International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12s,2025
https://theaspd.com/index.php

Early Detection Of Glomerular Dysfunction In Beta Thalassemia Major Patients Undergoing Chelation Therapy

Younis Hassan Mohammed Ali[1], Hashim Abdulsattar Jabbar[2], Entedhar Rifaat Sarhat[3],
[1]MSc at Department of Clinical Biochemistry, College of Medicine, Tikrit University, Tikrit, Salahaadeen, Iraq,Younis.h@st.tu.edu.iq
[2]Assistant Professor at Department of Clinical Biochemistry, College of Medicine, Tikrit University, Tikrit, Salahaadeen, Iraq,hashim_sattar@tu.edu.iq
[3]Professor at Department of Clinical Biochemistry, College of Medicine, Tikrit University, Tikrit, Salahaadeen, Iraq,entedharr@tu.edu.iq

Abstract
Background: *Thalassemia is one of the prevalent hemoglobinopathies caused by gene mutation leading to chronic anemia. Iron overload and direct nephrotoxic effects of chelators are the largely accepted explanations for the mechanisms of renal glomerular impairment in BTM. The aim of this study was to early detection of glomerular dysfunction in BTM patients receiving iron chelators using novel biomarker.* ***Methods:*** *The study included 60 patients with BTM receiving iron chelators: group 1 receiving Deferoxamine (DFO) and group 2 receiving Deferasirox (DFX). Control group include 30 healthy people where matched age and sex with patients. Serum Cystatin C, serum creatinine, blood urea, albumin/creatinine ratio (ACR) in urine was measured in patients and controls.* ***Results:*** *There is no statistically significant difference in blood urea levels between patients and controls. Patients' and controls' serum creatinine levels are statistically significantly different. Serum Cystatin C shows a significant difference between patients and the control group (higher in patients) and is slightly higher in the Deferoxamine group than the Deferasirox group. Patients in the study had higher Albumin-Creatinine Ratio (ACR) values than controls, which is statistically significant.* ***Conclusions:*** *Serum Cystatin C and urinary ACR consider as early markers of glomerular dysfunction. Both Deferasirox and Deferoxamine can produce changes in glomerular function markers.*
Keyword: *beta thalassemia major, glomerular dysfunction, iron chelators, Cystatin C.*

INTRODUCTION

Thalassemia is one of the most prevalent hemoglobinopathies worldwide, caused by gene mutations [1]. Blood transfusions are necessary for patients with β-thalassemia major, which is caused by mutations in two beta globin genes and leads to chronic anemia [2]. Patients with β-thalassemia had a higher survival rate due to blood transfusions and iron chelation treatment. Nonetheless, a number of problems, including renal complications, continue to affect many individuals [3]. Renal glomerular damage is known to be caused by persistent anemia and hypoxia, iron deposits in the kidney tissue and toxicities related to iron chelators [4]. For patients with β-TM, iron chelation therapy is required to eliminate excess iron. Iron is eliminated when circulating iron and iron-chelating agents bind together [5]. Prompt dose modifications based on weight fluctuations can be achieved with appropriate chelation therapy beginning in early childhood [6]. The first chelating medication to receive a license is deferoxamine (DFO). After binding to iron in parenchymal liver cells, it enters bile and plasma, and then the iron is eliminated from the body [2]. Acute kidney damage caused by high DFO dosages, renal glomerular and tubular abnormalities, and local injection responses are typical adverse effects of this medication [7]. Deferasirox (DFX), the newest oral iron chelator, is the second medication. Deferasirox has the ability to raise hepcidin levels, which causes ferroportin to degrade and then decreases the iron in the body [8]. Blood creatinine levels may increase in persons receiving it. After beginning large doses of DFX medication, nephrotoxicity may appear rapidly. Increasing the dosage

