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Assessment of renal tubular impairment in beta-thalassemia major patients by using a novel biomarker

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Abstract

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Thalassemia is a group of hereditary blood disorders caused by gene mutations. Beta-thalassemia major is a more severe type because the individuals require blood transfusions for survival. The renal tubular disorder is caused by anemia, iron overload and receiving specific iron chelators, which all of them can lead to renal tubular impairment. The aim of case control study was to assess the role of serum NGAL as an early marker for the diagnosis of tubular dysfunction in BTM patients. Also to investigate the effect of iron chelator on tubular function. The study includes 60 beta-TM patients. The control group includes 30 healthy individuals. Patients were divided into 2 groups: group A treated with Deferoxamine (DFO) and group B treated with Deferasirox (DFX). Blood iron, Serum NGAL and albumin/creatinine ratio (ACR) in urine were measured in patients and controls. Patients in the study had higher Albumin-Creatinine Ratio (ACR) values than controls, which is statistically significant. S.NGAL shows a statistically significant difference between the patients and control group.Urinary ACR and serum NGAL are considered an early marker of renal tubular dysfunction in BTM. Both Deferasirox and Deferoxamine produce changes in the tubular function.

1. Introduction

Thalassemia is a class of inherited blood disorder caused by gene mutations leading to low or absent α and β globin proteins, which result ineffective erythropoiesis and chronic anemia [1]. Beta-thalassemia major is more severe because the patients require blood transfusion for survival [2]. B-thalassemia patients now have a higher survival rate because of blood transfusions and iron chelation therapy. But many patients continue to suffer from a number of complications including renal dysfunction [3]. Numerous factors such as anemia which contribute to the renal tubular dysfunction by increasing the volume in the peri-tubular regions and causing the proximal tubules to be destroyed [4]. Persistent hypoxia promotes tubular renal cells to differentiate into myofibroblasts, which in turn activates fibroblasts and causes peritubular capillaries to be obliterated [5, 6]. Functional problem in tubular cells are associated with

oxidative stress and lipid peroxidation [7, 8]. Both proximal and distal tubules showed hemosiderin deposits which can cause interstitial fibrosis, tubular necrosis and cortical atrophy. Tubulo-interstitial fibrosis is caused by growth factors and cytokines released by injured tubular cells [7, 9]. Acute kidney injury, proteinuria, and renal tubular abnormalities can also result after iron chelation therapy, such as Deferoxamine and Deferasirox [10, 11]. Numerous biomarkers can be used to identify renal tubular dysfunction; however serum Neutrophil Gelatinase Associated Lipocaline (NGAL) is the most significant. The proximal and distal tubules, the loop of Henle and the intercalated cells of the collecting duct all express this 25-kDa lipocalin iron-carrying protein [7]. The primary function of NGAL's bacteriostatic activity is to stop bacteria from absorbing iron [7, 12]. NGAL is filtered by the glomerulus and subsequently reabsorbed by the proximal tubule. NGAL increases noticeably as early as (3) hours after the injury and peaks (6-12) hours later. After the initial trauma, the elevation may persist for up to 5 days if the injury is severe [12]. The NGAL gene is extremely sensitive and reacts rapidly to damage and stress in tubular cells [13]. Proteinuria (albuminuria) is another crucial indicator for tubular dysfunction and can be brought on by tubulointerstitial illness [14, 15]. The tubules over secrete certain proteins which results in secretory proteinuria. Assessing the urine albumin creatinine ratio is the most effective way to identify albuminuria early. [16]. The aim of the current study was to assess the role of serum NGAL in early detection of tubular impairment in BTM patients and correlate it with iron overload. Also to evaluate the effect of iron chelators on renal tubular function.

2. Materials and Methods

2.1 Setting and time of study

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

2.2 Subjects

The Case control study includes 60 BTM patients they need regular blood transfusions and treatment with iron chelators and for both genders. Their ages ranged were between (7-55) years and the median (Q1, Q3) is 17.50 (14.50, 21.00). The patients were divided into two classes according to the type of chelator: class A received DFO and class B received DFX. The cases were handled in the Nineveh Governorate's Al Hadbaa Specialist Hospital in Iraq. The control group consists of 30 people who appear to be in good health. They were matched to the patient groups based on age and sex. The controls ranged in age from 7-54 years with a median age of 19.50 (15.00-33.00). Direct interviews with patients and control participants were used to gather the data. The aim 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, among other details.

