

Evaluation of NGAL Concentration in UTIs among Beta-Thalassemia Major Patients

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Abstract

Background: UTIs are among the most common complications in beta-thalassemia patients, and the most serious problems due to iron overload, necessitating iron chelation, leading to renal dysfunction, increased UTI susceptibility, such as pyelonephritis, which can all lead to renal tubular impairment. This study aimed to evaluate the diagnostic value of NGAL protein as a biomarker for detecting UTIs in patients with thalassemia major.

Methodology: There were 90 people in this prospective case-control study. There were three groups of people: group one had 30 beta-thalassemia major patients with urinary tract infections (UTIs), group two had 30 beta-thalassemia major patients without UTIs, all of whom were between the ages of 10 and 50, and group three had 30 healthy controls between the ages of 10 and 30. All participants' urine samples were collected in sterile wide-mouth vials from all groups and subsequently centrifuged at 3000 rpm to differentiate between the cases and the control groups. The supernatant was then refrigerated at -20°C until the study began, and enzyme-linked immunosorbent assays (ELISA) were performed.

Results: showed a significant increase in NGAL levels in patients compared to the control group ($p < 0.001$). Analyses also revealed a significant correlation between elevated NGAL levels and the occurrence of urinary tract infections ($p = 0.002$). No statistically significant differences in NGAL levels were observed between males and females across any of the studied groups ($p > 0.05$).

Conclusion: Urine NGAL can be used as a useful biomarker for detecting kidney involvement in patients with thalassemia major; however, its ability to differentiate between patients with and without urinary tract infections appears to be limited.

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تقييم تركيز الليبوكالين المرتبط بإنزيم الجيلاتيناز العدلي (NGAL) في التهابات المسالك البولية لدى مرضى التلاسيميا الكبرى بيتا

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الخلاصة

المقدمة: تُعد التهابات المسالك البولية من المضاعفات الشائعة لدى مرضى التلاسيميا الكبرى بيتا، كما تمثل إحدى المشكلات المهمة المرتبطة بفرط حمل الحديد، الأمر الذي يستدعي استخدام خالبات الحديد. وقد يسهم كلٌّ من فرط الحديد والعلاج الاستخلافي في إحداث خلل في الوظيفة الكلوية وزيادة القابلية للإصابة بالتهابات المسالك البولية، بما في ذلك التهاب الحويضة والكلية، مما قد يفضي في النهاية إلى أذية النبيبات الكلوية. هدفت هذه الدراسة إلى تقييم القيمة التشخيصية لبروتين الليبوكالين المرتبط بإنزيم الجيلاتيناز العدلي (NGAL) بوصفه مؤشراً حيويًا للكشف عن التهابات المسالك البولية لدى مرضى التلاسيميا الكبرى بيتا.

المرضى وطرق العمل: أُجريت هذه الدراسة المستقبلية من نوع الحالات والشواهد على 90 مشاركًا، جرى توزيعهم على ثلاث مجموعات. ضُمَّت المجموعة الأولى 30 مريضًا مصابًا بالتلاسيميا الكبرى بيتا مع التهاب في المسالك البولية، بينما ضُمَّت المجموعة الثانية 30 مريضًا مصابًا بالتلاسيميا الكبرى بيتا من دون التهاب في المسالك البولية، وتراوح أعمار أفراد هاتين المجموعتين بين 10 و50 سنة. أما المجموعة الثالثة، فقد شملت 30 شخصًا سليمًا ظاهريًا تراوحت أعمارهم بين 10 و30 سنة. جُمعت عينات البول من جميع المشاركين في حاويات معقمة واسعة الفوهة، ثم خضعت للتردد المركزي بسرعة 3000 دورة/دقيقة. وبعد فصل الجزء الفوقاني، حُفظت العينات عند درجة حرارة -20°م لحين إجراء التحاليل. وقد تم قياس مستويات NGAL باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA).

النتائج: أظهرت النتائج ارتفاعًا ذا دلالة إحصائية في مستويات NGAL لدى المرضى مقارنةً بالمجموعة الضابطة ($p < 0.001$). كما بينت التحليلات وجود ارتباط ذي دلالة إحصائية بين ارتفاع مستويات NGAL وحدوث التهابات المسالك البولية ($p = 0.002$). في المقابل، لم تُظهر النتائج فروقًا ذات دلالة إحصائية في مستويات NGAL بين الذكور والإناث في أيٍّ من المجموعات المدروسة ($p > 0.05$).

الاستنتاج: يمكن اعتماد NGAL البولي بوصفه مؤشرًا حيويًا مفيدًا للكشف عن الإصابة الكلوية لدى مرضى التلاسيميا الكبرى بيتا، إلا أن قدرته على التمييز بين المرضى المصابين بالتهابات المسالك البولية وغير المصابين بها تبدو محدودة.