of deferasirox results in a marked increase in proteinuria [8, 9]. The conventional markers for glomerular involvement, such as urea and creatinine in thalassemia, have remained unchanged for a number of decades. However, in order to identify glomerular dysfunction in these individuals early, the additional biomarkers must be developed [10]. The protein known as cysteine proteinase inhibitor Cystatin C has a molecular weight of 13 kDa, is secreted by all nucleated cells at a constant rate, is easily filtered by the renal glomerulus, undergoes nearly total reabsorption and catabolism in the proximal tubule, and is not significantly excreted in the urine in healthy individuals [11]. Unlike creatinine, its level is independent of age, gender, and muscle mass, making it the ideal marker for early diagnosis of glomerular dysfunction [12]. Another excellent indicator for early albuminuria detection is the urinary albumin creatinine ratio (ACR), which may be brought on by modifications in the glomerular basement membrane's permeability [13]. The primary cause of proteinuria, which is associated with early kidney injury, is a disruption in the kidney filter [14]. Proteinuria is a sign of early renal dysfunction [15, 16]. Microalbuminuria is defined as a urine albumin creatinine ratio (UACR) of greater than 30 mg/g, whereas macroalbuminuria is defined as a UACR of greater than 300 mg/g [17].The aim of the current study is the early diagnosis of glomerular impairment in beta thalassemia major patients receiving iron chelator therapy by using novel biomarkers. And to evaluate the effect of DFX and DFO on the glomerular function and compare between them.

MATERIALS AND METHODS

Setting and time

This study was conducted at Al Hadbaa Specialist Hospital in Nineveh Governorate, Iraq. The study period was from October 2024 to February 2025.

Subjects

The current study includes 60 patients of both sexes with beta thalassemia major who undergo routine blood transfusions and take specialized iron chelators. Their ages range from 7 to 55 years, and the median (Q1, Q3) is 17.5 (14.5, 21.0). Based on the type of chelator, the patients were split into two groups: group 1 was given DFO, while group 2 was given DFX. Using hematological and genetic techniques, the hematologist determined that they had beta thalassemia major. The control group includes 30 apparently healthy individuals. The control group was matched for sex and age with the patients. The age range of the controls was 7-54 years, and the median (Q1, Q3) was 19.5 (15.0-33.0). Direct interviews with participants were used to gather the data. The purpose of the questionnaire was to collect data from both patients and controls, such as their name, sex, age, height, weight, and kind of iron chelator, etc.

Sample collection

About 5 ml of venous blood was collected from the controls and patients, placing 2 ml in an EDTA tube and 3 ml into a gel tube and allowing it to clot. The serum after centrifugation is transferred into Eppendrof tubes and stored until the time of analysis. Also, about a 10 ml urine sample was collected and, after centrifugation, examined under the microscope for hematuria.

Measured parameters

Estimation of pretransfusion hemoglobin (Hb) level by Automated Hematological Analyser (Erma Inc.). measurement of serum Cystatin C by ELISA technique (BT LAB kit 202411019 Lot), blood urea (302003 Lot), serum creatinine (307708 Lot) by Dry Chemistry Analyzer Fujifilm. measuring the creatinine by spectrophotometer (Biolabo kit 012301B2 Lot) and albumin by Cobas c 311 (ALB2 kit 81773401 Lot) in urine for calculating the urinary ACR.

Data analysis

The programming language R v4.4.2 was used for all studies. To check for a normal distribution, the Q-Q plots and Shapiro-Wilk tests were employed. Due to the lack of a normal distribution, continuous variables were displayed as medians with interquartile ranges (IQR), while categorical

variables were displayed as numbers. Pearson's chi-squared test was used for categorical variables and Mann-Whitney U tests (Wilcoxon rank sum test) for continuous variables to look for differences between unpaired groups (patients and controls). In order to determine the cut-off for biomarkers with the highest sensitivity and specificity, Receiver-operator characteristic (ROC) curves were computed using Medcalc software. Youden's J statistic was utilized to determine the ideal threshold.

RESULTS

The study includes 60 BTM patients and 30 controls. The study shows the median (Q1, Q3) among participants. The Wilcoxon rank sum test revealed a statistically significant association in body mass index, iron chelator therapy DFX (47%), DFO (20%), and hematuria between patients and control groups (p value < 0.05). The Wilcoxon rank sum test revealed no significant association in age and gender between two groups (p value > 0.05). The normal range considered for blood urea (12.8 - 42.8 mg/dL), serum creatinine (male = 0.9 - 1.3 mg/dl, female = 0.6 - 1.1 mg/dL), serum Cystatin C (1 - 1.3 mg/L), and UACR < 30 mg/g. The parameters of a research case (60) that was divided into two treatment groups, DFX (n = 42) and DFO (n = 18), and also (30) controls are displayed in Table 1.

