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Detection of Early Renal Damage using Serum Cystatin-C in Transfusion Dependent Thalassemia Syndrome Patients

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ABSTRACT

Objective

This study investigates the frequency of early renal damage among TDT patients using serum cystatin-C. The relationship between serum cystatin-C and serum ferritin levels is also elucidated.

Methodology

Descriptive cross-sectional study, Place & duration of research Khyber Medical University, May 2019 to January 2020.Known TDT patients, 3 to 16 years of age, were enrolled in the study. Biochemical tests were conducted on the blood and urine samples of the patients. Data was entered and analyzed for statistical significance.

Results

In pediatric TDT patients, serum cystatin-C was high but serum creatinine and estimated glomerular filtration rate (eGFR)normal when compared to controls. A positive correlation was identified between serum cystatin-C and serum ferritin. Similarly, Serum cystatin-C was found to have a positive correlation with pretransfusion hemoglobin. The interval of transfusion was, however, inversely associated with serum cystatin-C levels.

Conclusion

Asymptomatic renal dysfunction is found in TDT patients. This may be attributed to iron overload.

Key words: Transfusion dependent thalassemia, Thalassemia Major, Renal functions, Serum cystatin-C

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INTRODUCTION

Thalassemia is the most common type of hemoglobinopathy and its inheritance is autosomal recessive. It has a variable prevalence around the globe.Approximately 5000-6000 children are born with thalassemia per year[1]. The estimated carrier rate is 5-7% in Pakistan[2].The use of iron chelators has improved the overall wellbeing andlife expectancy among thalassemia (TDT) patients. Iron accumulation has been found to have deleterious effects on the heart, liver, and endocrine systemof the body; effects on renal health, however,still remains obscure and have not been sorted in-depth[3].Limited data is available on the mechanism of involvement of the renal system in thalassemia patients. Chronic anemia and iron overload followed by chelation therapy for iron overload are assumed to be the principal causes of renal epithelial and glomerular injury[4].Renal injury can occur in thalassemia patients without any obvious clinical symptoms and before being detected through routine renal function tests and urine analysis[5]. Cystatin-C is a proteinase inhibitor of cysteine protease that is produced at a constant rate by all nucleated cells and is presentin all body fluids& serum. It has free filtration and complete reabsorption from glomeruli and renal tubules. This means that levels of cystatin-C are only being obtained by its glomerular filtration; therefore, its serum concentration providesan excellent estimation of glomerular filtration rate (GFR)[6].Studies performed in the pediatric population suggest that renal injury can occur in thalassemia patients. A study conducted in Iran suggested reduced GFR was observed in 51% of pediatric thalassemia patients[7]. In another study, 36% of patients showed high serum cystatin-C & proteinuria in 24% of thalassemia patients[8]. Iron overload is common among local TDT patients. Studies depicting assessment of renal health are, however, lacking. The present study aims to detect renal function depletion among TDT patients using a serum cystatin-C biomarker. Findings from the study will also provide further insight into the renal pathophysiology of the disease in local settings.

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METHODOLOGY

The study commenced after it was approved from the ethical review board of Khyber Medical University (DIR/KMU-EB/DE/000611 on 07-05-2019), and patients were enrolled from Fatimid Foundation Peshawar. Diagnosed TDT patients, ranging in age from 3 to 16 years and a comparable group of healthy controls were enrolled into the study. The control group comprised healthy siblings of study patients. All the participants were enrolled on the investigation after the acquisition of informed written consent from guardians of the patients. The participants were randomly selected, excluding those with urinary tract infections, systemic renal disease, family history of inherited renal disease and use of antibiotics like cephalosporins, trimethoprim and corticosteroids in the last seven days. This study was conducted from May 2019 to January 2020.

