Urinary neutrophil gelatinase-associated lipocalin as a marker of kidney injury in Egyptian patients with thalassemia

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Background
Renal disease is a long-term complication that should be recognized in thalassemia, especially with the rise in the average age of this population. Proper assessment of renal function abnormalities in thalassemia can be challenging because of the increased use of iron chelators, which themselves can affect renal function. There is a trend toward earlier detection of glomerular and tubular abnormalities, using early biomarkers of renal dysfunction. However, conflicting data make conclusive correlations difficult to achieve and strong diagnostic statistical parameters for these biomarkers are still lacking. Examples include albumin/creatinine ratio which can predict the initial signs of glomerular impairment, serum cystatin-C, and serum or urinary β-2 microglobulin have received attention as better tools to assess sensitive changes in glomerular filtration rate (GFR) and creatinine clearance in thalassemia as compared with serum creatinine and eGFR. Neutrophil gelatinase-associated lipocalin (NGAL) excretion in urine occurs when there is proximal tubular injury that disrupts NGAL reabsorption or increases NGAL synthesis. Significant correlations between the levels of urinary neutrophil gelatinase-associated lipocalin (uNGAL) and the degree of proteinuria in patients with chronic renal disease from diverse etiologies have been reported.

Aim
The study was to assess the markers of renal injury in β-thalassemia (β-TM) patients and to detect the significance of urinary albumin/creatinine ratio and uNGAL levels, as markers of kidney injury in Egyptian patients with thalassemia.

Materials and methods
This case–control study was conducted on 40 β-TM patients and 45 age-matched and gender-matched healthy controls, 27 women and 13 men, with a mean age of 29±9 years and for controls 28±5 years.
The majority of enrolled patients (22) were thalassemia intermediate. One-third of the patients were receiving iron chelation therapy, 78.6% on deferasirox, and 21.4% on deferiprone. All patients were subjected to through history taking, physical examination, and routine laboratory investigation. Tests were done for the diagnosis of thalassemia and to identify the type of thalassemia. Type and dose of Iron chelation therapy was recorded treatment: type and dose of chelation. Measurement of uNGAL and calculation of uNGAL/creatinine ratio were done. eGFR was calculated according to the original modification of diet in renal disease equation eGFR (ml/min/1.73 m^2). Chronic kidney disease was defined and graded according to KDIGO 2012.

Results
The results show that there is a statistically significant difference between cases and controls regarding uNGAL level as median was 498.0 ng/ml for cases compared with controls 423.3 ng/ml with a *P* value of 0.034. There is a statistically significant difference between cases and controls regarding uNGAL/creatinine ratio as the median was 481.75 ng/mg for cases compared with 333.79 with a *P* value of 0.003. There is no significant correlation between uNGAL (ng/ml) and dose of iron chelator, duration of iron chelator, eGFR, hemoglobin (Hg) level, serum ferritin, and albumin/creatinine ratio. There is no significant correlation between uNGAL/creatinine ratio (ng/mg) and eGFR, Hg level, serum ferritin, and albumin/creatinine ratio. eGFR was significantly higher (hyperfiltration) in cases compared with controls, with a median of 169.8 ml/min/1.73 m^3 compared with 119.6 ml/min/1.73 m^3, with a *P* value of less than 0.001. There was a statistically significant difference between cases and controls regarding albumin/creatinine ratio (μg/mg creatinine) as the median was 29.4 μg/mg creatinine for

cases compared with controls 10.0 μg/mg creatinine with a *P* value of less than 0.001.

Conclusion

This study showed that assessment of albumin/creatinine ratio, uNGAL, and uNGAL/creatinine ratio, can be regarded as potential early biomarkers of renal dysfunction in β-TM patients.

Keywords:

chelation therapy, neutrophil gelatinase-associated lipocalin thalassemia, renal disease

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Introduction

β-Thalassemia (β-TM) is regarded as a serious public health problem in the Mediterranean region, Southeast Asia, and the Middle East. In Egypt, homozygous thalassemia was first described by Diwani 1944, who suggested that thalassemia is the most common type of chronic hemolytic anemia in Egypt [1].

Thalassemias are defined as a heterogeneous group of genetic disorders of hemoglobin (Hg) synthesis, all of which result from a reduced rate of production of one or more of the globin chains of Hg. This basic defect results in imbalanced globin chain synthesis, which is the hallmark of all forms of thalassemia. β-TM is due to impaired production of β-globin chains, leading to a relative excess of α-globin chains [2].

The survival and quality of life of patients with β-TM in developed countries have improved markedly in recent decades. The availability of blood transfusion and iron chelation strategies for patients with severe forms of β-TM now allow long-term disease control and improved quality of life [3].

