterioration, highlighting the need for continuous monitoring in high-risk groups.

INTRODUCTION

Beta-thalassemia is a group of inherited blood disorders caused by diminished or absent production of the beta-globin chains of hemoglobin, therefor resulting in chronic hemolytic anemia, ineffective erythropoiesis, and numerous clinical complications (Taher et al., 2018) It is most commonly found in individuals from mediterranean , middle Eastern, and southeast Asian regions (Taher, Musallam & Cappellini et al., 2018). Moreover, Beta thalassemia is the result of mutations to the HBB gene (hemoglobin beta gene) on chromosome 11 which leads defective hemoglobin synthesis (Origa et al., 2021).

Furthermore, disease severity is dependent on whether a patient has one or two defective alleles:

For instance, Beta thalassemia minor: Mild anemia, heterozygotes

In comparison, Beta thalassemia intermedia : Moderately severe, frequent blood transfusions.

Finally, Beta thalassemia major (Cooley's Anemia): Homozygous form that causes severe anemia and needs lifelong transfusions (Taher, Weatherall & Cappellini et al., 2021).

As a result, This mismatch of synthesis of the beta-globin chain results in an ineffective erythropoiesis ,chronic hemolytic anemia and dependence on regular blood transfusions (Taher et al., 2018).

Excess unpaired α-globin chains accumulate in erythroid precursors causing oxidative damage and apoptosis, thus leading to ineffective erythropoiesis. Moreover, lysis of defective red blood cells in the spleen leads to hemolytic anemia and splenomegaly.

Transfusions indeed correct anemia and enhance quality of life however, add a major complication in the form of iron overload. Humans have no physiological mechanism to eliminate excess iron, and each unit of stored blood contains about 200–250 mg of iron, which consequently contributes to accumulation in organs including liver, heart, endocrine glands and kidneys (Musallam et al., 2021)

Furthermore, the consequent ineffective erythropoiesis will also lead to hepcidin suppression ,thereby enhances iron absorption from the gastrointestinal tract—As a result, this creates a dual fistula overload by hepcidin suppression (Taher et al., 2018)

In order to manage the excess iron, iron chelation therapies—deferoxamine (parenteral), deferiprone (oral), and desferosirox (oral) are consider the best options. Nevertheless, the nephrotoxic effects of these agents add a substrate for renal risk (Bareli et al.,2019). Renal complications in β-TM have traditionally been underappreciated in comparison to cardiac and hepatic morbidity. But with improvements of chelation leading to prolonged life expectancy, renal dysfunction has emerged as a common and insidious complication, with studies since 2015 estimating that 20–60% of patients have either glomerular or tubular abnormalities (Mahmoud et al.,2021; Romadhon et al., 2022).

β-TM is associated with a complex and multifactorial renal pathology. Abnormal iron accumulation in renal tissue leads to the production of reactive oxygen species (ROS) that induces lipid peroxidation and induces cell injury to glomerular endothelium and proximal tubular cells (Hashemieh et al., 2017; Romadhon et al., 2022). Chronic anemia also plays a role through the development of renal hypoxia with the activation of hypoxia-inducible factors (HIFs) leading to tubule interstitial fibrosis with time (Musallam et al., 2021). Adding to this complexity are chelating agents: deferoxamine can lead to proximal tubular dysfunction, while deferasirox is associated with dose-dependent decrements in GFR, proteinuria, and increased urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) (Badeli et al., 2019).

Clinical manifestations of these insults include early glomerular hyperfiltration—often as a compensatory response to anemia—progressive GFR decline, tubular proteinuria, and aminoaciduria, frequently
detectable only with sensitive markers prior to the onset of frank renal failure (Mahmoud et al.,2021). This asymptomatic advancement requires early diagnosis tools to avoid chronic kidney disease (CKD), a burgeoning entity since β-TM patients currently survive to their fourth and fifth decades (Musallam et al., 2021).

Standard renal markers such as serum creatinine fail in this setting. In β-TM, creatinine, a product of muscle metabolism, is not a good marker due to low muscle mass due to chronic illness, poor growth and endocrine dysfunction resulting in abnormally low readings even in the presence of significant kidney injury as creatinine level typically increases only after 50% or more of GFR as been lost, failing to provide opportunities for early intervention (Shlipak et al., 2017). This limitation is especially severe in children and adolescents with β-TM, in whom discordance between muscle mass and growth is evident (Filler et al., 2016).

These shortcomings are addressed by Cystatin C, a 13-kDa cysteine protease inhibitor secreted by all nucleated cells at constant rates. Unlike creatinine, cystatin C nor is it reabsorbed or secreted by proximal tubular cells, making it a more sensitive and reliable biomarker for early detection of kidney dysfunction in B-TM patients.