1. Introduction

Beta-thalassemia major(β -TM) is the most serious kind of beta-thalassemia. It lowers the formation of β -globin chains, which causes changes in both β -globin alleles. (Ali et al., 2021). The incidence of β -thalassemia is particularly high in countries of the Middle East (Iraq and Iran) and parts of Asia (India, Pakistan, and Bangladesh). Moreover, the distribution of β -thalassemia in Northern Europe and North America. (Kattamis et al., 2020; Hasan et al., 2025a) In Iraq(β -TM), there is a big public health problem. For example, in Sulaymaniyah (northeastern Iraq), 4.1% of people are carriers, and the disease is thought to affect 37.1 out of every 100,000 people in Iraq (Amin et al., 2020). A complete blood count and hemoglobin electrophoresis test showed that the person had b-TM. (Al-Abidy and Al-Aidy, 2025). The most serious long-term consequence of a urinary tract infection (UTI) might result in accelerated nephrosclerosis, which may induce hypertension and chronic renal failure, resulting from infection with Gram-negative bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae*. Early detection and treatment of urinary tract infections (UTIs) are crucial; failure to diagnose or treat a UTI promptly may result in long-term complications, including renal scarring, hypertension, and chronic renal failure. Recent investigations have found glomerular and tubular abnormalities in thalassemia patients, as well as renal failure. This cohort is more susceptible to urinary tract infections and antibiotic resistance. (Al et al., 2025; El-Hawy et al., 2023). Iron overload and secondary hemosiderosis begin in early childhood as a result of treating (β -TM) with frequent transfusions of packed red blood cells. Resulting in organ damage, including renal, cardiac, and hepatic damage (Şen et al., 2015). Some patients have low-grade proteinuria and albuminuria, whereas others present with tubular diseases such as hypercalciuria, hyperuricosuria, aminoaciduria, and glucosuria. In people with β -TM, several factors may cause their kidneys not to work properly: iron chelation therapy, iron deposits in tissues, and decreased erythrocyte lifespan (Stoyanova and Chervenkov, 2021). When ferritin levels exceed 1000 ng/ml or following 10–20 blood transfusions, patients are typically recommended to begin iron chelation treatment. If serum iron levels are elevated and not properly managed, they may lead to greater illness and death (Hashemieh, 2020). Traditional indicators like Neutrophil gelatinase-related lipocalin (NGAL) have been used to find kidney injury. which is a twenty-five-kDa iron-containing protein that active neutrophils release. People first discussed it in 1993. NGAL is an extracellular protein that is part of the lipocalin family. It binds tiny, hydrophobic molecules and helps keep cells in balance. (Danquah et al., 2023). There is an immediate necessity for initial prognostic biomarkers of renal damage in(β -TM), as prompt organization can markedly enhance the prognosis (Amal et al., 2018). It is a highly sensitive and early sign of kidney injury. The origin and temporal progression of NGAL were evaluated in numerous investigational and clinically pertinent renal disorders. (NGAL) It is a differentiation inducer for the epithelial cells of the loop of Henle in the embryonic kidney, with its expression significantly elevated in the context of kidney damage. The proximal tubule epithelial cells also make NGAL, and some people think that measuring NGAL levels in urine early on can help anticipate kidney damage. (Karaman et al., 2019).In recent years, some studies has shown that renal damage or defect is an underappreciated consequence in people with β -TM. UTIs or Chronic anemia, iron excess from repetitive transfusions, and some iron chelators are the primary contributors to renal impairment in β -thalassemia. Recognizing this illness early on lets us slow down renal impairment and UTI in the last few decades. (Arian et al., 2025) Another investigation on the urine of mice with pyelonephritis revealed an early increase in GAL levels. Additionally, urine NGAL levels were elevated in both juvenile and adult patients with UTI. (Pamuk et al., 2022). Diagnosing a urinary tract infection (UTI) usually takes 48 to 72 hours and depends on a urine culture. For early

detection, NGAL has become one of the most significant biomarkers. NGAL comes from the kidney and shows inflammation caused by infection without the presence of neutrophils, which is different from standard markers. (Shaikh et al., 2023). One of the most important drug chelators is Deferasirox (DFX). Long-term use can have substantial side effects, like bone marrow destruction, renal tubular, and glomerular abnormalities. (Hasan et al., 2025b).