2.3 Sample collection

About 5 cc of venous blood were drawn from the patients and controls and 2 cc were placed in an EDTA tube and 3 cc were placed in a gel tube and left to clot. After centrifugation, the serum is transferred into Eppendrof tubes and kept in a deep freezer at -20 degrees until the time of analysis. Collect about 10 ml of urine sample and centrifuge to examine under the microscope for hematuria.

2.4 Measured parameters

Measuring pretransfusion Hb, serum iron (Biolabo 062335A1 Lot) by spectrophotometers and serum NGAL by ELISA (BT LAB 202411019 Lot). Measuring the creatinine by spectrophotometer (Biolabo kit 012301B2 Lot) and albumin by Cobas c 311 (ALB2 kit 81773401 Lot) in urine sample for calculation of the urinary ACR.

2.5 Statistical analysis

All investigations were conducted using R v4.4.2, a computer language. Shapiro-Wilk tests and Q-Q plots were used to determine whether the data was distributed normally. Since there was no normal distribution, categorical data were shown as numbers (%) and continuous variables as medians with interquartile ranges (IQR). To look for differences between the unpaired groups (Controls and Patients), the Mann-Whitney U tests (Wilcoxon rank sum test) were employed for continuous variables and Pearson's Chi-squared test for categorical variables. Using Medcalc software, Receiver-operator characteristic (ROC) curves were calculated to find the cut-off for biomarkers with the highest sensitivity and specificity. The optimal threshold was found using Youden's J statistic.

3. Result

The current study shows the median (Q1, Q3) among the group study. Which demonstrates a statistically significant association in iron chelator therapy and hematuria between patients and control groups (p value < 0.05). No significant association in age, gender between two groups (p value > 0.05) as shown in Table 1.

Table 1. General characteristics of the study groups

Characteristic	**Controls N = 30**	**β thalassemia major N = 60**	**p-value**
Age (years)	19.50 (15.00,33.00)	17.50 (14.50,21.00)	0.3
Gender			0.4
Male	14 (47%)	34 (57%)	
Female	16 (53%)	26 (43%)	
Name chelator			<0.001
Deferasirox	0 (0%)	42 (70%)	
Deferoxamine	0 (0%)	18 (30%)	
Not applicable	30 (100%)	0 (0%)	
Hematuria			<0.001
No	30 (100%)	10 (17%)	
Yes	0 (0%)	50 (83%)	

Our study reported a statistically significant association (p value < 0.05) for the Blood iron (ug/dl), Urinary Albumin-Creatinine Ratio (ACR) (mg/g) and serum NGAL (ng/dl) as present in the Table 2.

Table 2. Laboratory parameters among the study groups

Biomarkers	**Controls N = 30**	**β thalassemia major N = 60**	**p-value**
Hb level	13.00 (12.00,14.00)	8.00 (7.00, 8.00)	<0.001
Blood Iron	102.0 (95.0, 111.0)	307.0 (246.5, 391.0)	<0.001
Urinary (ACR)	4.67 (2.96, 6.27)	97.00 (47.04,149.3)	<0.001
Serum NGAL	26.50 (20.0, 34.0)	389.50 (211.0,511.5)	<0.001

Weakly positive no significant correlation between blood iron and serum NGAL. ACR and serum NGAL also have a moderately positive, statistically significant correlation (R = 0.35, p = 0.005). Serum NGAL was slightly greater in the Deferasirox group than in the Deferoxamine group and the difference between DFO and DFX in S.NGAL was not statistically significant (p-value = 0.9). The Deferoxamine group has a lower ACR than the Deferasirox group, but the difference is not statistically significant. Receiver Operating Characteristic (ROC) curve for S.NGAL as a diagnostic marker shows the Sensitivity = 69.4%, Specificity = 81.8% at the ideal cutoff threshold (>306), 94.4% PPV. 37.5% NPP. The Area under the Curve (AUC) of 0.757. The normal range considered for serum iron (children=50-120 ug/dl, Men=65-175, Women=50-170), serum NGAL 20-105 ng/ml and UACR < 30 mg/g.