Table 1: The effect of DFX and DFO on renal glomerular function parameters

Parameter	Group 1 DFO N[1]=18	Group 2 DFX N=42	Control n=30	P-value[2]		
				1 versus C	2 versus C	1 versus 2
Blood urea (mg/dl)	21.40 (19.50, 26.10)	25.05 (20.00, 33.30)	24.15 (19.80, 27.10)	0.443	0.239	0.165
Serum creatinine (mg/dl)	0.42 (0.38, 0.53)	0.49 (0.39, 0.57)	0.75 (0.65, 0.85)	<0.001	<0.001	0.248
UACR mg/g	66.53 (26.31, 150.69)	111.34 (51.44, 147.90)	4.67 (2.96, 6.27)	<0.001	<0.001	0.279
Serum Cystatin C (mg/l)	3.19 (1.90, 3.87)	2.78 (1.61, 3.69)	0.75 (0.31, 0.85)	<0.001	<0.001	0.493

[1]Median (Q1, Q3)
[2]Wilcoxon rank sum test

The study displays the interquartile ranges (Q1, Q3) as well as the median values of a number of laboratory parameters. There is no statistically significant difference (p value > 0.05) between patients and controls according to the Wilcoxon rank sum test for blood urea as shown in Fig. 1.

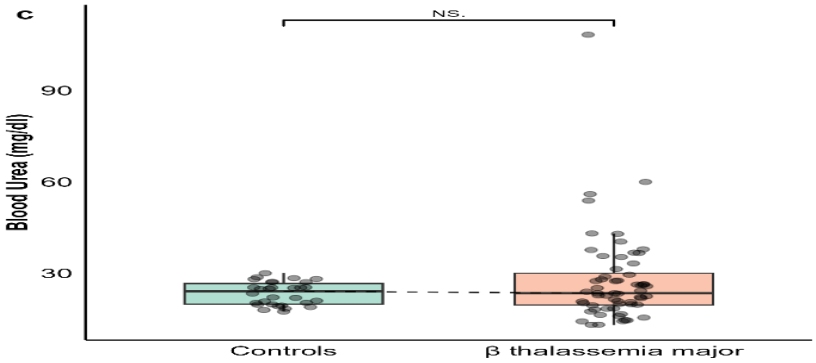

Fig. 1. Box plot comparing the blood urea among the study group

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12s,2025
https://theaspd.com/index.php

Between patients and controls, there is a statistically significant difference in serum creatinine levels as shown in Fig. 2. The median blood creatinine levels in the DFO group are a little lower than DFX (0.42 mg/dl vs. 0.49 mg/dl), and the difference is statistically not significant.

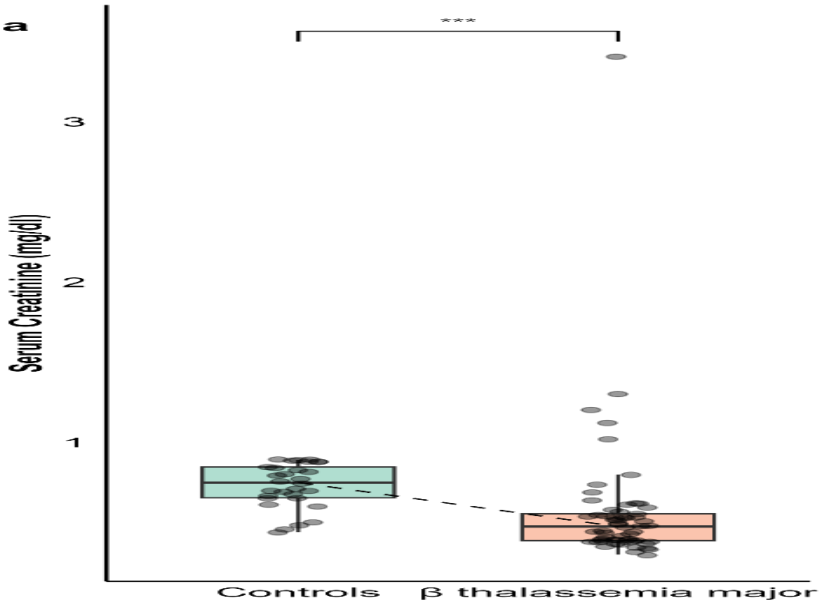

Fig. 2. Box plot comparing serum creatinine among the study group

Although there is no statistically significant difference between DFX and DFO, serum Cystatin C shows a significant difference between patients and controls (higher in patients) as present in Fig. 3. and is slightly higher in the Deferoxamine group than the Deferasirox group.

