DOI: 10.24287/1726-1708-2024-23-4-107-111

Serum neutrophil gelatinase-associated lipocalin in children with β-thalassemia major as a promising marker for predicting renal tubular impairment

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To assess the usefulness of serum neutrophil gelatinase-associated lipocalin (NGAL) in children with β-thalassemia major as an early indicator of kidney damage. A case-control study was conducted on 140 children. Two main groups were identified: 70 patients with transfusion-dependent beta thalassemia major and 70 age- and gender-matched healthy controls. All the participants were evaluated for medical history and underwent a thorough physical examination, clinical assessments, and laboratory tests for complete blood count, serum ferritin, renal function, and serum NGAL. A significant increase in serum NGAL levels was observed in the patients compared to the controls ($p = 0.001$). Moreover, NGAL showed a positive correlation with serum urea ($r = 0.257$; $p < 0.001$), creatinine ($r = 0.389$; $p < 0.001$), and ferritin levels ($r = 0.635$; $p < 0.001$), and a negative correlation with hemoglobin level ($r = –0.608$; $p < 0.001$), MCV ($r = –0.0480$; $p < 0.001$), MCH ($r = –0.433$; $p < 0.001$), and eGFR ($r = –0.346$; $p < 0.001$). NGAL had an AUC of 0.914, a cut-off value of 1370 ng/mL, 86.7% sensitivity, and 90% specificity. The Ethical Committee of the Faculty of Medicine, Menoufia University, reviewed the study protocol and gave approval (No. 191219 PEDI 28). Written informed parental consent was obtained in all cases. Renal dysfunction in thalassemia can start as a hidden damage with no apparent symptoms or complaints. Hence, NGAL may serve as an early indicator of renal tubular and glomerular dysfunction in patients with beta-thalassemia.

Key words: *beta thalassemia major, iron overload, neutrophil gelatinase-associated lipocalin, renal dysfunction*

Fahima M. Hassan, et al. Pediatric Hematology/Oncology and Immunopathology 2024; 23 (4): 107–11.
DOI: 10.24287/1726-1708-2024-23-4-107-111

Received 14.10.2024
Accepted 15.11.2024

EDN: LZDZVO

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Beta thalassemia major (β-TM) belongs to a diverse group of hereditary hemoglobin (Hb) disorders characterized by abnormal production of beta globin chains of Hb resulting in an excess of α-globin chains. The premature breakdown of red blood cells in the bone marrow and spleen leads to faster red blood cell replacement, inefficient production of red blood cells, and severe anemia [1].

Improved survival rates in patients with thalassemia major are attributed to the use of effective iron chelators and new imaging techniques that can detect excessive iron in the body. However, an increase in the survival rates of thalassemia patients has brought up the problem of numerous organ failures [2].

Many factors can be involved in kidney damage in children with thalassemia. Long-term lack of oxygen, anemia, frequent blood transfusions, continuous hemolysis, excessive iron from blood transfusions, and potential kidney damage from iron-removal medications can all contribute to a decline in renal function [3].

Diagnosing advanced kidney damage with standard biomarkers (serum creatinine, creatinine clearance, or serum urea) may be inaccurate due to various factors that may influence creatinine and urea concentrations such as age, gender, muscle mass, protein intake, inflammation, and the presence of liver disease [4].

One of the most promising indicators of initial renal damage is neutrophil gelatinase-associated lipocalin (NGAL), a 25-kDa iron-carrying protein secreted by activated neutrophils and expressed in epithelial cells. It is significantly elevated in kidney injury. After renal injury caused by toxins or lack of blood supply, the levels of NGAL significantly rise in kidney cortical tubules, blood, and urine. NGAL could potentially have predictive value in determining both immediate kidney damage and a long-term decline in kidney function in patients with chronic kidney disease. This research sought to confirm serum NGAL as an early predictor of kidney damage in children with β-thalassemia.