4. Discussion

Serum NGAL and UACR, two early indicators for renal tubular functioning, were not evaluated in the majority of papers. Chronic anemia, iron overload, and the use of particular iron chelators, which can result in nephrotoxicity and acute kidney injury (AKI), are some of the main causes of renal tubular abnormalities in BTM. Discuss tubular dysfunction in the current investigation, beginning with the value of the urine Albumin-Creatinine Ratio (ACR). As seen in Fig. 1, our results demonstrate that the β-thalassemia major group's urinary ACR is much greater than that of the control group. The statistically significant difference (***) demonstrates that patients with β-thalassemia major have albuminuria. In our investigation, there were 6 patients with macroalbuminuria and 43 with microalbuminuria. Our findings differ from that of Şen V et al., who did not find that thalassemic individuals had an elevated urine protein/Cr ratio [17]. There was no discernible difference between beta TM and controls in ACR, according to a study done by Mahmoud Ahmed El-Hawy et al. [18]. Our findings are consistent with those of a study conducted in Iraq by Shaalan M. G. et al., which found that patients had a significantly higher ACR level [3]. In individuals with β-TM, Mahmoud A et al. found significantly higher urine ACR [8]. Mahdieh Arian et al. showed that β-TM patients had a considerably higher albumin/creatinine ratio (ACR) than healthy people [19]. A greater rate of microalbuminuria was found by Mohamed Abd Elrazek Mostafa et al. [20]. Additionally, Muhammad Tammadondar et al. was discovered microalbuminuria in beta TM patients [21]. Ashraf Sayed Kamel et al. observed the A/C ratio between beta-TM and controls was not differs significantly [22]. In beta TM patients, a greater ACR can be a sign of early tubular deterioration.

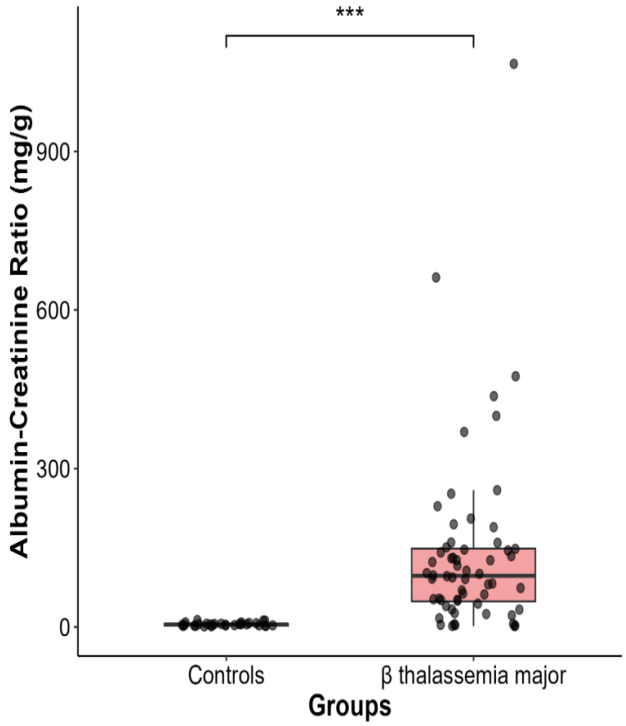

Fig. 1. Box plot comparing the urinary ACR between patients and controls

Our study demonstrates that the β-thalassemia major group's serum NGAL levels are much greater than those of the control group as seen in Fig. 2, indicating a statistically significant difference (***). The present study is comparable to research conducted by Mozhgan Hashemieh MD et al., which found that the S.NGAL was significantly greater in the β-thalassemia major group than in the control group [7]. Mohamed Abd Elrazek Mostafa et al. was also found a very statistically significant

rise in serum NGAL levels in patients relative to controls [20]. According to Fahima M. Hassan et al., patients' serum NGAL levels were significantly higher than those of the controls [23]. According to Marwah Mohammed et al., patients with beta TM showed a substantial rise in serum NGAL levels [24]. All of these findings suggest that renal tubular damage occurs in β-TM patients and is early detected by serum NGAL.