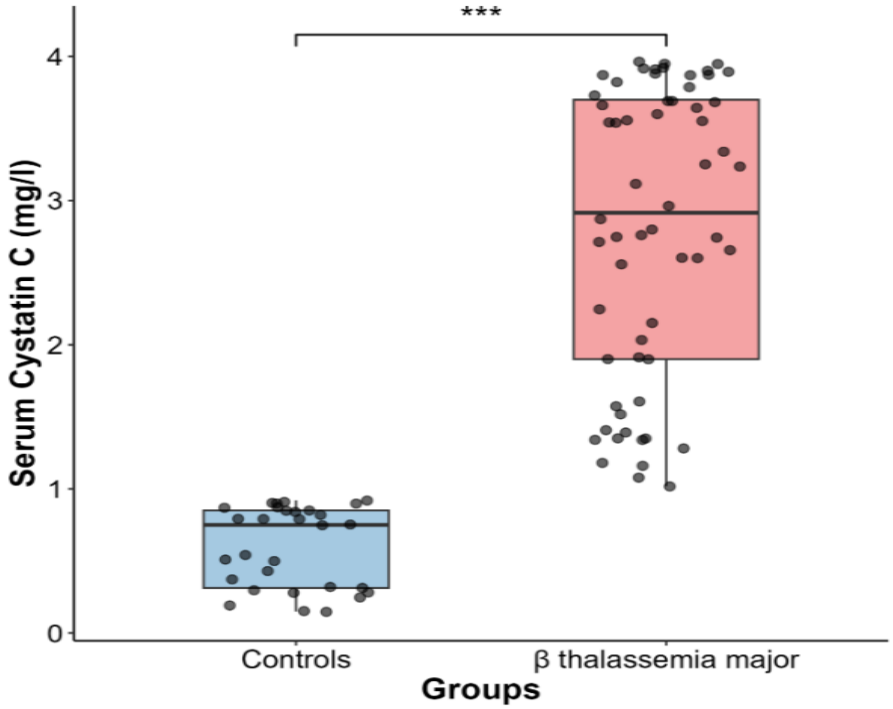

Fig. 3. Box plot comparing serum Cystatin among the study group

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12s,2025
https://theaspd.com/index.php

Patients in the study had higher Albumin-Creatinine Ratio (ACR) values than controls, which is statistically significant as present in Fig. 4. In our investigation, there were (6) patients with macroalbuminuria and (43) with microalbuminuria. The DFO group has a lower ACR than the DFX group; however, this difference is not statistically significant.

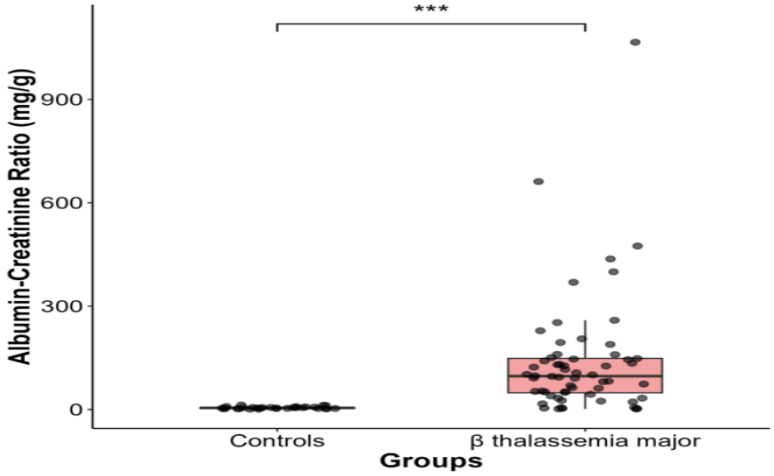

Fig. 4. Box plot comparing the urinary ACR among the study group

The Hb level between patients and controls is a statistically significant difference, which is very low in patients. A Receiver Operating Characteristic (ROC) curve for serum Cystatin C is displayed in Fig. 5. to assess its diagnostic ability to distinguish between sick and non-diseased states. At the optimal threshold (>1.39), the sensitivity of 93.9%, specificity of 63.6%, area under the curve (AUC) of 0.758, positive predictive values (PPV) of 92, and negative predictive values (NPV) of 70 are all displayed by the Cystatin C ROC.