In addition to these advantage, Cystatin C is a high-fidelity measure of GFR independent of muscle mass, age, gender, or nutritional status (Shlipak et al., 2017). Its widespread implementation in pediatric and chronic disease settings since 2015 has also been accompanied by evidence that its sensitivity to early GFR changes in diseases related to oxidative stress and systemic inflammation, classical features of iron-overloaded β-TM (Romadhon et al., 2022).

Cystatin C is a 13 kDa cysteine protease inhibitor produced by all nucleated cells at a fairly steady rate. It is freely passed through the glomerular basement membrane, and although the proximal renal tubules reabsorb and metabolize almost all of it, no significant re-secretion into the bloodstream occurs (Grubb., et al., 2015). For example, this is an ideal endogenous marker for glomerular filtration rate (GFR), particularly when compared to serum creatinine, which is substantially affected by factors such as age, sex, race, muscle mass and dietary protein intake (Shlipak., et al., 2016).

In contrast to creatinine, cystatin C is independent of muscle mass and diet, rendering it particularly useful in specific populations with inaccurate estimates of the glomerular filtration rate (GFR) using creatinine, including children, the elderly, and patients with chronic disease or poor nutritional state (Inker., et al., 2016). Adjustments to serum creatinine measurement, such as creating several equations using cystatin C alongside serum creatinine, were performed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) in an attempt to enhance the accuracy of estimated GFR and improve the accuracy of staging for kidney disease (Levey., et al., 2020).

Clinically, raised serum cystatin C has been associated with early renal functional decline as well as cardiovascular events, stroke and mortality, providing the reasoning for its use as a marker for risk stratification in patients with and without overt kidney disease (Shlipak., et al., 2021). Cystatin C has been recognized as a sensitive and early biomarker of glomerular dysfunction in hematological disorders like beta-thalassemia major; these patients are at high risk for renal injury due to chronic anemia, hypoxia, iron overload and nephrotoxic chelation therapies (Mahmoud., et al., 2021).

Cystatin C has indicated the potential to detect unrecognized renal injury prior to irreversible damage (Romadhon., et al., 2022); this assertion is especially true in thalassemia cohorts, where it has been shown to rise against normal eGFR and serum creatinine. Because of the cumulative effect of transfusion-induced iron overload and nephrotoxicity caused by chelators, cystatin C should be routinely monitored in these patients to start timely interventions and prevent progression to chronic kidney disease.

Objectives

The objective of this study was to assess the value of cystatin c as an early marker of renal dysfunction in pediatric patients with iron overload Beta thalassemia major and investigates the related clinical factors.

PATIENTS AND METHOD

Study Design, Setting and Time

This is a cross sectional study which was conducted in Babylon center for hereditary blood disorders in Babylon governorate between 2nd of January to 1 st of April (2025)

Sample Size Calculation

The sample size (61) was calculated according to the equation below: $N=Z^2 *P(1-P)/d^2$ (Harris et al .,2019).

Where:

N: Sample size.

Z: Level of confidence interval which equals to 1.96.

P: The prevalence of beta thalassemia major.

The prevalence of was 4.1% (Amin, S. et al. 2020)

d: Estimated error which equals to 5%.

This study included a sample of 74 patients with iron overloaded beta thalassemia major were recruited from Babylon center for hereditary blood disorders in Babylon governorate.

Inclusion Criteria

1. Pediatric age group (1_14Y) had definitive diagnosis (beta thalassemia major) whose parants accept to engage in this syudy.
2. Receiving regular blood transfusion and had history of iron overload.
3. B-TM Patients attending the hospital for routine consultations or management.

Exclusion Criteria

B-TM patients coinheritance with other types of hemoglobinopathy.

Data Collection

Data collection was done by direct interview with parents. And by using a predesigned questionnaire by which the parents were interviewed after giving their verbal consent. Each parent was interviewed for about 15_20 minutes (parents who refused to participate were excluded). The questionnaire included the following items:

Sociodemographic factors: include Age, sex, age of diagnosis, age of 1st blood transfusion, duration of disease, duration of

receiving blood, chelation therapy use , type of chelation therapy dose and adherence with therapy , take blood sample and measure the length or height
Questions about demographic characteristics: -
1-Age
2-Sex
3-Age at diagnosis
4-Age at first blood transfusion
5-Duration of receiving blood transfusion
6-Frequency of blood transfusion per month
7-compliance with therapy / did he or she take oral therapy daily / how many time per week he or she take chelation therapy .
Adherence to medication was based on Morisky 8 Items (Morisky., et al,.2008) Medication Adherence Scale (MMAS-8). The MMAS-8 was designed to evaluate the patient's adherence to their medical treatment. It consists of eight questions. From questions 1 to 7, the answer choices are "yes" or "no". The eighth questions is answered according to a scale of five options: never, almost never, sometimes, frequently, and always. The total score of this scale is from 0 to 8.
The classification of patients according to their scores is as follows: less than 6 (low adherence), 6 or 7 (moderate adherence), and 8 (high adherence).
A questionnaire form had been filled for each patient through direct interview. The time needed for each interview was nearly 15-20 minutes, then estimated height/lenghth and blood sample was taken .