2. Materials and Methods

2.1. Study Design and Participants

The case–control research included 90 people. Conducted from December 2025 to March 2026 at the Thalassemia Center, Al-Zahra Hospital, Najaf Al-Ashraf. The members were separated into three groups: the first group comprised 30 beta-thalassemia major patients with urinary tract infections (UTIs) (12 males and 18 females), who, upon microscopic examination, had pus cells above 10 in number. Group two consisted of 30 beta-thalassemia major patients without urinary tract infections (14 males and 16 females), all aged between 10 and 50 years, and 30 healthy control patients aged between 10 and 30 years (14 males and 16 females). Every three to four weeks, all of the patients get blood transfusions, packed red blood cell transfusions, and iron chelation treatment. For example, deferasirox and deferoxamine. Urine samples were placed in sterilized containers. Then we put them in sterile tubes and spun them around in a centrifuge. After that, we put them into four Eppendorf tubes. for uNGAL, which we spun at 3000 rpm for around 20 minutes. The supernatant was carefully collected and then kept in the refrigerator at -20°C until the investigation began. They employed enzyme-linked immunosorbent assays (ELISA) to examine the biomarker Urinary NGAL. They used ELISA kits from (Milson, China), according to manufacture to find out how much NGAL was in random urine samples.

2.2. Inclusion & Exclusion criteria

Patients with β -TM with or without UTI infection required signed informed consent for the age between 10 and 50 years, and the control group aged 10 to 30 years. Any other forms of anemia, concurrent conditions that may impair kidney function or potentially cause renal damage, including diabetes, rheumatic heart disease, thyroid or hepatic disorders, and sepsis, pregnancy, cancer disease, taking nephrotic drugs, thalassemia minor, and sickle cell anemia.

2.3. Ethical approval and regulatory considerations

Patients gave their permission for this study and were told what it was about. Information and data were kept private, and the scientific research ethics committee of the College of Medicine / Jabir Ibn Hayyan University of Medical and Pharmaceutical Sciences 1207 gave its clearance on (22/2/2026). The Najaf Health Directorate gave permission for the sample and data of the participating patients to be collected. The number 144 on (26/11/2025) was an unethical Approval.

2.4. Statistical Analysis

The data were entered and analyzed using Statistical Package for the Social Sciences (SPSS), version 26. A range of statistical methods was applied to ensure appropriate analysis of the study data. Descriptive statistics, including the mean, standard deviation (SD), median, and interquartile range (IQR), were used to summarize the variables. Comparisons among the three study groups were performed using the Kruskal–Wallis test, followed by the Mann–Whitney U test for pairwise comparisons when appropriate. Associations between categorical variables were assessed using the chi-square test. A p-value < 0.05 was considered statistically significant.

3. Results

This case–control research was executed from December 2025 to February 2026. including a total number of 90 participants. The individuals were split into three groups: group one, 30 beta-thalassemia major patients with urinary tract infections (UTIs) (male 12 and female 18), group two, 30 beta-thalassemia major patients without UTIs (male 14 and female 16), all of whom were between the ages of (10 and 50), and 30 healthy control age between of (10 and 30) (male 14 and female 16). For infection B-TM with UTI, the mean ages of participants were 16.8 ± 6.1 and 35.2 ± 4.1 years in the 10–30 and 31–50-year groups, respectively, when the p-value is considered statistically significant ($p = 0.002$), the mean ages of participants were 15.9 ± 5.3 and 34.8 ± 3.7 years, in the 10–30 and 31–50-year groups, respectively, for the non-infection group, when the p-value is considered statistically significant ($p = 0.001$), while the mean age in the control group was 21.8 ± 6.5 years. (Table1, Fig.1.)

Table1: Mean Age According To Age Among Study Groups.

Group	Age Group (years)	n	Age Mean $\pm$ SD	P-value
Without UTI infection	10–30	23	16.8 ± 6.1	0.001
	31–50	7	35.2 ± 4.1	
With UTI Infection	10–30	26	15.9 ± 5.3	0.002
	31–50	4	34.8 ± 3.7	
Control	10–20 / 21–30	30	21.8 ± 6.5	—