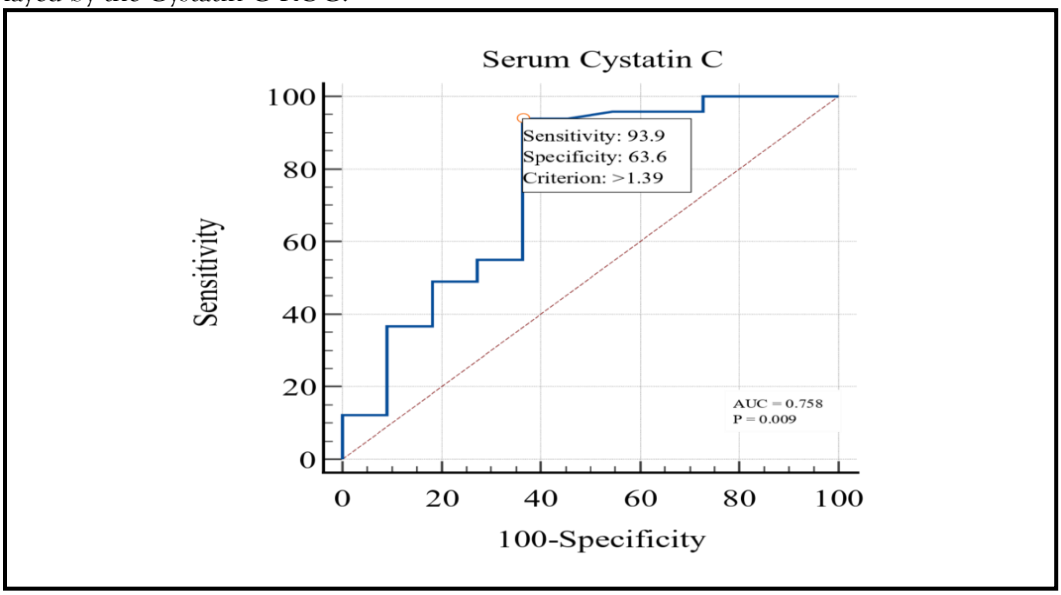

Fig. 5. ROC curve of Serum Cystatin C in patients with BTM.

DISCUSSION

Serum cystatin-C and UACR, the two early indicators of glomerular function, were not evaluated in the majority of reports. The use of particular iron chelators, which may result in nephrotoxicity and acute kidney damage (AKI), is one of the main causes of the renal abnormalities seen in BTM. In the current study, talk about the glomerular dysfunction, starting with blood urea, which indicates that