A comprehensive questionnaire containing age, gender, weight, height, splenectomy and transfusion history, history of intake of iron chelators and clinical examination was filled out for each study patient. Patients were advised for an overnight fasting and avoiding intake of any medicine 24-hours prior to sample collection. Venous blood samples and urine samples were collected before transfusion. Complete blood count (CBC) was performed within two hours of sample collection. Serum was extracted within two hours of sample collection and immediately frozen at -80 °Ctill further biochemical analyses. Urine routine examination and microscopy were performed on the same day of sample collection. Serum creatinine was performed by Creatinin-jeffe® enzyme assay on Cobas c111 and eGFR was calculated using Schwartz formula for children:

eGFR (ml/min/1.73 m^2) = height (cm) × constant/ serum creatinine (mg/dL)

height was expressed in "cm" and constants was 0.44.

Serum cystatin-C was measured according to manufacturer's guidelines using Human Cys-C ELISA Kit®(Elabsciences USA; Catalog No: E-EL-H3643).The data was analyzedon SPSS version 20. Numerical variables were presented by mean ±SD. Categorical variable was presented by percentage& frequency. Independent sample T-test was used to compare numerical data across subgroups, while chi-square test was used to compare categorical data across subgroups. A p-value of ≤0.05 was considered significant. To determine the bivariate relation between continuous predictor variables like ferritin, age, and Cystatin-C Pearson's correlation and to compare means between two groups for different variables independent T-test was done.

RESULTS

A total of 88 participants, 69 TDT patients and 19 healthy controls, were enrolled in the study. Mean ages of participants (study and control groups) were 7.6±3.5 and 7.2±3.2 years, respectively. There was no statistically significant difference between the patients and controls regarding age, gender, weight, and height (Table 1).

Table. 1. Demographic Characteristic of Case and Control (n=88)

Demography	Case (N=69) Mean (±SD)	Control (N=19) Mean (±SD)	*p*-value
Age (Years)	7.6(3.5)	7.2(3.2)	0.64
Height (Cm)	114(16.0)	112.05(10.3)	0.51
Weight (Kg)	20(7.0)	25(9.3)	0.05
Gender	n (%)	n (%)	
Male	33 (48%)	10(52.6%)	
Female	36(52%)	9 (47.3%)	

Renal function tests, eGFR and serum cystatin c were performed in both patients and the control group (Table 2).

Biochemical Parameters	Cases(N=69) Mean (±SD)	Controls(N=19) Mean (±SD)	*p*-value
Serum Na	137.38 (±13.02)	140.86(±3.74)	0.255
Serum K	4.343 (±0.59)	4.38(±0.47)	0.773
Serum Cl	115.44 (±114.84)	100.56(±3.45)	0.575
Urea	19.97 (±5.75)	17.78(±4.47)	0.130
Creatinine	0.355(± 0.718)	0.405(±0.524)	0.006
Serum ferritin	2627.15(±235.89)	79.42(±13.95)	< 0.001
eGFR	134.26(±135.43)	113.21(±11.1)	0.013
Cystatin-C	25.78 (±9.5110)	9.44 (±7.338)	0.000

Table 2. Results of biochemical parameters in both groups

The mean eGFR was found to be 134.2 ml/mi/1.73m for the TDT patients, whereas, among the control group, it was 113.21(±11.1) (p=0.013). In the current study, eGFR positively correlated with age, weight) and height and serum cystatin-C significantly correlated with serum ferritin (Table 3).

Parameters	eGFR		Cystatin-C	
	R	*p* value*	R	*p* value*
Age	0.450	< 0.001*	0.149	0.166
Weight	0.406	< 0.001*	-0.054	0.619
Height	0.5400	< 0.001*	0.136	0.206
Age at diagnosis in months	0.245	0.021*	0.509	<0.001
Serum ferritin	0.272	0.010*	0.322	0.002

Table 3. Correlation of eGFR with different parameters for patients. *Significant at 0.05

Serum cystatin-C of cases was 25.78±9.51, and controls are 9.44±3.380 (p<0.01). There was a statistically significant and positive correlation between serum cystatin-C and pre-transfusion Hb. A positive Pearson correlation was found between serum cystatin-C and other laboratory tests performed (Table 4).