MATERIALS AND METHODS

In this case-control study, 70 patients with β-TM were recruited along with 70 age- and gender-matched healthy controls. The cases visited hematology outpatient clinic at the Pediatric Department of Menoufia University to receive regular blood transfusions.

The Ethical Committee of the Faculty of Medicine, Menoufia University, reviewed the study protocol and gave approval (No. 191219 PEDI 28). Written informed parental consent was obtained in all cases.

The results of medical assessments and laboratory tests for CBC, ferritin levels, kidney function, and NGAL levels were documented for every subject.

The estimated glomerular filtration rate (eGFR) was calculated using the Schwartz equation [6]. Creatinine clearance (mL/min/1.73m^2) = *κ* × *height* (cm)/*serum creatinine* (mg/dL).

The study included children with transfusion-dependent β-TM who met specific requirements at the time of first diagnosis (age less than 2 years, Hb levels between 6–7g/dL, HbF > 50%, and HbA2 < 3%).

Patients with various hemoglobinopathies or hemolytic anemia, systemic illnesses like heart failure, hepatic diseases, or diabetes mellitus, and those exhibiting clinical or laboratory signs of other renal diseases were excluded from the study.

Analytical methods

Venous blood samples were collected from the cases (prior to blood transfusion) and controls in sterile conditions. The following laboratory tests were performed: complete blood count (analyzed using the Sysmex XN-10 Automated Hematology Analyzer, Sysmex, Kobe, Japan); kidney function tests (analyzed using the AU680 chemistry analyzer, Beckman Coulter Inc., Brea, California, USA); and serum ferritin levels (measured using the mini VIDAS Automated Immunoassay Analyzer (Biomerieux, Marcy-l'Etoile, France).

In addition, serum samples for NGAL assays were isolated after centrifugation at 3000 rpm for 10 min and kept frozen at –20°C until analysis. Serum NGAL levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Sun Red Biological Technology Co., Ltd, Shanghai, China, Catalog No. 201-12-1720) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences software (SPSS version 25; SPSS Inc., Chicago, Illinois, USA). Numerical data was presented as means with standard deviations, and ranges, as needed. Numbers and percentages were used to display categorical data. The Student's independent t-test was used to compare two groups of quantitative variables. Pearson's correlation coefficients were computed to evaluate the degree of correlation between normally distributed numerical measurements. The ROC curve was used to find the best cut-off point, sensitivity, specificity, and area under the curve. *p*-values lower than 0.05 were considered significant.

RESULTS

The demographic data and clinical information of the subjects is presented in *table 1*.

There were significant differences in the levels of Hb, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), red cell distribution width (RDW), serum creatinine and urea levels, eGFR, and serum NGAL concentrations between the two groups ($p = 0.001$) (*table 2*).

NGAL levels showed a positive correlation with creatinine ($r = 0.389$; $p < 0.001$), urea ($r = 0.257$; $p < 0.001$), and ferritin ($r = 0.635$; $p < 0.001$), and a negative correlation with Hb ($r = –0.608$; $p < 0.001$) and eGFR ($r = –0.346$; $p < 0.001$), according to correlation analysis findings provided in *table 3*.

The utility of serum NGAL in children with β-TM as an early predictor of kidney damage was evaluated using the ROC analysis. The AUC for NGAL was 0.914, with a cutoff value set at 1370 ng/mL, showing 86.7% sensitivity, 90% specificity, and a 95% CI of 0.908–0.968 (*table 4*, *figure 1*).

DISCUSSION

Kidney iron overload associated with frequent blood transfusions in beta thalassemia patients causes damage to kidney components, leading to atrophy of tubules, scarring of glomeruli, and kidney interstitial fibrosis [7].

Regarding the hematological parameters, our patients with β-TM had lower levels of Hb, MCV, and MCH than the controls. Origa [8] demonstrated that children with thalassemia major exhibit low levels of Hb, MCV, and MCH. Moreover, Vehapoglu et al. [9] found that the average Hb level was 10.39 ± 0.69, and the average MCV and MCH levels were 60.11 ± 3.49 and 18.9 ± 1.37, respectively. This aligns with Ali et al. [10], who reported abnormal Hb levels and anemia due to excessive red blood cell destruction.