there is no statistically significant correlation between the patients and controls. The fact that blood urea is not a reliable predictor of renal function because it is greatly influenced by the body's nitrogen balance and level of hydration may help to explain these findings. Our research differs from that of Mohammad G. Shaalan et al., who found that patients receiving deferoxamine had significantly higher blood urea levels [4]. When compared to the control group, Zeina A. Althanoon et al. found no significant difference in the blood urea levels of the DFO group. In contrast, the DFX group's blood urea significantly increases when compared to the control group. Furthermore, a comparison of the DFX and DFO groups reveals a notable rise in DFX's blood urea levels, both of which are within the normal range [9].Serum creatinine shows a statistically significant association between patients and controls, which is lower in patients due to low muscle mass, and the patients may be in the early stage of the disease. According to Mohammad G. Shaalan et al., there was no discernible difference in serum creatinine levels between the DFO-treated subjects and the control group [4]. Additionally, Osama Tanous et al. discovered no discernible difference in blood creatinine levels between the DFX and DFO groups, nor between the control and cases (both within normal limits) [18]. The serum creatinine is affected by a number of factors, such as muscle mass, and its level does not increase until the kidney loses 50% of its function, so that is not a good parameter for early detection of glomerular impairment.Patients' serum levels of Cystatin C differ significantly from those of the control group (higher in patients). Our results are consistent with those of Pradana Zaky R. et al., who found a substantial difference in S. Cystatin C between the control group and the chelation therapy-treated subjects [19]. Additionally, Hamidreza Badeli et al. discovered a significant difference in S. Cystatin C between the control group and the chelation therapy-treated cases (greater in DFO than DFX) [20]. Halima Sadia et al. show that the chelation therapy-treated cases (higher in DFX than DFO) and the control group differ significantly in S. Cystatin C [21]. This finding implies that if administered in an inappropriate dosage, both DFO and DFX may result in glomerular damage and early detection by serum Cystatin C. Patients in the study had higher Albumin-Creatinine Ratio (ACR) values than controls, which is statistically significant. In our investigation, there are (6) patients with macroalbuminuria and (43) with microalbuminuria. Ahmed M. Mahmoud and Basma A. Ali found a significant difference in ACR between the control group and the cases treated by DFO [22]. When Hamidreza Badeli et al. compared the subjects treated with iron chelators with the control group, they found no discernible difference in ACR. However, DFO has a higher ACR than DFX [20]. Maha Y. Kamal et al. discovered a noteworthy difference in ACR between the control group and the subjects treated with iron chelators. Nevertheless, DFX has a higher ACR than DFO [23]. The results indicate both DFX and DFO can produce effects on glomerular function in comparable ways as present in Table 1. At the optimal threshold (>1.39), Cystatin C's ROC indicates a sensitivity of 93.9%, a specificity of 63.6%, and an AUC of 0.758. It is judged that the diagnostic performance is fair to good. At a threshold of 0.74 mg/L, Ola Galal Behairy et al. discovered that the ROC curve analysis for cystatin-C had a 91.4% sensitivity, 90% specificity, 97% PPV, 75% NPV, and an area under the curve of 0.989 [24]. The ROC curve analysis for cystatin-C at cutoff 1.03 mg/L was published by Basma A. Ali and Ahmed M. Mahmoud. The area under the curve was 0.84, the sensitivity was 65%, and the specificity was 91% [25]. All these findings indicate that serum Cystatin C is considered a novel biomarker for early detection of glomerular impairment in BTM.

CONCLUSIONS

The renal glomerular impairment is common even in the early age of patients with BTM. Compared to the traditional renal function tests (blood urea and serum creatinine), the use of early indicators for glomerular function is far more beneficial and appropriate for screening and monitoring of potential glomerular dysfunction. It is appropriate and most likely economical to use urine ACR for microalbuminuria as a predictor of glomerular function. A new novel biomarker for the early

detection of glomerular dysfunction is serum Cystatin C. Both deferasirox and deferoxamine produce changes in the renal glomerular function.

Acknowledgment

We would like to thank the Ministry of Health, the Ministry of Higher Education and Scientific Research and Tikrit University/College of Medicine for giving us this chance to complete this research. We would like to extend our deepest thanks and appreciation to the staff of Al Hadbaa specialist Hospital for their assistance they provided to us during the collection of samples. We extend our sincere thanks to all those who donated their samples used in this research, both patients and healthy individuals. We wish them good health and wellness.

REFERENCES

[1] Jawad NN, Al-Terehi MN, 2024. Variation of DNA repair genes APE1 and RAD18 in β-thalassemia patients. *Med J Babylon*, 21, pp.844-887.

[2] Abd IK, Zainal IG,2020. Assessment of biochemical parameters and study its correlation in ß-thalassemia major patients and healthy controls in Kirkuk City, Iraq. *Med J Babylon*,17, pp.172-176.

[3] Shaalan MG, Hassan MK, Al-Shanoof HJ, Al Naama LM, 2022. Renal Dysfunction in Pediatric Patients in Iraq With β-Thalassemia Major and Intermedia. *Cureus*. September.

[4] Arman Bilir Ö, Kirkiz S, Fettah A, Ok Bozkaya İ, Kara A, Çakar N, Yaralı N, 2020. Renal function and the oxidative status among children with thalassemia major and healthy controls: A cross-sectional study *Transfus Apher Sci*,59(4)

[5] Hasan BK, Saud WH, 2023. Zinc and copper in transfusion-dependent thalassemia patients on different iron chelators in Basrah: A case-control study. *Med J Babylon*, 20, pp.124-129.

[6] Pinto VM, Forni GL, 2020. Management of iron overload in beta-thalassemia patients: Clinical practice update based on case series. *Int J Mol Sci*, 21(22), pp.1–20.

[7] Bashi AYD, Younus ZM, Al-Hamdani MSY, 2024. Compliance with deferoxamine therapy among transfusion-dependent β-thalassemia patients in Nineveh Province, Northern Iraq. *Med J Babylon*.,21, pp.315-317.