Historically, renal disease has not been a major issue in patients with thalassemia because survival was limited by severe cardiac iron loading from chronic transfusion therapy leading to premature early death. With the introduction of effective chelating agents that can reduce the iron burden and its consequences, and extending patients' survival, renal disease has become a more common occurrence [4].

However, the extent of intrinsic renal injury in patients with thalassemia is poorly defined. Damage to tubular cells may manifest in several ways from simple protein leak in the urine on dipstick to more severe damage leading to proximal tubular dysfunction and potentially acute tubular necrosis. Several studies report increased urinary excretion of markers of proximal renal damage in a considerable number of patients with β-TM major. Examples include albumin/creatinine ratio, serum cystatin-C serum or urinary β-2 microglobulin and neutrophil gelatinase-associated lipocalin (NGAL) [5].

One of the most promising biomarkers of early kidney injury is the NGAL. Upon nephrotoxic and/or ischemic injury, NGAL levels are highly increased in kidney cortical tubules, blood, and urine. Induction of NGAL after kidney injury precedes the elevation of classical markers for kidney damage, for example, serum creatinine, urinary *N*-acetyl glucosaminidase, and β2 microglobulin levels [6].

The aim of the current study was to assess the markers of renal injury in β-TM patients and to detect the significance of urinary albumin/creatinine ratio and urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels, as markers of kidney injury in Egyptian patients with thalassemia.

Materials and methods

The study was conducted on 40 adult Egyptian patients with thalassemia and 45 age-matched and gender-matched healthy controls. The study protocol was revised and approved by the Medical Research Committee in the Internal Medicine Department, Faculty of Medicine Cairo University.

Informed consent from each participant was obtained after proper orientation regarding the study objectives, data confidentiality, as well as the impact of the study. Only those who agreed were included and those who refused were excluded from the study. All procedures for data collection were treated with confidentiality according to the Helsinki Declaration of Biomedical Ethics [7].

Inclusion criteria included any patient with thalassemia of more than 18 years of age with normal kidney function tests (urea and creatinine). Exclusion criteria included patients with concomitant diseases that could affect kidney functions or potentially lead

to renal damage, such as diabetes, rheumatic heart disease, thyroid disease, hepatic diseases, sepsis, consumption of nephrotoxic drugs, recurrent urinary tract infections, family history of hereditary renal diseases, any active infection, renal stones, hydronephrosis, and urinary reflux.

All patients were subjected to through history and physical examination, diagnosis of thalassemia, type of thalassemia, the frequency of blood transfusion, whether the patient had splenectomy or not and comorbid conditions (e.g. cardiac, hepatitis). Iron chelation treatment: type and dose of chelation, compliance to chelation and duration.

Laboratory investigations included complete blood picture, total and direct bilirubin, serum urea, and creatinine level. Urine analysis, dipstick proteins in urine, albumin/creatinine ratio, serum sodium, potassium, uric acid, calcium and phosphorus level, and serum ferritin level.

Complete blood count was done using an automated cell counter (Cell Dyn-1700, Abbott Diagnostics, Abbott Park, IL, USA) and Leishman-stained peripheral blood films were examined. Serum creatinine, uric acid, urea nitrogen, calcium, and phosphorus were analyzed by a chemistry autoanalyzer. Serum sodium and potassium levels were determined by ion selective electrode on Dimension EXL (Siemens Healthcare Germany). Levels of urinary microalbumin were determined by immunoturbidimetry assay on Mindray BS-200 chemistry analyzer (Mindray, China). Serum ferritin was determined by electrochemiluminescence immunoassay on cobas e411 (Roche Diagnostics, North America).

Estimated glomerular filtration rate (eGFR) was calculated according to the original modification of diet in renal disease equation eGFR (ml/min/1.73 m^2). Chronic kidney disease was defined and graded done according to KDIGO 2012.

The urinary biomarker: the immunodiagnostic enzyme-linked immune sorbent assay (ELISA) kit was used for the quantitative determination of human NGAL. The ELISA kit was supplied by Shanghai Korain Biotech Co. Ltd (catalog no: E1719Hu; Shanghai, China). Biomarker levels were normalized by dividing by urine creatinine.

Fresh second morning urine samples collected into sterile cups were centrifuged into sterile tubes and then immediately divided into two different aliquots in Eppendorf tubes and stored at −20°C until analysis, one for creatinine and microalbumin, and another one for uNGAL which was centrifuged at 3000 rpm for ~20 min. The supernatants were carefully collected. Repeated freeze–thaw cycles were avoided and when sediments occurred during storage, centrifugation was performed again before work.

This kit uses ELISA based on the Biotin double-antibody sandwich technology to assay the human NGAL. Add NGAL to the wells, which are precoated with NGAL monoclonal antibody and then incubate. After that, add anti-NGAL antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing. Add substrates A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of the solution and the concentration of human NGAL are positively correlated. The assay range was 10 ng/ml→3000 ng/ml with a sensitivity of 5.01 ng/ml.