Characteristics	Cystatin-C	
	R*p* value	*p*-value
Pre-transfusion Hb	0.331	0.005*
Creatinine (mg/dL)	0.135	0.270
Urea(mg/dL)	0.122	0.320
eGFR ml/min1.73m^2	0.185	0.084
Serum ferritin(ng/mL)	0.322	0.002*
Interval of transfusion	-0.095	0.440

Table 4. Correlation of serum cystatin-C with other laboratory parameters.*Significant at 0.05

All the patients in the current study had high serum ferritin levels, with a mean of 2625.15±2357.89ng/ml. These patients were divided into categories of mild (500-999ng/ml), moderate (1000-2499 ng/ml) and severe iron overload (above 2500 ng/ml).A serum ferritin level of ≤500ng/ml was taken as normal. Among study patients,45 had severe, 23% had moderate, and 26% had a mild iron overload. The mean eGFR and serum cystatin-C was also calculated in these categories of iron overload. Patients with severe iron overload had high levels of mean eGFR and mean serum cystatin-C (Table 5).

	(N=69)	eGFR (ml/min/1.73m^2)	Cystatin -C ng/ml
Severe iron overload	31	140.57((±41.7)	27.88(10.42)
Moderate iron overload	16	127.31((±26.5)	23.89(±8.53)
Mild iron overload	18	134.44((±32.8)	23.38(±7.27)

DISCUSSION

Overall quality of life and wellbeing of thalassemia major patients have been improved by regular blood transfusion and iron chelation at the same time.Regular transfusion consequently led to a myriad of complications due to iron overload.Studies have demonstrated that cystatin-C is proving to be a good tool than serum creatinine to detect early renal impairment. The present study is the first to observe cystatin-C in thalassemia patients in Pakistan to the best of our research efforts. In the current study, it was observed that, among thalassemia patients, the mean value of serum cystatin-C was 25.77±9.51 in comparison to 9.44±7.33 for the control group. The difference between the two values was statistically significant (p<0.001). These findings are favoured by the previous studies performed by Hanna et al. in thalassemia major patients[9]. Another researchin Greece showed similar findings where cystatin-C was higher in thalassemia major than controls[5].Hamid et al. identified similar findings; their study was conducted on thalassemia major patients of Egypt[10].However, it must be made clear that the causes and mechanism of renal damage have not been explored in this study which could be chronic anemia, hypoxia and iron overload. There is no correlation of serum cystatin-C with weight and height and age (r=0.054, r=0.136,r=0.149,) which is in accordance with HamedElmelgy (Age r=0.101, r=0.046)[10]. In contrast,Macdonaldset al.differ in this regard by stating that cystatin-C is influenced by the weight and height of the person[11]. The plausible explanation for these findings is that 60% of the body cells are skeletal muscles, and the skeletal muscle mass is variable for different demographics,races and nutritional intake[12].

There was statistically no significant correlation between creatinine, urea and serum cystatin-C, creatinine and urea (r=0.135 p=0.270), (r=0.122 p>0.005), which is similar to Kacaret al.[13]and contrary to Behairy et al. showing statistically significant positive correlation of serum cystatin-C with urea (r=+0.661,p=0.03) and creatinine (r=+24, p<0.005)[14].

There is no correlation between eGFR and cystatin-C is r=0.043 and p=0.73, which is contrary to Asma et al. who stated a robust negative correlation between cystatin-C and eGFR which is statistically significant (r=-0.151, p=0.001)[15]. The most reasonable argument for this may be because the biological variation of serum cystatin-C is low, it may give a better assessment of GFR during follow-ups[15].

There is a fair, positive correlation of serum cystatin -C with the serum ferritin, which is highly significant r=0.322, p<0.005, similar to Asmaet al. (r=0.519, p=0.001)[16]. It can be explained based on chronic life-long blood transfusions, which leads to iron deposition and increasediron absorption, causing secondary iron deposition and ongoing renal dysfunction[17, 18]. However, Annayev did not find any correlation between the two[19].