Laboratory Investigations

Table (3-1): Analytic Methods and Assays Used for Biomarker Measurements.

Parameter	Method	Notes
Cystatin C	Fluorescence immunoassay (FIA)	Marker for GFR, unaffected by muscle mass
Serum Creatinine	Fluorescence immunoassay (FIA)	For comparison with traditional marker
Serum Ferritin	ELISA	Marker of iron overload
Pcv	Baseline labs	Baseline metabolic and renal status

Table (3-2): Laboratory Kits assays Were Used .

Kits	Company	Origin
Cystatin C kit	**Ichroma**	**Korea**
Creatinine kit	**Ichroma**	**Korea**
S.Feittin kit	**VIDAS Biommerieux**	**Italy**

Table(3-3): The Apparatuses Were Used.

Istrument and equipement	Manufacturers company	Country
Capillary tube	Sigma	United kingdom
Disposable syring	Al-pharabi	China
Centrifuge	Kokusan	Japan
Tube holder	Kokusan	Japan
Tube reader	Kokusan	Japan
Hematocrit reader	Kokusan	Japan
EDTA tube	Sigma Aldrich	United state
Face mask	SAMA	China
Cotton	Mecho	China

Sample Collection and Handling

Blood samples: Collected randomly
Serum separated and stored at -20°C until analysis

Estimated eGFR

Estimated GFR by creatinine to assess the degree of renal dysfunction by new bedside formula
eGFR=0.43 * height in cm/serum creatinine in mg/dl (nelson et al .,2024)

Ethical Approval and Consent

Ethical clearance was obtained from the Institutional Ethic committee in Babylon university college of medicine(family and comuinty department) also from Babylon center for hereditary blood disorders in Babylon governorate and informed verbal consent was taken from all participants or their legal guardians.

Statistical Analysis

Data was be analyzed using SPSS-23
Descriptive statistics was summarize patient characteristics
The mean and standard deviation were used to represent each result. To compare continuous variables, an independent t-test was employed. Pearsons(r) correlation coefficient were used to determine the relationship between various variables..
Receiver Operating Characteristic (ROC) curve analysis will evaluate the predictive value of Cystatin C for early renal dysfunction.
A p-value < 0.05 was be considered statistically significant and higly
significant when the p-value 0.001 (khanna et al.,2020)

RESULTS

Seventy-four patients previously diagnosed with iron overloaded beta thalassemia major were analyzed for demographic variables associated with clinical outcome. Variables that were addressed in the analysis include age, gender, age at diagnosis, duration of disease, and adherence to therapy..

Statistical analyses were also performed to determine the significance of associations between the main variables of interest described in table (4-1).

Table (4-1) Demographic Characteristic of Studied Patients

Variable	Categories	Frequency	No. and %
Age/years	less than 5	14	18.91%
	5-10	18	24.32%
	10-14	42	56.75%
Sesx	Male	32	43.24%
	Female	42	56.75%
Age at diagnosis/years	Less than 1	48	64.86%
	1-3	16	21.62%
	3-5	10	13.51%
Duration of disease/ years	Less than 5	16	21.62%
	5-10	35	47.29%
	10-14	23	31.08%

More than half of the participants (56.75%) were aged between 10–14 years, while the smallest proportion (18.91%) were below 5 years. Females represented a slightly higher proportion (56.75%) compared to males (43.24%). Regarding age at diagnosis, the majority (64.86%) were diagnosed before the age of one year. Regarding disease duration, nearly half of the patients (47.29%) had a disease duration between 5–10 years, followed by 31.08% with a duration of 10–14 years, and 21.62% with less than 5 years,(Table 4-1)