Statistics

Data management and analysis were performed using the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, Illinois, USA) version 25. Numerical data were summarized using means and SD or medians and/or ranges, as appropriate. Categorical data were summarized as numbers and percentages. Estimates of the frequency were done using the numbers and percentages. Numerical data were explored for normality using the Kolmogrov–Smirnov test and the Shapiro–Wilk test.

χ^2 or Fisher's tests were used to compare between the groups with respect to categorical data, as appropriate. Comparisons between two groups for normally distributed numerical variables were done using the Student's *t*-test while for non-normally distributed numeric variables, comparisons were done by Mann–Whitney test. Comparisons between more than two groups were performed by Kruskal–Wallis for non-normally distributed variables. To measure the strength of association between the normally distributed numerical measurements, Pearson's correlation coefficients were computed. Spearman's correlation coefficients were calculated for non-normally distributed variables. All tests were two-sided. *P* values less than 0.05 were considered significant.

Receiver operating characteristic curve (ROC) was done to determine the best cutoff point, sensitivity, specificity, and area under the curve.

Results

This case–control study was conducted from May to October 2018 including a total number of 40 thalassemia patients and 45 age-matched and gender-matched healthy controls, who were attending the hematology outpatient clinic at Internal Medicine Department of Cairo University during the study period (Table 1).

About two-third of the patients 27 (67.5%) were women. The mean±SD values of age for cases were 29±9 years and for controls 28±5 years, of weight (kg) for cases was 60±13 and for controls 78±10.

The majority of enrolled patients were thalassemia intermediate [8]. The mean±SD values of blood transfusions per year was 5.5±4.6. One-third of our patients were receiving iron chelation therapy. Of these, 78.6% were on deferasirox oral iron chelator and 21.4% were on deferiprone. About 17.5% of the patients were hepatitis C virus (HCV) positive. Less than half of the patients (45%) have a history of splenectomy.

Table 1 Characteristics of the total studied group with β-thalassemia at the time of study enrollment

Factors	*n*=40 [*n* (%)]
Thalassemia	
Thalassemia major	12 (30.0)
Thalassemia intermediate	21 (52.5)
Thalassemia trait	7 (17.5)
HTN	
Yes	2 (5.0)
No	38 (95.0)
Virology	
HCV (positive)	7 (17.5)
HCV (negative)	33 (82.5)
Splenectomy	
Yes	18 (45.0)
No	22 (55.0)
Iron chelators	
Yes	14 (35.0)
No	26 (65.0)
Drugs used for iron chelation	*n*=14
Deferasirox	11 (78.6)
Deferipone	3 (21.4)

HCV, hepatitis C virus; HTN, hypertension.

All controls were not hypertensive; HCV negative and dipstick proteins were negative in all controls.

In our study, the mean and SD of serum Hg was 8.4 ±1.3 in cases and 13.8±1.1 in controls with a statistically significant difference, *P* value less than 0.001. The median serum ferritin was 859 ng/ml in cases and 58.5 ng/ml in controls with a statistically significant difference, *P* value less than 0.001. The median albumin/creatinine ratio μg/mg creatinine was 29.4 with a range from 6.8 to 850 μg/mg creatinine. The mean±SD values of serum creatinine was 0.6±0.2 mg/dl compared with controls 0.8±0.2 mg/dl. The mean of serum calcium was 8.8±0.6 mg/dl compared with controls 9.1±0.4 mg/dl. Our patients had normal values of serum sodium, potassium, calcium, and phosphate and uric acid and no significant differences were found between patients and controls.

There is a statistically significant difference between cases and controls regarding uNGAL level as the median was 498.0 ng/ml for cases compared with controls, 423.3 ng/ml with a *P* value of 0.034 (Table 2 and Figure 1). There is a statistically significant difference between cases and controls regarding uNGAL/creatinine ratio as the median was 481.75 ng/mg for cases compared with 333.79 with a *P* value of 0.003. There is a statistically significant difference between cases and controls regarding albumin/creatinine ratio (μg/mg creatinine) as the median was 29.4 μg/mg creatinine for cases compared with controls, 10.0 μg/mg creatinine with a *P* value of less than 0.001 (Table 2). eGFR was higher (hyperfiltration) in cases with a median of 169.8 ml/min/1.73 m^3 compared with controls (119.6 ml/min/1.73 m^3), with a statistically significant *P* value of less than 0.001 (Table 2 and Figure 2).