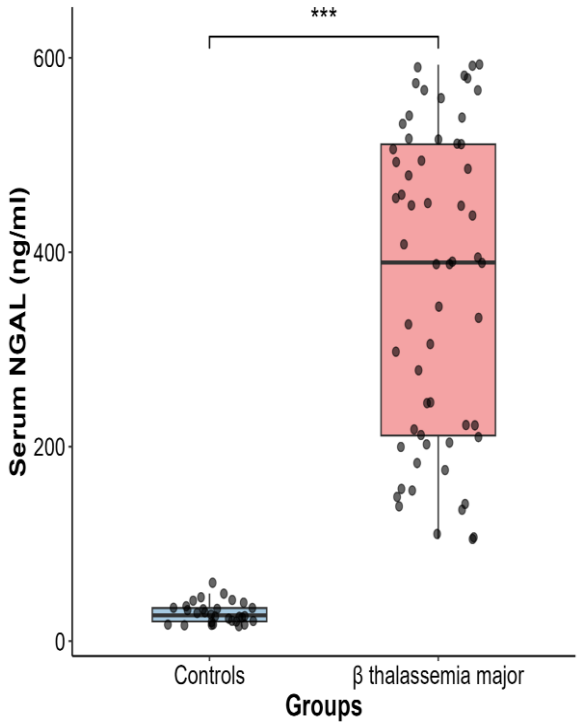

Fig. 2. Box plot comparing serum NGAL among the study group

Fig. 3 illustrates the correlation between serum NGAL and ACR for two groups. Patients with beta thalassemia major have a moderately positive correlation that is statistically significant (R = 0.35, p = 0.005). Our findings are consistent with those of Mohamed Abd Elrazek Mostafa et al., who similarly found a positive correlation between urine ACR and serum NGAL in the patient with BTM [20]. In patients with β-thalassemia major, higher serum NGAL is associated with elevated ACR, indicating a relationship between tubular dysfunction and NGAL levels.

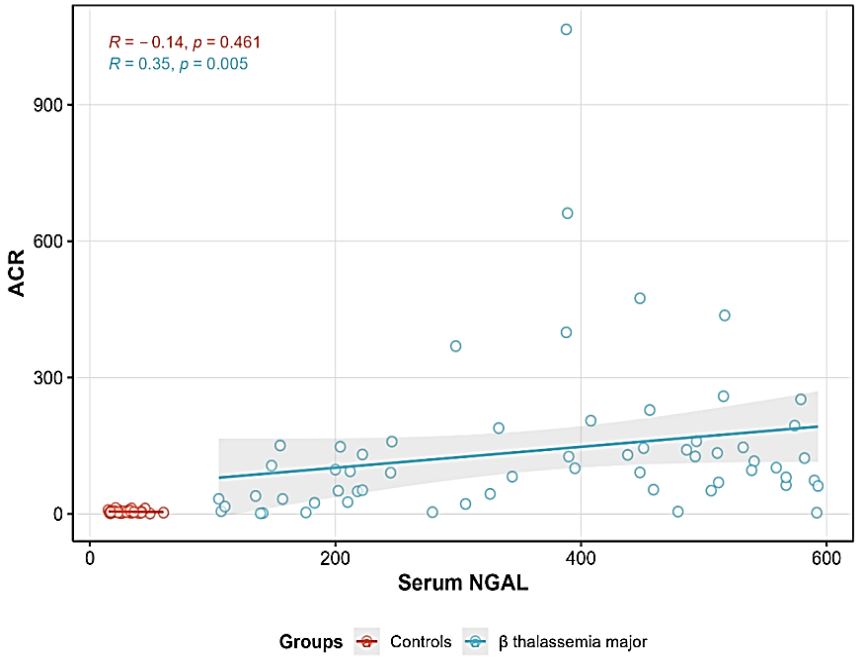

Fig. 3. Scatter plot of serum NGAL and ACR among the study groups

Our research showed a weak positive but non-significant relationship between blood iron and serum NGAL. This may indicate that increased iron deposits in the renal tubular tissue can cause tubular damage, which in turn raises blood levels of NGAL. Özlem Arman Bilir et al. found a strong association between ferritin and serum NGAL [6], which is different from our results. Mozhgan Hashemieh MD et al. found no correlation between ferritin and serum NGAL [7]. Table 3 illustrates how iron chelation therapy affects tubular function. According to the recent study, the S.NGAL was statistically not significantly different between DFO and DFX (p-value = 0.9) and somewhat higher in the Deferasirox group than in the Deferoxamine group (both significantly higher than the control group). Our research is comparable to that of Pradana Zaky R. et al., who found that patients on Deferasirox had significantly higher levels of uNGAL and sNGAL than those on Deferoxamine [25]. Additionally, u.NGAL is higher in DFX than DFO (both above normal level), according to Osama Tanous et al. [26]. These findings showed that if not administered in a suitable dose, both medications may result in renal tubular damage.The ACR is not statistically significant, but it is lower in the Deferoxamine group than in the Deferasirox group (both significantly higher than in the control group). When comparing the cases treated by DFO with the control group, Shaalan et al. identified a substantial difference in ACR, which is consistent with our results [3]. Additionally, Basma A. Ali and Ahmed M. Mahmoud showed a significant difference in ACR between the DFO-treated subjects and the control group [20]. 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 [27]. When Maha Y. Kamal et al. compared the subjects treated with iron chelators with the control group; they discovered a substantial difference in ACR. However, DFX has a higher ACR than DFO [28]. The findings might suggest that DFX and DFO have an impact on tubular which raise urine ACR.