The mean e-GFR was 134.26 (±35 SD) ml/mi/1.73m^{2}in the case of thalassemia patients and 113.21 (±11.1 SD) for controls, the p<0.005, which is statistically significant. But the eGFR was higher than the normal range for children, which is calculated to be 107.3ml/min/m^2 [8]. It may be because the current study participants mainly were of younger age groups; therefore, it is assumed that renal damage was at an early stage and was insufficiently detectable by routine markers and formula using the same serum creatinine levels. Osama Tanous et al. presented that their patients had normal eGFR 100.9 ±17 ml/min/1.73m^2[20]. eGFR was elevated in a study conducted by Annayev et al. in Turkey; the mean value of eGFR was 216±57.2 in the Turkish population[19].The most suitable explanation for glomerular hyperfiltration is attributed to underlying anemia ,reducing systemic vascular resistance and increasing renal plasma flow and GFR. Milo et al .conducted a study in Israel that found a low eGFR in 58% of the patients[21]. Another study done by Ebru et al. et showed similar results with patients having low GFR[22].

The eGFR also positively correlates with serum cystatin- C in this study, which is not statistically significant. Ebru Uzun et al. have shown similar results, where the mean eGFR was normal

thalassemia patients than controls, and there was no statistical difference[22]. Ali et al. stated that eGFR and cystatin-C had a negative correlation which is statistically significant[18]. Hamed et al. also found eGFR and cystatin-C had a strong positive and significant correlation[11].

Previous studies were done by Zafari et al .also described that routine markers of renal functions (urea and creatinine) were normal in beta-thalassemia major children and their healthy controls[23]. In accordance with these previous studies, the observation of this study showed that the mean serum creatinine is 0.355 (± 0.0718) of thalassemia patients. In contrast, the mean creatinine of controls was (mean= 0.40±0.05) and the $p>0.005$, which is statistically not significant. In contrast ,Mahmoud et al. described the higher level of serum creatinine in beta-thalassemia major children[16].

The majority of thalassemia patients in this study had high serum ferritin levels (67%) and a mean serum ferritin level of 2625.15(±2357.89). Similar to this, Ali et al. also had high serum ferritin in thalassemia patients than controls with $p<0.005$[18]. There is a fair, positive correlation of serum cystatin -C with the serum ferritin, which is highly significant r=0.322, $p<0.005$ supporting Uzun et al.[22]. This may explain that early glomerular dysfunction is associated with iron accumulation in glomerular cells. In the present study the complete blood count values like Hb, RBC count, MCV and MCH, were significantly different for patient and controls with the $p<0.001$. The study conducted by Ebru et al. stated similar findings that pretransfusion Hb of thalassemia patients and controls were significantly different ($p<0.001$)[22].

CONCLUSION

To summarize the current study, serum cystatin-C is raised in 71% of transfusion-dependent thalassemia syndrome patients. The percentage of patients with serum ferritin more than 1000ng/ml is 90%. This finding concludes that these thalassemia patients have early renal damage which is not being detected by routine renal function tests, and eGFR is also being observed higher than the normal range; however, at the same time, it also raises a few unanswered quarries about the actual mechanism of renal damage in thalassemia patients and the outcome of iron overload and iron chelation therapy on the renal system. The limitation is a small sample size, and extensive researches are recommended to elucidate mechanism of renal disease in thalassemia population on a large sample size involving multiple ethnic groups and populations involving other provinces as well.