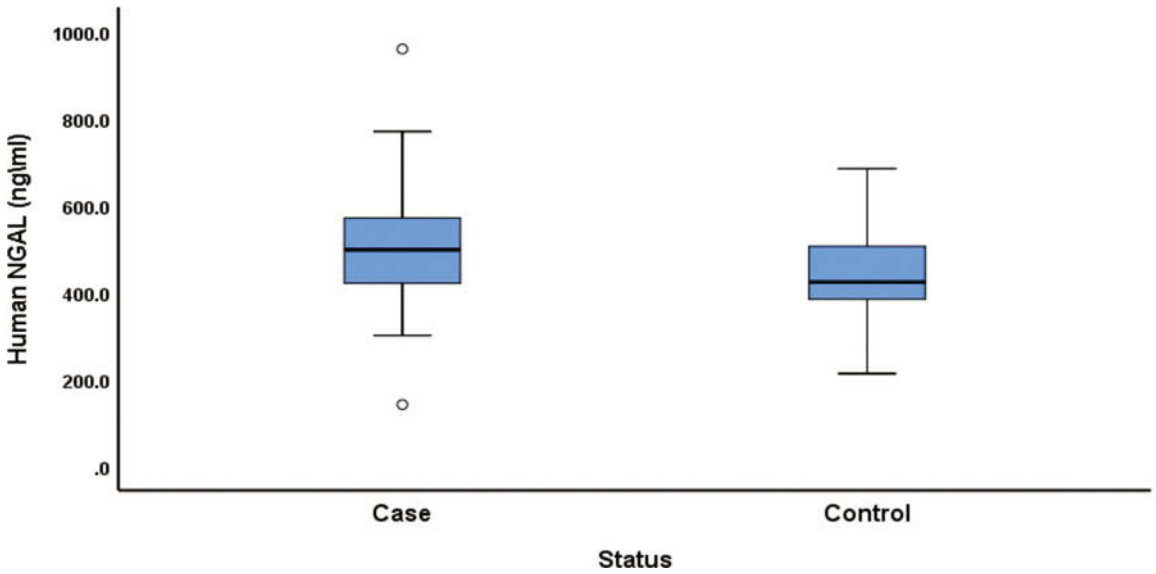
Table 3 shows albumin/creatinine ratio among different thalassemia cases according to median and range which revealed that thalassemia major patients tend to have the highest range (8.2–850 μg/mg creatinine) in contrast to thalassemia trait

Table 2 Significant laboratory investigations of β-thalassemia patients and controls

Factors	Case		Control		
	Median	Range	Median	Range	*P* value
Albumin/creatinine ratio (μg/mg creatinine)	29.4	6.8–850	10.0	6–27.9	<0.001
eGFR (ml/min/1.73 m^3)	169.8	84.5–727.9	119.6	84.8–203.4	<0.001
NGAL (ng/ml)	498.0	141.6–959.6	423.3	212.9–684.6	0.034
UNGAL/creatinine ratio (ng/mg)	481.75	80.3–1599.3	333.79	106.5–823.5	0.003

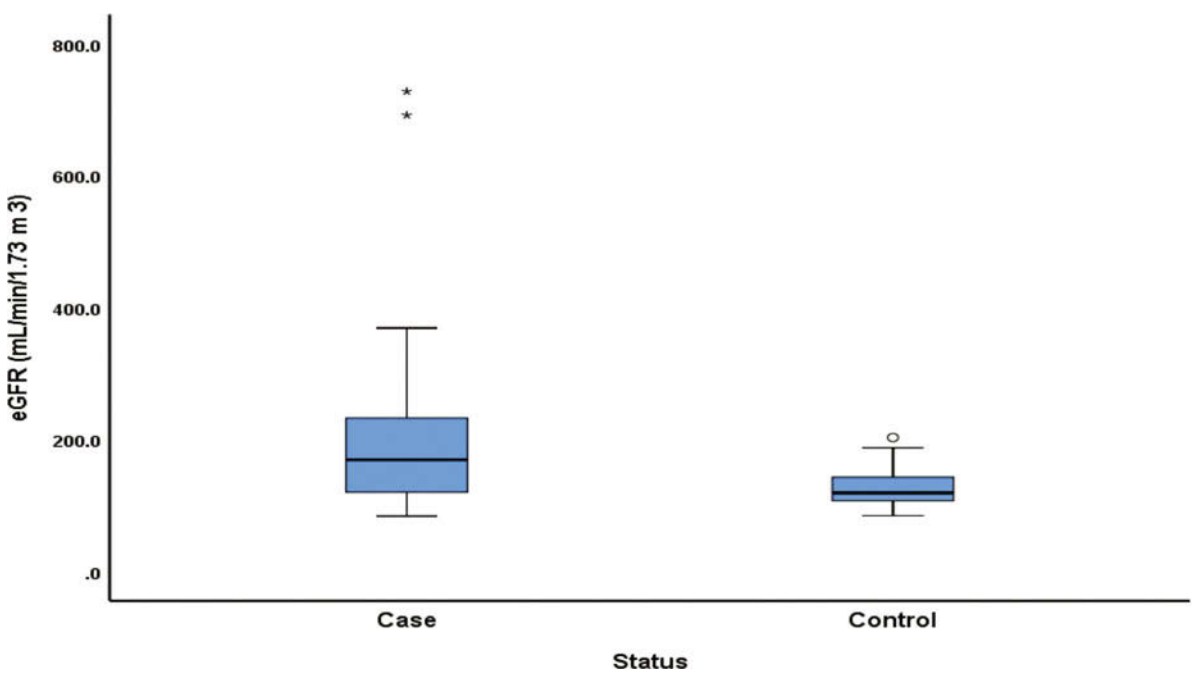
eGFR, estimated glomerular filtration rate; UNGAL, urinary neutrophil gelatinase-associated lipocalin.