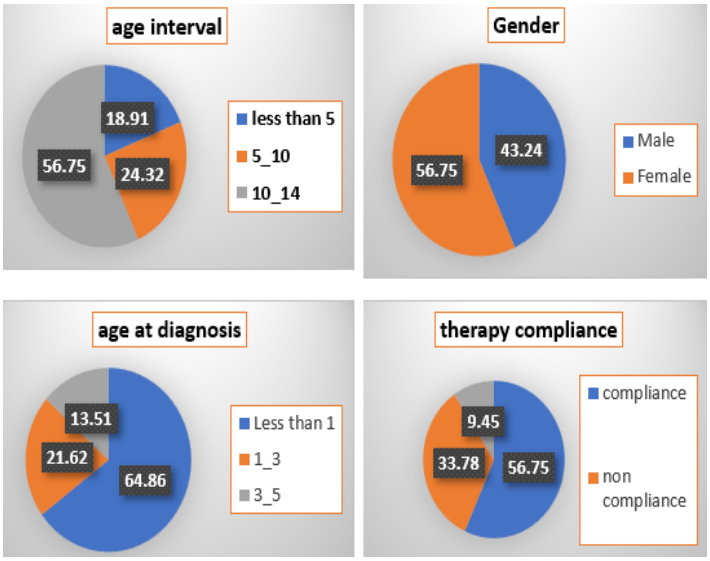


Figure (4-1): Demographics data

Table (4-2): Association Between Age Groups and Levels of Hemoglobin, Serum Ferritin, Creatinine, and Cystatin c .

Age interval/ years	Less than 5	5-10	10-14	P value
Hb level (g/ dl)	8.05±0.95	7.72±1.44	7.56±0.85	0.07
S ferritin ng/ ml	1547.3±851.06	1677.5±941.09	3537.2±1847.3	0.001**
S creatinine mg/dl	0.32±0.04	0.36±0.05	0.43±0.75	0.008**
Cystatin c mg/l	1.11±0.21	1.10±0.26	1.16±0.27	0.476
eGFR by creatinine ml/ min/1.73m2	117.6±21.1	145.6±27.2	160.7±22.2	<0.0001**

* Significant association at P<0.05 ** highly Significant association at P<0.01

The mean HB level declined with age: 8.05±0.95 in less than 5 years, 7.72±1.44 in 5–10 years and 7.56±0.85 in the 10–14 years age group.

Mean serum ferritin progressively increased with age: Importantly, this finding was significant (P = 0.001) suggesting age of patients as a crucial factor in iron overload. Mean serum creatinine also positively correlated with age (0.324±0.04 to 0.368±0.05; P = 0.036, (Table 4-2)

Table (4-3) Association Between Adherence to Chelation Therapy and Levels of Hemoglobin, Serum Ferritin, Creatinine, and Cystatin c

Variable	High adherence	Low adherence	p-value
HB level (g/dl)	8.30±0.43	7.41±1.02	0.025*
S ferittin (ng/ml)	1139.05±575.1	2729.5±1667	0.00**
S creatinine (mg/ dl)	0.94±0.18	1.12±0.25	0.148
S cystatin c (mg/l)	1.25±0.2	1.55±0.3	0.032*
eGFR by craitinine ml/min/1.73m2	98.5±15.2	85.4±12.7	0.01*

* Significant association at P<0.05**highly Significant association at P<0.01

Patients who were high Adherence to therapy had a significantly higher mean HB level (8.30 ± 0.43 g/dL) compared to low adherent patients (7.41 ± 1.02 g/dL)

The difference was statistically significant (P = 0.025), indicating that therapy compliance positively influences hemoglobin levels, likely through better disease management.

Serum ferritin levels were markedly lower in the high adherent group (1139.05 ± 575.1 ng/mL) than in low adherent patients (2729.5 ± 1667 ng/mL)
This difference was highly significant (P = 0.001), highlighting the critical role of compliance in controlling iron overload in chronically transfused patients.
Although serum creatinine levels were slightly higher in low adherent patients(1.12±0.25) compared to low adherents patients(0.94±0.18),The difference did not reach statistical significance (P = 0.148).
Also Cystatin C levels were slightly higher in low adherents patients (1.25±0.2)compare to patients were high adherents chelating therapy(1.55±0.3), the differences were statistically significant (P = 0.032). Therefore, strong correlation between compliance with therapy and cystatin c level .
Compliant patients had significantly higher eGFR (98.5 ± 15.2 vs. 85.4 ± 12.7 mL/min/1.73m², *p* = 0.01*),(Table 4-3)

Table (4-4) : Correlation of Laboratory Parameters.