We observed elevated levels of serum urea in the group of cases compared to the control group, but these values still fell within the range of normal values. Our results are in line with the study by Mahmoud et al. [11], where a marked rise in serum urea levels was observed in β-TM patients. In the Egyptian study by Shfik et al. [12], it was found that the average urea level in 45 patients was 26.6 ± 8.5, showing a significant statistical variance compared to the control group. In addition, Hamed et al. [13] demonstrated that chronic lack of oxygen, severe anemia, frequent blood transfusions, hemolysis, excessive iron from blood transfusions, and potential kidney damage from iron

chelators are known factors contributing to a decline in renal function.

As regards serum creatinine, in the studies by Naderi et al. [14] and Mohkam et al. [15] children with β-TM had higher creatinine levels than controls. On the other hand, Thongsaen et al. [16] discovered that β-TM patients had typical serum creatinine levels and did not show any significant association in the patient group when compared to the control group.

Table 1
Demographic data and anthropometric measurements of the study subjects

Variables	Patient group (*n* = 70)	Control Group (*n* = 70)	*p*-value
Age, year: mean ± SD range	8.18 ± 3.49 1–14	8.53 ± 3.43 2–14	0.697
Gender, *n* (%): male female	39 (55.7) 31 (44.3)	45 (64.3) 25 (35.7)	0.584
Weight, kg: mean ± SD range	19.46 ± 7.49 11–35	36.3 ± 14.66 15–65	0.001*
Height, cm: mean ± SD range	114.16 ± 20.17 60–145	136.06 ± 22.51 88–170	0.001*
BMI, kg/cm^2: mean ± SD range	14.81 ± 1.38 12.50–19.44	17.47 ± 3.81 15.90–23.50	0.001*

Note. BMI – body mass index.

Table 2
A comparison of the laboratory test results of the study subjects

Variables	Patient group (*n* = 70), mean ± SD	Control Group (*n* = 70), mean ± SD	*p*-value
Hb, g/dL	7.58 ± 1.17	13.09 ± 1.02	0.001*
MCV, fL	71.78 ± 4.49	81.84 ± 1.02	0.001*
MCH, pg	23.21 ± 3.21	29.08 ± 0.64	0.001*
RDW, %	15.28 ± 1.36	13.0 ± 0.92	0.001*
Serum Creatinine, mg/dL	0.83 ± 0.13	0.45 ± 0.23	0.001*
Serum Urea, mg/dL	28.26 ± 6.45	23.3 ± 3.28	0.001**
eGFR, mL/min	107.73 ± 13.09	123.66 ± 10.47	0.001**
Serum ferritin, ng/mL	2119.93 ± 208.28	102.86 ± 48.09	0.001**
Serum NGAL, ng/mL	1864.2 ± 549.51	1202.86 ± 165.18	0.001**

Table 3
A correlation between serum NGAL and laboratory parameters in the patient group

Variables	Serum NGAL	
	r	*p*-value
Hb, m/dL	–0.608	< 0.001**
MCV, fL	–0.480	< 0.001**
MCH, pg	–0.433	< 0.001**
RDW, %	0.422	< 0.001**
Serum Ferritin, ng/mL	0.635	< 0.001**
Serum Creatinine, mg/dL	0.389	< 0.001**
Serum Urea, mg/dL	0.257	< 0.001**
eGFR, mL/min	–0.346	< 0.001**

In our study, there was a noticable decline in the average eGFR values in the group of patients. Milo et al. [17] discovered that individuals with thalassemia had lower GFR and suggested to use more precise methods for early identification and prevention of further GFR decline. In addition, Shoeib et al. [18] reported impaired glomerular function in 50 individuals with β-TM, with 41.9% of them having decreased GFR. Naderi et al. [14] demonstrated that children with β-TM had lower eGFR levels than healthy controls. This is in agreement with Ponticelli et al.'s [19] explanation that anemia, iron overload, and treatment with specific iron chelators can lead to kidney damage and reduced eGFR levels.