Figure 1



Neutrophil gelatinase-associated lipocalin among cases and controls. There is a statistically significant difference between cases and controls regarding urinary neutrophil gelatinase-associated lipocalin level as the median was 498.0 ng/ml for cases compared with 423.3 with a *P* value of 0.034.

Figure 2

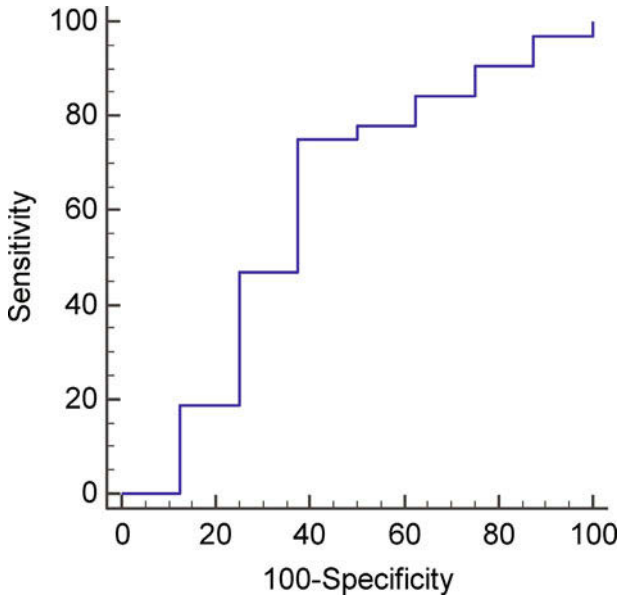


Estimated glomerular filtration rate among cases and controls. This figure shows that the estimated glomerular filtration rate tend to be higher (hyperfiltration) in cases with a median of 169.8 ml/min/1.73 m^3 compared with 119.6 ml/min/1.73 m^3, with a statistically significant *P* value of less than 0.001.

Table 3 Albumin/creatinine ratio among different thalassemia cases

	Thalassemia major		Thalassemia intermediate		Thalassemia trait		*P* value
	Median	Range	Median	Range	Median	Range	
Albumin/creatinine ratio (μg/mg creatinine)	34.7	8.2–850	35.0	6.8–633.3	12.5	11.9–43.1	0.051

Figure 3



Receiver operating characteristic curve for urinary neutrophil gelatinase-associated lipocalin. AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

(11.9–43.1 μg/mg creatinine) yet with no significant *P* value.

Although serum levels of urea and creatinine were within normal range in patients and controls, there was a statistically significant difference in eGFR and creatinine values, between patients and controls with a *P* value of less than 0.001.

Figure 3 shows that there is no significant correlation between uNGAL (cutoff point by ROC) and eGFR (classified by median), albumin/creatinine ratio (μg/mg creatinine), iron chelators, and serum ferritin.

There was no significant correlation between urinary human NGAL (ng/ml) and dose of iron chelator, duration of iron chelator, eGFR, Hg level, serum ferritin, and albumin/creatinine ratio. There was no significant correlation between urinary human NGAL/creatinine ratio (ng/mg) and eGFR, Hg level, serum ferritin, and albumin/creatinine ratio. There is no significant correlation between eGFR and weight, dose of iron chelator, duration of iron chelator, Hg level, and serum ferritin. There was no significant correlation between uNGAL (cutoff point by ROC) and eGFR (classified by median), albumin/creatinine ratio (μg/mg creatinine), iron chelators, and serum ferritin.

There was no significant correlation regarding NGAL among different thalassemia groups. There was no significant correlation between oral iron chelators (DFX and DFP) and eGFR, Hg level, albumin/creatinine ratio, and uNGAL.

There was no significant correlation between urinary albumin/creatinine ratio and weight, dose of iron chelator, duration of iron chelator, Hg level, and serum ferritin.

Discussion

β-TM causes defective Hg synthesis leading to ineffective erythropoiesis, chronic hemolytic anemia, and subsequent clinical complications. The availability of blood transfusion and iron chelation strategies for patients with severe forms of β-TM now allow long-term disease control. Despite important improvements in the management of β-TM, there are still many challenges to overcome before global disease control is achievable [2].