Parameter	P	HB level (g/dl)	S ferritin (ng/ml)	S creatinine (mg/dl)	Cystatine c (mg/l)	eGFR by creatinine ml/min/1.73m2
Hb	P		0.048*	0.809	0.034*	0.27
	R		-0.08	-0.02	-0.09	-0.12
S ferritin (ng/ml)	P	0.048*		0.552	0.041*	0.01
	R	-0.08		0.07	0.06	0.27
S creatinine (mg/dl)	P	0.809	0.552		0.047*	0
	R	-0.02	0.07		0.23	-0.46
Cystatine c (mg/l)	P	0.034*	0.041*	0.047*		0.02*
	R	-0.09	0.06	0.23		
eGFR by creatinine	P	0.27	0.01	0	0.02*	
	R	-0.12	0.27	-0.46	0.23	

*Significant association at P<0.05 **highly Significant association at P<0.01

The results demonstrated a significant negative correlation between Hb and serum ferritin (P = 0.048, R = –0.08) as well as between Hb and cystatin C (P = 0.034, R = –0.09). Serum ferritin was positively correlated with cystatin C (P = 0.041, R = 0.06). Serum creatinine also showed a significant positive correlation with cystatin C (P = 0.047, R = 0.23). Moreover, a significant negative correlation was found between cystatin C and eGFR (P = 0.020, R = –0.46).Table 4-4).

Table (4-5) Correlation between Patient age, Age at diagnosis, Duration of Disease ,other Clinical Variable and Laboratory Parameters.

Parameter	P	Hb level (g/dl)	S ferritin (ng/ml)	S.creatinine (mg/dl)	Cystatin c (mg/l)	eGFR by creatinine ml/min/1.73m2
Age/year	P	0.07	0.001**	0.008**	0.476	0*
	R	-0.21	0.38	0.30	0.08	0.61
Age at diagnosis/year	P	0.218	0.752	0.77	0*	0.18
	R	-0.15	0.36	0.33	-0.04	0.54
Duration of disease/ year	P	0.187	0.001**	0.004**	0.7	0**
	R	-0.08	0.33	0.29	-0.01	0.54
Age at 1 st blood trsnsfusion/year	P	0.37	0.66	0.8	0.001**	0.29
	R	-0.14	0.3	-0.03	0.42	0.15
Duration of receiving blood transfusion/year	P	0.5	0.005**	0.011*	0.88	0**
	R	-0.10	0.05	0.03	0.51	0.12

*Significant association at P<0.05 **highly Significant association at P<0.01

The results showed that age was significantly correlated with serum ferritin (P = 0.001, R = 0.38) and serum creatinine (P = 0.008, R = 0.30), but not with Hb, cystatin C, or eGFR.
Age at diagnosis was significantly associated with cystatin C

(P = 0.040, R = –0.04), while no significant correlations were found with other variables.

Duration of disease was significantly associated with serum ferritin (P = 0.001, R = 0.33), serum creatinine (P = 0.004, R = 0.29), and eGFR (P = 0.000, R = –0.54), but not with Hb or cystatin C.

Age at first transfusion showed a significant positive correlation with cystatin C (P = 0.001, R = 0.42), while no significant associations were found with Hb, ferritin, creatinine, or eGFR.

Duration of transfusion demonstrated significant positive correlations with serum ferritin (P = 0.005, R = 0.05) and serum creatinine (P = 0.011, R = 0.03), and a significant negative correlation with eGFR (P = 0.000, R = –0.12).,(Table 4-5)

Table(4-6) Comparison of laboratory parameters between patients with Hb ≥ 7 g/dL and Hb < 7 g/dL

Variable	Hb>7 n=(57)	Hb<7 n=(17)	P value
HB level	0.655±8.121	0.84±6.27	0.001**
S ferritin	1752.77±2723.68	1529.75±3627.85	0.059
S Creatinine	0.05±0.35	0.06±0.36	0.5
S Cystatin C	0.265±1.10	0.22±1.24	0.05

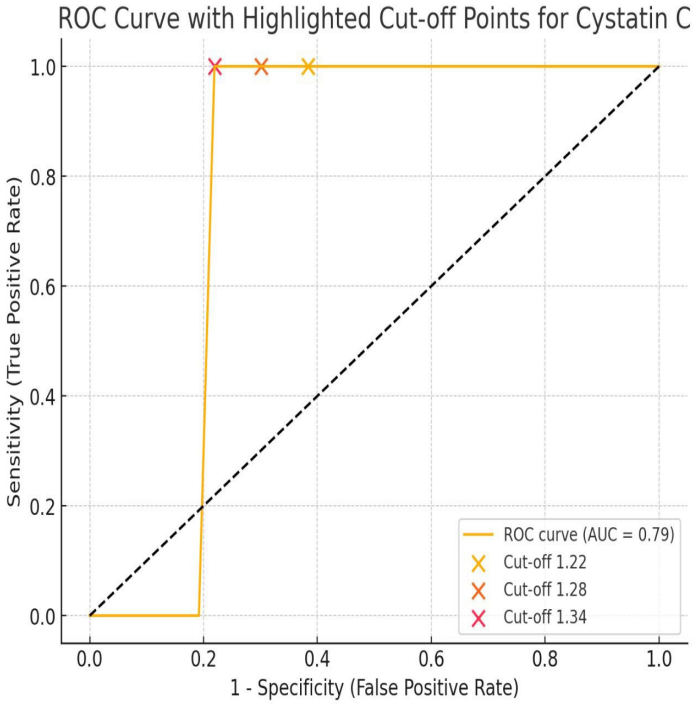
*Significant association at P<0.05 **highly Significant association at P<0.01