Conflict of Interest: None

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REFERENCES

1. Goonasekera H, Paththinige C, Dissanayake VJArog, genetics h. Population screening for hemoglobinopathies. 2018;19:355-80.
2. Fazal F, Arshad M, Mustafa H, Rehman MEU, Fazal UTF, Arshad M, et al. Assessment of level of Awareness regarding Thalassemia Major among Parents of Affected Children. 2021;25(1).
3. Origa RJGiM. β-Thalassemia. 2017;19(6):609-19.
4. Deveci B, Kurtoglu A, Kurtoglu E, Salim O, Toptas TJAoh. Documentation of renal glomerular and tubular impairment and glomerular hyperfiltration in multitransfused patients with beta thalassemia. 2016;95:375-81.
5. Yaghobi M, Miri-Moghaddam E, Majid N, Bazi A, Navidian A, Kalkali AJIjoh-o, et al. Complications of transfusion-dependent β-thalassemia patients in Sistan and Baluchistan, South-East of Iran. 2017;11(4):268.
6. Duarte DB, Lacerda MCSR, Ribeiro YJP, Ribeiro MZD, Frederico MdA, Oliveira MJCJP, et al. Kidney biomarkers in tropical infections: an update. 2020;114(6):302-8.
7. Quinn CT, Johnson VL, Kim HY, Trachtenberg F, Vogiatzi MG, Kwiatkowski JL, et al. Renal dysfunction in patients with thalassaemia. 2011;153(1):111-7.
8. Mian AN, Schwartz GJJAickd. Measurement and estimation of glomerular filtration rate in children. 2017;24(6):348-56.
9. Hanna D, Beshir M, Khalifa N, Baz E, Elhewala AJPH, Oncology. Assessment of cystatin C in pediatric sickle cell disease and β-thalassemia as a marker of subclinical cardiovascular dysfunction: a case-control study. 2021;38(7):620-32.
10. Demosthenous C, Vlachaki E, Apostolou C, Eleftheriou P, Kotsiafti A, Vetsiou E, et al. Beta-thalassemia: renal complications and mechanisms: a narrative review. 2019;24(1):426-38.
11. Hamed EA, ElMelegy NTJIjop. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. 2010;36:1-10.
12. Macdonald J, Marcora S, Jibani M, Roberts G, Kumwenda M, Glover R, et al. GFR estimation using cystatin C is not independent of body composition. 2006;48(5):712-9.
13. Forbes GB. Human body composition: growth, aging, nutrition, and activity: Springer Science & Business Media; 2012.
14. Kaçar AG, Şilfeler İ, Kacar A, Pekun F, Türkkan E, Adal E. Levels of beta-2 microglobulin and cystatin C in beta thalassemia major patients. 2015.
15. Behairy OG, Abd Almonaem ER, Abed NT, Abdel Haiea OM, Zakaria RM, AbdEllaty RI, et al. Role of serum cystatin-C and beta-2 microglobulin as early markers of renal dysfunction in children with beta thalassemia major. 2017:261-8.
16. 16. Mahmoud AA, Elian DM, Abd El Hady NM, Abdallah

HM, Abdelsattar S, Khalil FO, et al. Assessment of subclinical renal glomerular and tubular dysfunction in children with beta thalassemia major. 2021;8(2):100.

17. Hilderink JM, van der Linden N, Kimenai DM, Litjens EJ, Klinkenberg LJ, Aref BM, et al. Biological variation of creatinine, cystatin C, and eGFR over 24 hours. 2018;64(5):851-60.

18. Ali BA, Mahmoud AMJSQUMJ. Frequency of glomerular dysfunction in children with beta thalassaemia major. 2014;14(1):e88.

19. Annayev A, Karakaş Z, Karaman S, Yalçıner A, Yılmaz A, Emre SJTJoH. Glomerular and tubular functions in children and adults with transfusion-dependent thalassemia. 2018;35(1):66.

20. Tanous O, Azulay Y, Halevy R, Dujovny T, Swartz N, Colodner R, et al. Renal function in β-thalassemia major patients treated with two different iron-chelation regimes. 2021;22(1):1-11.

21. Milo G, Nevo RFG, Pazgal I, Gafter-Gvili A, Shpilberg O, Gafter U, et al. GFR in Patients with β-Thalassemia Major. 2015;10(8):1350.

22. Uzun E, Balcı YI, Yüksel S, Aral YZ, Aybek H, Akdağ BJRf. Glomerular and tubular functions in children with different forms of beta thalassemia. 2015;37(9):1414-8.

23. Zafari M, Aghamohammady A, Mosavy MJBJoMS. Renal function in Thalassemia major patients who treated by Desferal. 2018;17(1):58.

CONFLICT OF INTEREST

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