While functional abnormalities have been well studied in many systems of patients with β-TM, including cardiac, endocrine, pulmonary, and hepatic disorders, the renal effects and manifestations of the disease have been poorly investigated [9].

In thalassemia major, there are no longitudinal or prospective studies examining the renal disease. Historical data have shown that thalassemia has represented a small proportion of dialysis patients. Indeed, significant renal involvement is not a frequent complication in children and young adults suffering from thalassemia. Clearly, however, these estimates have changed with the aging thalassemic population and use of medications, which may potentially affect the kidneys [9].

The extent of intrinsic renal injury in patients with thalassemia remains poorly defined. The detection of renal damage has been made by traditional biomarkers such as serum creatinine and blood urea nitrogen, and this has remained unaltered for a few decades. However, these biomarkers have several shortcomings as early and sensitive diagnostic markers of kidney injury [10].

Some studies have investigated renal proximal tubular damage using biomarkers including b2 microglobulin, *N*-acetyl-d-glucosaminidase (NAG), proteinuria, and aminoaciduria in thalassemia patients. In this regard, biomarkers are needed to allow detection of early renal damage. The most promising biomarkers tested are urine NAG, NGAL, kidney injury molecule-1, and liver-type fatty acid binding protein [11].

NGAL is a 25-kDa lipocalin iron-carrying protein secreted by activated neutrophils and expressed in epithelial cells such as those in the proximal tubule, distal tubule, and loop of Henle segments. It is upregulated in renal tubular injury. NGAL excretion in urine occurs when there is proximal tubular injury that disrupts NGAL reabsorption or increases NGAL synthesis. NGAL rises dramatically with acute kidney injury (AKI). However, NGAL is also elevated in non-AKI patients such as lupus nephritis, immunoglobulin A nephropathy, and urinary tract infection. Significant correlations between the levels of uNGAL and the degree of proteinuria in patients with chronic renal disease from diverse etiologies were reported [12].

In the current study, the total number of eligible patients was 40 and there were 45 healthy controls. Twelve of them had thalassemia major (30%), 21 (52.5%) thalassemia intermediate, and seven (17.5%) thalassemia trait. The mean age and SD of the studied patients was 29±9 and that of controls was 28±5 years and 67.5% of the studied patients were women. The mean and SD of blood transfusion over the last year for the studied patients was 5.5±4.6; 17.5% of patients are HCV positive and 35% of cases were receiving iron chelation therapy (78.6% were on deferasirox and 21.4% were on deferiprone).

Results show that there is a statistically significant difference between cases and controls regarding the uNGAL level as the median was 498.0 ng/ml for cases compared with controls 423.3 ng/ml with a *P* value of 0.034. There is statistically significant difference between cases and controls regarding uNGAL/creatinine ratio as the median was 481.75 ng/mg for cases compared with 333.79 with a *P* value of 0.003. There is statistically significant difference between cases and controls regarding albumin/creatinine ratio (μg/mg creatinine) as the median was 29.4 μg/mg creatinine for cases compared with controls, 10.0 μg/mg creatinine with a *P* value of less than 0.001. eGFR was higher (hyperfiltration) in cases with a median of 169.8 ml/min/1.73 m^3 compared with controls 119.6 ml/min/1.73 m^3, with statistically significant difference, ($P<0.001$). Although serum levels of urea and creatinine were within normal range in patients and controls, there was a statistically significant difference in eGFR and creatinine values, between patients and controls with a *P* value of less than 0.001.

Studies of renal affection in various thalassemia populations have shown that 3.1% of patients progressed to dialysis therapy and 8% had a reduced creatinine clearance and 21% had an increased creatinine clearance [6].

A cross-sectional study in all thalassemic groups including thalassemia major has confirmed these findings with an increased creatinine clearance in 20.8%, impaired renal function in 7.8%, and albuminuria in up to 59% of cases [13].

Although in patients with thalassemia, hyperfiltration has been noted in several studies, some researchers demonstrated decreased eGFR levels in β-TM patients. They hypothesized that large variations in Hg levels were also associated with an increased risk of decline in eGFR. Other authors suggest that despite the existence of severe anemia combined with iron deposition in β-TM patients, renal injury did not reach clinically detectable abnormalities in serum urea and creatinine or eGFR in their patients [14].

Hyperfiltration is driven by several factors including: hemolysis which in turn affects nitric oxide-dependent vasodilatation. The effects of anemia are reduced peripheral and renal vascular resistance and hence increased renal plasma flow. Renal hyperfiltration, if untreated, leads to progressive tubulointerstitial injury and glomerulosclerosis from disruption of the renal architecture, through effects on mesangial cell function and increased proteinuria [15].