The comparison between patients with Hb ≥ 7 g/dL and those with Hb < 7 g/dL showed a highly significant difference in hemoglobin level (P < 0.001), as expected due to the classification criteria. Serum ferritin levels tended to be higher in the low-Hb group, but the difference did not reach statistical significance (P = 0.059). Similarly, no significant differences were found for serum creatinine, or cystatin C levels (P > 0.05 for all), although patients with Hb < 7 g/dL tended to have and higher cystatin C values. (Table 4-6)

Table (4-7): Sensitivity and Specificity of Different Cystatin C Cut-off Values in Predicting Kidney Dysfunction

Cut-off Cystatin c	Sensitivity (%)	Specificity (%)	AUC	P value
1.34	100%	71%	0.79	<0.0001

Receiver Operating Characteristic (ROC) analysis demonstrated that Cystatin C has a good predictive ability for early detection of kidney dysfunction, with an area under the curve (AUC) of 0.79. The optimal cut-off value was identified at 1.34 mg/L, which yielded a sensitivity of 100% and a specificity of 71%. This cut-off reflects an excellent balance between identifying true positive cases and minimizing false positives., the p-value was <0.001, indicating strong statistical significance of the results.(Table 4-7)



(Figure 4-2)ROC curve with highlighted cut off point for cystatin C

DISCUSSION

This study include 74 patients previously diagnosed with iron overloaded beta thalassemia major were analyzed for demographic variables .. Key demographic characteristics observed include a majority of participants (56.75%) aged between 10–14 years, with a smaller proportion (18.91%) below 5 years. Females constituted a slightly higher proportion (56.75%) than males. A significant finding was that most patients (64.86%) were diagnosed before the age of one year. Furthermore, nearly half of the patients (47.29%) had a disease duration between 5–10 years .this agree with local study (Shwayel, Jewad and Abdulsattar., et al.,2023) reported an equal sex distribution (50.0% males, 50.0% females) among 60 children aged 1–14 years. Similarly, (Mahmoud et al., 2021) examined 100 children with β-TM (62.0% males, 38.0% females).The most important result in our study is age at diagnosis showed strong correlation with cystatin C.

Such association between age of diagnosis and cystatin C levels is a new finding, not highlighted in the past literatures. While (Behairy., et al., 2017) and (Mahmoud., et al.,2021) wrote that cystatin C can be used as new risk factor for renal features in thalassemic child but they did not focus on this question or limited to the role of time of diagnosis in cystatin C levels. Our results further the existing knowledge by providing evidence that early diagnosis could have a role in limiting renal involvement.

Patients diagnosed at later age (3-5 years) showed significantly increased levels of cystatin C compared

to younger child. This implies that late diagnosis of beta thalassemia major might raise the degree of renal glomerular dysfunction manifested by increased cystatin C levels. This finding is consistent with the pathophysiology of untreated disease; oxidative stress and tissue injury occur prior to commencement of therapeutic intervention. Chronic anemia, hypoxia, and increased oxidative stress pre-diagnosis are probably underlying the pathophysiology of this association.

Regarding to age and duration of disease our study reveals absence of correlation between cystatin C with age or disease duration in our study, in contrast to the significant associations with creatinine this go with the finding of other reports (Mahmoud., et al., 2021) who found serum creatinine rose slightly but significantly with age, presumably due to somatic growth and cumulative exposure to transfusions. This is consistent with a characteristic that cystatin C rather than creatinine is less age-dependent after infancy, which results in a better early maker of renal dysfunction.

The study further elucidated the associations between these demographic factors and various laboratory parameters. For instance, mean hemoglobin (Hb) levels were found to decline with increasing age, while mean serum ferritin progressively increased with age, indicating that age is a crucial factor in iron overload this finding supported by study (Jing et al., 2021) (Fairweather-Tait et al., 2014)

In contrast, serum ferritin concentrations generally increase with age. A cross-sectional study of 441 men and women reported that serum ferritin concentration was positively associated with increasing age in women, although this association was not observed in men.