Table 3. Effect of iron chelator on renal tubular markers

parameter	Group A DFO N=18	Group B DFX N=42	Control C N=30	P value		
				A versus C	B versus C	A versus B
UACR mg/g	66.53(26.31,150.69)	111.34(51.44,147.9)	4.67(2.96,6.27)	<0.001	<0.001	0.279
SNGAL ng/ml	347.00(202.30,517.00)	392.50(218.00,506.00)	26.50(20.00,34.00)	<0.001	<0.001	0.853

A Receiver Operating Characteristic (ROC) curve for serum NGAL as a diagnostic marker is displayed in Fig. 4. At the optimal cutoff threshold (>306), the sensitivity is 69.4%. 81.8% specificity, 94.4% PPV, and 37.5% NPP. The AUC of 0.757 indicates that the diagnostic capacity is good. According to Fahima M. Hassan et al., the ROC curve study for S.NGAL at cutoff 1370 ng/ml showed an area under the curve of 0.914, 86.6% sensitivity, 90% specificity, 89.6% PPV, and 87.1% NPV (23). NGAL seems to be a rather helpful biomarker for tubular damage, with a value of 0.757. The statistical significance (p) of 0.007 indicates that the results are statistically significant**.**

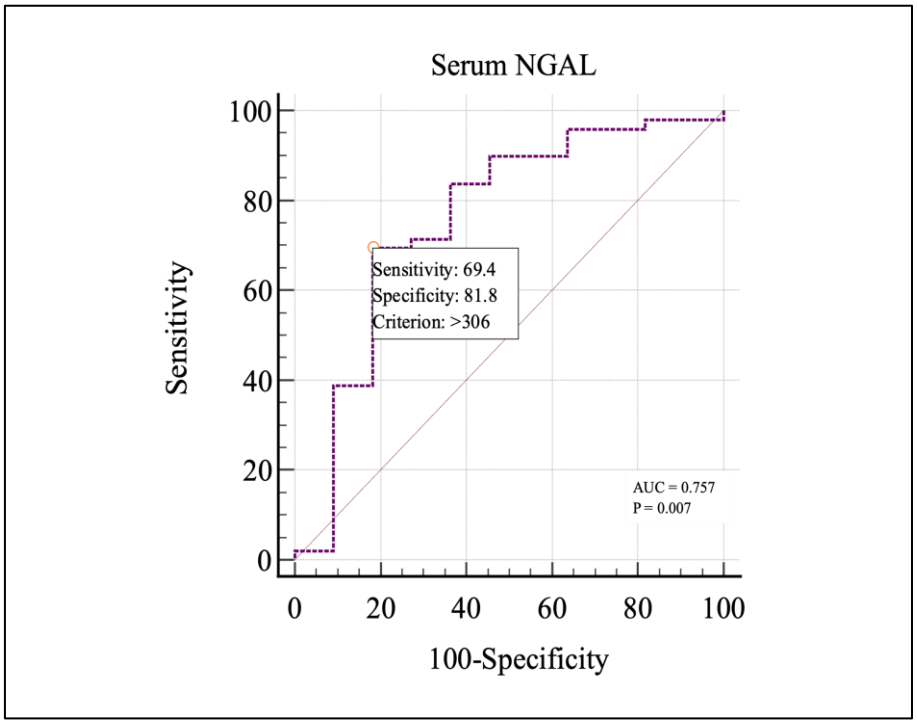

Fig. 4. Receiver operating characteristic (ROC) curve of serum NGAL in patients with β-thalassemia major

5. Conclusions

The renal tubular dysfunction is prevalent in BTM even in early age. The urinary ACR and serum NGAL are considered early markers of renal tubular dysfunction in BTM. Weak positive no significant correlation between sNGAL and blood iron which indicate the iron overload can cause tubular injury. Both Deferasirox and Deferoxamine produce changes in the tubular function especially when given in high dose.

6. Acknowledgments

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