Glomerular disease in thalassemia is related either to GFR or albuminuria. Glomerular hyperfiltration might be an early hallmark of renal manifestations. A retrospective study of 50 β-TM patients revealed glomerular hyperfiltration to be present early in the course of the disease with nearly half of the patients exhibiting this finding. Importantly, within years, around 60% of these patients had abnormal urine protein to creatinine ratio of greater than or equal to 200 mg/g, and 14% developed proteinuria with urine protein to creatinine ratio of greater than 500 mg/g [16].

In a cohort of 127 β-TM patients, 4% developed end-stage renal disease that required regular hemodialysis. A review of their medical charts over a 10-year observation period revealed that elevations in serum creatinine and dipstick-positive albuminuria were the first manifestations of renal disease [10].

In our study, the mean and SD of serum Hg was 8.4 ±1.3 in cases and 13.8±1.1in controls with a statistically significant difference, *P* value less than 0.001. The median serum ferritin was 859 ng/ml in cases and

58.5 ng/ml in controls with a statistically significant difference. *P* value is less than 0.001.

In considering the potential mechanisms of renal injury, anemia and associated potential chronic hypoxia could be important. Anemia can lead to activation of the oxidative stress cascade once again with the end result of lipid peroxidation and cell damage and eventual functional change of the tubules [11].

Renal tubular cells can equally be exposed to oxidative stress and lipid peroxidation from states of chronic hypoxia and anemia, even without iron overload [17].

The severity of anemia correlated well with markers of tubular abnormalities; the latter were reduced in the thalassemia major group with hypertransfusion regimens. Anemia brings about a state of glomerular hypertension, where glomerular capillary walls stretch and endothelial/epithelial injury ensues. Macromolecular leaks occur to relieve the intraglomerular pressure, but end up causing glomerulotubular dysfunctions like increased albuminuria and may lead to end-stage renal disease [18].

The aftermath can vary between tubulointerstitial injury, glomerulosclerosis, and kidney fibrosis, especially in the setting of iron overload [10]. For example, increases in NAG and β2-M are higher in patients with high serum ferritin levels, and chelation therapy showed reversal of the findings [19].

In our study, there is a statistically significant difference between cases and controls regarding albumin/creatinine ratio (μg/mg creatinine) as the median was 29.4 μg/mg creatinine for cases compared with controls 10.0 μg/mg creatinine with a *P* value of less than 0.001 (Table 2). This proteinuria might be explained primarily by the impairment of proximal tubular reabsorption, which may be due to severe iron overload in the tissues where it also stimulates the production of reactive oxygen radicals and results in cellular injury [19].

In our study, although our patients had normal values of serum sodium, potassium, calcium, and phosphate and uric acid which can all be affected by renal dysfunction, no significant differences were found between patients and controls. Albumin/creatinine ratio ranges from 6.8 to 850 (μg/mg creatinine), and the significantly elevated uNGAL and uNGAL/creatinine ratio levels in patients may both be indicators of renal proximal tubular damage. Many previous studies also presented strong clinical evidence consistent with proximal tubular injury in β-TM [19].

Studies of renal affection in various thalassemia populations have shown that 0.5% of patients developed renal tubular dysfunction [6]. Clinical studies confirm that mild tubular dysfunction and abnormalities in GFR are common in patients with β-TM [20].

Damage to tubular cells may manifest in several ways from simple protein leak in the urine on dipstick to more severe damage leading to proximal tubular dysfunction and potentially acute tubular necrosis [21].

Evidence of proximal tubular damage is observed in patients with β-TM major. Low molecular weight proteinuria is found in almost all patients. Moreover, several studies report increased urinary excretion of several markers of proximal tubular damage in patients with β-TM major [5].

Other studies show that the secretion of such markers is significantly reduced in patients with β-TM major who have undergone curative hematopoietic stem-cell transplantation compared with age-matched patients who have not had transplantation [6].

A probable mechanism for this outcome may be that thalassemia itself leads to proximal tubular dysfunction either through chronic hypoxia from persistent anemia, through iron deposition or iron chelation. Both iron overload and chronic anemia could explain tubular dysfunction in patients with β-TM, although the two often coexist and the independent contribution of each risk factor has not been widely investigated. Rats subjected to chronic iron loading develop iron deposits that are clearly evident in glomeruli and in proximal but not distal tubules, as well as signs of significant glomerular sclerosis, tubular atrophy, and interstitial fibrosis.

Similarly, autopsy series of patients with β-TM major show hemosiderin deposits in both the terminal portion of proximal tubules and the distal tubules [8].