In our study, individuals with β-TM had elevated levels of serum ferritin as was shown by Ayulinda et al. [20].

The level of serum NGAL was found to be higher in the cases than in the controls, as in our study, as reported by Roudkenar et al. [21] Patsaoura et al. [22] suggest that there are numerous factors that can contribute to increased NGAL levels in thalassemia patients, such as anemia/hypoxia, kidney injury, and disruptions in iron homeostasis. Additionally, El-shall et al. [23] found that levels of serum NGAL were higher in β-thalassemia patients when compared to controls. Similarly, Fouad et al. [24] suggests that urinary NGAL excretion is associated with proximal tubular injury, which can lead to a disruption in NGAL reabsorption or an increase in NGAL synthesis.

The present research demonstrated a positive correlation between NGAL and serum levels of urea, creatinine, ferritin, RDW, and platelet counts. Conversely, a negative correlation was found between NGAL and Hb level, MCV, MCH, and eGFR. In the event of no apparent kidney disease, our findings suggest that NGAL levels are able to predict a hidden kidney damage.

Our results support the findings of El-shall et al. [23] as regards a positive correlation between serum NGAL and serum urea, creatinine, and ferritin

Figure
ROC curve of serum NGAL in the children with β-TM

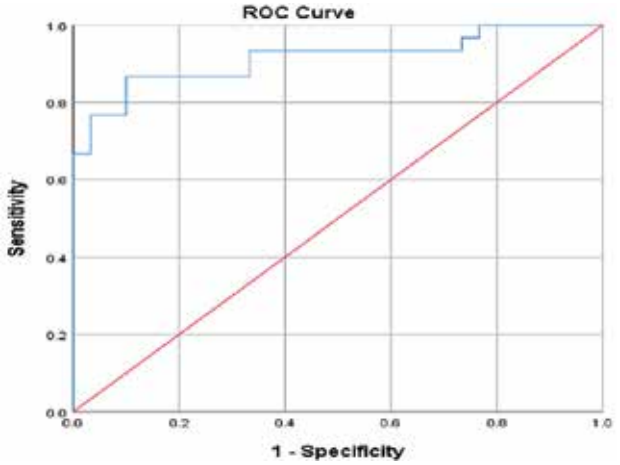

Table 4

Receiver operating characteristic (ROC) curve of serum NGAL in the children with β-TM

Parameter	AUC	*p*-value (95% CI)	Cutoff value	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Serum NGAL, ng/mL	0.914	< 0.001 (0.838–991)	1370	86.6	90	89.6	87.1

Note. CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value.

levels; and a negative correlation between NGAL and Hb levels. Furthermore, Bolignano et al. [25] demonstrated a strong positive correlation between NGAL and hematocrit, MCV, MCH, serum iron, and ferritin.

Our research showed that NGAL had an AUC of 0.914 at a cut-off of 1370 ng/L, 86.7% sensitivity, and 90% specificity, with a 95% confidence interval of 0.838-0.991. El-shall et al. [23] demonstrated an AUC of 0.976 for NGAL, with 88% sensitivity, 91% specificity, and a 95% CI of 0.908–0.968.

Our findings support the results of the study by Romejko et al. [26], where NGAL is primarily recognized as a biomarker for acute kidney injury which is released after tubular damage and during renal regeneration processes. In their study, NGAL levels were also increased in individuals with chronic kidney disease and those undergoing dialysis. It could serve as an indicator of a decline in renal function along with complications and death caused by kidney failure.

CONCLUSION

Renal dysfunction in thalassemia can start as a hidden damage with no apparent symptoms or complaints. Hence, NGAL may serve as an early indicator of renal tubular and glomerular dysfunction in children with beta-thalassemia.

ACKNOWLEDGMENTS
The authors would like to thank the participants of the study and acknowledge the support of the data collection team.

FUNDING
No funding.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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