Regarding to adherence to therapy There was a significant correlation between chelation compliance and cystatin . Curiously, those non- compliance with therapy were the ones with higher levels of cystatin C (1.22 mg/L), pointing to more severe glomerular involvement and this result goes in line with (Tanous., et al., 2021), The effects of chelation therapy on the renal function are probably various. Chelating agents might minimize renal iron by depleting body iron sources or reducing renal iron retention and, thus, ameliorate oxidative damage related to iron overload in the kidney. However, nephrotoxicity has been reported in some patients with some chelation agents, mainly deferoxamine. The contrast between the positive impact of reducing iron load and the possible negative nephrotoxic impact of chelation agents may depend on the chelator used, dose and the individual patient.

This is consistent with this large nephroprotective thalassemia chelation therapy literature (Bilir., et al., 2020) also described increased cystatin C related to iron chelation in transfusion dependent beta thalassemia that supported the link between iron burden management and preservation of kidney function. The most important effect of therapy compliance on cystatin C in our study is to support adhering to iron chelation regimens to prevent renal injury.

Regarding to blood transfusion in our study, a significant difference was identified between age at first indication for blood transfusion and cystatin C levels, suggesting that timing of transfusion initiation may critically influence kidney health. Specifically, patients who began transfusions at an older age showed elevated cystatin C, indicative of more severe glomerular dysfunction—a relationship that remained significant even after adjusting for age and disease duration. This underscores the independent impact of transfusion timing on renal outcomes. This finding supported by (Demosthenous., et al., 2019) revealed chronic anemia prior to transfusion may lead to sustained tissue hypoxia, promoting adaptive mechanisms in the kidney such as glomerular hyperfiltration and renin–angiotensin–aldosterone system activation, which, over time, contribute to glomerular hypertension, proteinuria, and progressive renal injury. Conversely, our study revealed a highly significant positive correlation was observed between serum ferritin and serum creatinine ,and a significant positive correlation with cystatin C. These findings strongly suggest a direct link between iron overload and renal function impairment, likely attributable to iron-induced nephrotoxicity agree with finding of other other reports (Tanous et al., 2021; Romadhon., et al., 2022) iron overload from repeated transfusions—each unit delivering ~200–250 mg of iron—is associated with oxidative stress, tubular injury, and glomerulosclerosis, accelerating renal decline .

Furthermore, a statistically significant positive correlation was noted between eGFR and serum ferritin , as well as eGFR and cystatin C. The inverse

relationships observed indicate that as iron load and renal injury increase, glomerular filtration capacity declines

This reinforces the notion that cumulative iron exposure over time, often due to chronic transfusions, significantly impacts renal health.

Importantly, literature supports our study finding (Romadhon et al.,2022) revered that elevated cystatin C reliably correlates with both iron overload and subclinical kidney dysfunction the utility of cystatin C as a sensitive biomarker of early renal dysfunction in β-thalassemia major. (Makmettakul., et al.,2020) described frequent tubular and glomerular abnormalities in thalassemia patients, mediated by hypoxia and iron deposition in renal tissue.

However, persistent iron dysregulation, whether deficiency or overload, can contribute to the progression of renal damage.

These align with our study, serum cystatin C exhibited a significant inverse correlation with hemoglobin levels , indicating that worsening anemia is associated with elevated cystatin C, a marker of early renal impairment. Although anemic patients (Hb < 7 g/dL) showed higher cystatin C levels than those with Hb ≥ 7 g/dL, the trend—although borderline—suggests that chronic anemia may contribute to early subclinical kidney dysfunction in thalassemia major, (Behairy., et al., 2017) support our finding this association

reflects known pathophysiologic mechanisms: sustained anemia can induce renal medullary hypoxia, activating compensator hyperfiltration and RAAS upregulation, which, if unrelieved, may progress to glomerular sclerosis and loss of nephron integrity. As cystatin C is highly sensitive to subtle declines in glomerular filtration, its elevation in anemic children signals early renal stress.

Regarding to s,creatinine and eGFR, Serum creatinine correlated significantly with cystatin C in our study , it is reasonable that they are both markers of renal function. However , the association of cystatin C with other clinical factors was not like those of the conventional markers. Serum creatinine was significantly correlated with the age and disease duration , whereas cystatin C did not show significant relationships with these. These finding supported by (Al-jefri,Alotaibi and Alanazi., et al., 2017) 94 β-TM patients revealed that 56.3% had elevated cystatin C levels, with a positive correlation to serum creatinine and urea, and a negative correlation with eGFR. This underscores cystatin C's role in detecting early renal issues before significant creatinine elevation .also, A study involving 70 children with β-TM found that serum cystatin C levels were significantly higher compared to healthy controls. Importantly, cystatin C levels were negatively correlated with estimated glomerular filtration rate (eGFR), indicating its potential as an early marker of glomerular dysfunction (Hamdy., et al.,2017).