In the acidic proximal tubular fluid, iron dissociates from transferrin, resulting in the production of reactive oxygen species with subsequent damage to the brush border of the renal tubular membrane. If iron enters proximal tubular cells along with transferrin, it can be released from transferrin inside the lysosomes to enter

the cytoplasm as free reactive iron, where it also stimulates the production of reactive oxygen species and cellular injury. The mechanism of injury is mediated by mitochondrial stress in proximal tubular cells, as evidenced by increased efflux of cytochrome C, release of lactate dehydrogenase, and reduction in adenosine triphosphate [22].

Chronic anemia and hypoxia are also associated with oxidative stress, lipid peroxidation, and functional abnormalities in tubular cells [17].

Damage to the proximal tubule and increases of the peritubular space volume were also demonstrated in one study on anemic rats. A good correlation between the severity of anemia and markers of tubular abnormalities are reported in patients with β-TM major [21].

Moreover, chronic hypoxia of tubular cells with increased metabolic demand causes apoptosis or epithelial–mesenchymal transition, leading to the development of tubulointerstitial injury and consequent glomerulosclerosis and kidney fibrosis. In addition, tubular cell damage from heavy iron overload may allow the injured cells to migrate into the interstitium, releasing cytokines and growth factors that can cause tubulointerstitial scarring and glomerular sclerosis and leading to further decrease in GFR [11].

De-novo renal disease may also occur and exacerbate glomerular dysfunction. Hepatitis B and C, which are relatively common in older thalassemia patients, should be remembered as the potential causes of renal disease (including membranous and mesangiocapillary glomerulonephritis). Immunoglobulin A nephropathy has previously been described in a patient with thalassemia [23].

Renal manifestations attributed to iron chelation therapy have probably received the most attention from investigators studying thalassemia. Acute renal failure attributed to drug use is rare but has also been reported. In some studies, deferoxamine overdose secondary to administration-pump malfunction or inadequate dosage monitoring resulted in acute renal failure necessitating dialysis [5].

Oral deferasirox has become a routine therapy for the treatment of iron overload in patients with transfusion-dependent anemia. Several renal side effects have been reported with increasing frequency [24].

Cases of reversible mild or even life-threatening Fanconi syndrome, in both children and adult patients, have mainly been associated with patients receiving prolonged (>6 months) courses of deferasirox. Recovery is usual upon complete withdrawal of deferasirox but recurrence of Fanconi syndrome even after initiation of lower doses of deferasirox may occur [25].

The rapid improvement in biochemical parameters after stopping the drug and the lack of other potential etiologies strongly point to deferasirox as the cause of Fanconi syndrome. Perhaps, the two iron (III) complexes of deferasirox, which are noncharged, penetrate membranes less readily in comparison with other chelators [9].

The mechanism of renal injury associated with chelation is not exactly known. Several mechanisms were proposed for deferasirox-induced tubular dysfunction. The toxic effect of deferasirox on tubular epithelial cells was suggested as a cause of kidney damage in β-TM patients. A drug hypersensitivity reaction might lead to kidney damage in patients with β-TM. In contrast, safety data with Deferasirox in patients with β-TM have been reported for up to 5 years of treatment and confirm the absence of progressive increases in serum creatinine over longer-term treatment [11].

Several authors have shown that in myoglobinuric AKI, there is a marked increase in labile iron content. They also have demonstrated that iron chelation had a protective effect on renal function and was also associated with a marked reduction in histological evidence of renal damage [26].

Our results indicate that glomerular and tubular dysfunctions exist in β-TM patients, these abnormalities are mainly subclinical, but, with protracted recurrent tubule injury, progression of renal damage may occur. A possible mechanism to account for these findings is perhaps that thalassemia itself may lead to proximal tubular dysfunction either through chronic hypoxia from persistent anemia or through haemosiderosis/iron deposition or iron chelation therapy [27].

According to the Thalassemia International Federation guidelines of 2014 for β-TM management, periodic chemistry panel, urea, and creatinine should be done every 3 months (monthly if on DFX). Urinalysis is to be done biannually. Since iron overload, chronic anemia, and improper iron chelation therapy use are all linked to renal complications, the judicial use of blood transfusion and iron chelators, along with proper follow-up testing, is recommended [28]. Definitions and

markers of kidney disease across the studies are highly heterogeneous, and most studies available are cross-sectional, involving a small number of patients. More longitudinal data are required to fully portray any possible renal abnormalities, in the era of new iron chelation and blood transfusion guidelines [4].

Progress of research in this topic will allow the detection of renal dysfunction in the hope to arrest the progress of renal injury, if not to reverse it.

Limitations

A potential limitation of our study was that it was a case–control study, so we did not follow individual participants over time. In addition, we did not obtain a 'gold standard' measurement of eGFR, including the clearance of iohexol or inulin. However, this is a preliminary study, which provided information regarding renal dysfunction in β-TM by evaluating early urinary biomarkers. Longitudinal studies are needed to understand the true burden of renal dysfunction in patients with β-TM.

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Conflicts of interest

There are no conflicts of interest.

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