[8] Sarbay H, Akbalık Kara M, 2023. The Effect of Deferasirox Dose and Treatment Duration on Frequency of Proteinuria and Renal Functions in Patients with Thalassemia Major. *Cyprus J Med Sci*,7(6), pp.801–805.

[9] Althanoon ZA, Alkazzaz NA, 2020. Comparison of the effects of deferasirox and deferoxamine on uric acid and renal function in patients with beta thalassemia. *Syst Rev Pharm*, 11(11), pp. 214–222.

[10] Hashemieh M, 2020. Early Detection of renal dysfunction in β thalassemia with focus on novel biomarkers. *Iranian Journal of Pediatric Hematology and Oncology*,10(1), pp.57–68.[11] Benoit SW, Ciccia EA, Devarajan P, 2020. Cystatin C as a biomarker ofchronic kidney disease: latest developments. *Expert Rev Mol Diagn.*,20(10), pp.19–26.

[12] Carrero JJ, Fu EL, Sang Y, Ballew S, Evans M, Elinder CG, et al, 2023. Discordances Between Creatinine- and Cystatin C–Based Estimated GFR and Adverse Clinical Outcomes in Routine Clinical Practice. *Am J Kidney Dis*,82(5), pp.34–42. [13] Benzing T, Salant D, 2021. Insights into Glomerular Filtration and Albuminuria. *N Engl J Med*,384(15), pp.1437–1446.

[14] Liu D, Lv LL, 2019. New Understanding on the Role of Proteinuria in Progression of Chronic Kidney Disease. *Adv Exp Med Biol*,1165, pp.487-500.

[15] Haider MZ, Aslam A. Proteinuria. [Updated 2023 Sep 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK564390/

[16] Bökenkamp A, 2020. Proteinuria-take a closer look. *Pediatr Nephrol*.,35(4), pp.533-541.

[17] Kamińska J, Dymicka V, Tomaszewska J, Matowicka J, Koper OM, 2020. Diagnostic utility of protein to creatinine ratio (P/C ratio) in spot urine sample within routine clinical practice. *Crit Rev Clin Lab Sci*,14;57(5), pp.345–364.

[18] Tanous O, Azulay Y, Halevy R, et al, 2021. Renal function in β thalassemia major patients treated with two different iron- chelation regimes. *BMC Nephrol*, pp.1–11

[19] Romadhon PZ, Ashariati A, Bintoro SUY, Thaha M, Suryantoro SD, Windradi C, et al, 2022. Markers of Renal Complications in Beta Thalassemia Patients with Iron Overload Receiving Chelation AgentTherapy: A Systematic Review. *J Blood Med*,13, pp.725–738.

[20] Badeli H, Baghersalimi A, Eslami S, et al, 2019. Early Kidney Damage Markers after Deferasirox Treatment in Patients with Thalassemi Major : A Case-Control Study. *Oxidative Medicine and Cellular Longevity*, 2019:8

[21] Halima S, Furqan S, Rehan R, et al, 2022. Effect of Iron Chelator on Renal Function in Thalassemia Patients who Undergo Regular Transfusion Iron Chelator Detail. *PJMHS*,16(01), pp.1403–1405.

[22] Abd M, Mostafa E, Hesham MA, Khalifa NA, Beshir MR, 2024. Evaluation of Novel Biomarkers for Early Detection of Acute Kidney Injury in Children with B-Thalassemia Major.*zagazig university mediacal Journal*, 30(4), pp.2282–2288

[23] Zeid MY, Hassab HM, Younan DN, et al, 2020. Study of the effect of different iron-chelating agents on early renal glomerular and tubular function markers in children with beta thalassemia. *Alexandria Journal of Pediatrics*, pp.116–123

[24] Ola GB, Eman RA, Neveen TA, et al, 2017. Disease R. Role of serum cystatin-C and beta-2 microglobulin as early markers of renal dysfunction in children with beta thalassemia major. *International Journal of Nephrology and Renovascular Disease*, pp.261–268.

[25] Basma A. Ali and Ahmed M, 2014. Mahmoud. Frequency of Glomerular Dysfunction in Children with Beta Thalassaemia Major. *Sultan Qaboos University Med J,*14(1),pp.88-94.