Regarding to sensitivity and specificity of cystatin c our date specifically highlights the predictive ability of cystatin c for early detection of kidney dysfunction, with an optimal cut-off value of 1.34 mg/L yielding 100% sensitivity and 71% specificity. Several study supported our finding(Hamdy., et al.,2017) reported that cystatin C had a sensitivity of 91.4% and specificity of 90.0%, compared to serum creatinine's 83.0% sensitivity and 100% specificity.Another study in Egypt found that cystatin C had a higher area under the receiver operating characteristic curve (AUC) than serum creatinine, indicating better diagnostic accuracy (Mahmoud and Ali., et al ., 2012)

The establishment of appropriate cut-off points for biomarkers is critical for their clinical utility in screening and diagnosis. While the specific optimal cut-off for cystatin C in β-TM patients may vary slightly across studies due to population differences and methodologies, For example, (Al-Tameemi and Altawry., et al., 2020) reported that the mean serum cystatin C level among their cohort of transfusion-dependent beta thalassemia major patients was 1.075 mg/L. While this suggests higher sensitivity, the study did not define a precise diagnostic threshold for cystatin C. In our study a value around 1.2 mg/L has been frequently discussed as indicative of early renal dysfunction. This finding aligns with the general understanding that values above 1.2 mg/L often signify a decline in GFR, even when creatinine levels are still within normal limits. This cut-off point of 1.2 mg/L for cystatin C is particularly relevant when related to eGFR by creatinine . A cystatin C level of 1.2 mg/L, especially when eGFR by creatinine is still seemingly normal, serves as a critical warning sign, prompting further investigation and potentially earlier initiation of nephroprotective measures. This early detection is vital in iron-overloaded β-TM patients, where progressive renal damage can be insidious and difficult to reverse once established. The sensitivity of 100% at a cut-off of 1.22 mg/L suggests that this threshold is highly effective in identifying all individuals with kidney dysfunction, making it an excellent screening tool.

Cystatin C has gained considerable attention as a superior biomarker for early detection of renal dysfunction in β-TM patients compared to serum creatinine. Its advantages stem from its stable production rate and exclusive elimination by glomerular filtration, making its serum concentration a direct reflection of GFR (Saghir., et al., 2020). Several studies have demonstrated that elevated serum cystatin C levels can identify subtle reductions in GFR even when serum creatinine remains within the normal range, thus providing an earlier indication of kidney impairment in iron-overloaded β-TM patients.For instance,(Hamdy., et al ., 2021) investigated the utility of cystatin C in detecting renal insufficiency in β-TM patients and found it to be a more sensitive marker than creatinine.

CONCLUSIONS

Chronic iron overload significantly impacts renal function in β-TM patients.

Cystatin C serves as a sensitive and early predictor of kidney dysfunction.

This cut-off point of 1.34 mg/L serves as a critical warning sign, prompting further investigation and potentially earlier initiation of nephroprotective measures.

Strong statistical correlations were observed between iron overload markers (ferritin), renal function parameters (creatinine, cystatin C, eGFR), and the positive effects of chelation therapy compliance.

RECOMMENDATIONS

1. Regular monitoring of cystatin C levels, particularly in patients with risk factors such as later diagnosis or poor therapy compliance, may allow for earlier detection of renal complications and timely intervention.
2. Screening programmes designed for beta-thalassemia so that early diagnosis and treatment can be implemented. In areas where the frequency of beta thalassaemia is high, newborn screening may be of benefit to ensure early diagnosis and treatment.
3. Educational and support programs for patients and their parents should address adherence to potentially maintain renal function and prevent complications. Interventions to

increase adherence might involve easier dosing schedules, side-effect management, ongoing monitoring and feedback, and psychosocial support for patients and caregivers.

4. New approaches that are patient focused for the enhancement of adherent to treatment should acknowledge the differences and challenges in the care of the pediatric patient and the family.

5. Transfusion protocols may need to consider potential renal effects when determining optimal timing for transfusion initiation. The development of evidence-based guidelines for transfusion initiation that balance the risks of anaemia-related hypoxic damage against the long-term complications of transfusion therapy, including iron overload, would be valuable for optimising outcomes in beta thalassemia major patients.

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