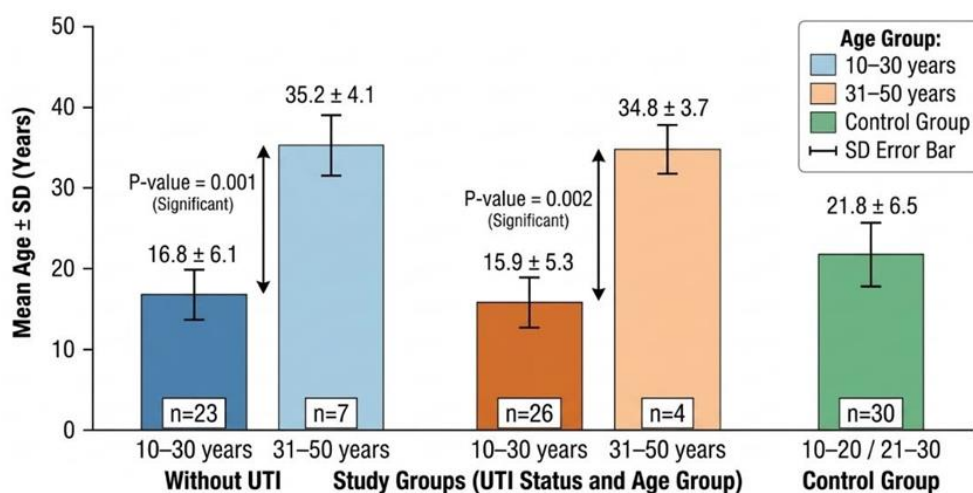
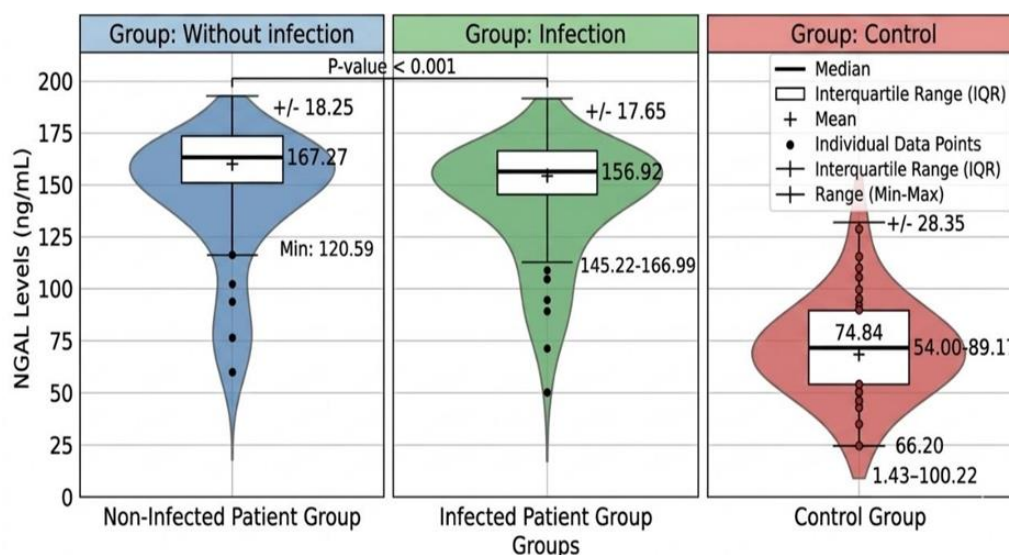


Figure1: Mean Age Distribution of Patients and Control Group Subjects.

In our study, NGAL in all groups shows a difference that is statistically significant between patients (with and without infection) and controls regarding uNGAL level, as the median was 156.98 ng/ml, 167.27 ng/ml, respectively, for cases in relation to controls, 74.84 ng/ml, with a P value of ($p < 0.001$). However, the difference between patients with infection and those without infection was relatively small. This indicates that NGAL may be useful for distinguishing patients from healthy individuals, but it has limited ability to differentiate between infected and non-infected individuals. Table2, Fig.2.

Table2: Descriptive Analysis of NGAL Levels in Patient and Control Groups

Group	n	Mean± SD	Median (IQR)	Min – Max	P value
Without infection	30	162.96±18.25	167.27(151.47-175.40)	120.59-192.64	<0.001
Infection	30	156.92 ± 17.65	156.98 (145.22 – 166.99)	116.19– 190.40	
Control	30	66.20 ± 28.35	74.84 (54.00 – 89.17)	1.43 – 100.22	

**Figure2: Mean NGAL Levels ± SD in Patient and Control Groups.**

Furthermore, the correlation of NGAL levels between groups, as determined by the Kruskal-Wallis and Mann-Whitney tests, revealed a statistically significant disparity in NGAL levels across all groups ($H = 52.8$, $p < 0.001$). Nonetheless, no statistically significant difference was detected between individuals with infection and those without infection ($p = 0.147$). In contrast, both patient cohorts had markedly elevated NGAL levels relative to the control group ($p < 0.001$). (See Table3 and Fig.3).

Table3: Comparison of NGAL Levels Between Groups (Kruskal-Wallis / Mann-Whitney Tests).

Comparison	Test	Statistic	df	P value
All groups	Kruskal-Wallis	$H = 52.8$	2	< 0.001
Without infection vs Infection	Mann-Whitney	$Z = -1.45$	–	0.147
Without infection vs Control	Mann-Whitney	$Z = -6.10$	–	< 0.001
Infection vs Control	Mann-Whitney	$Z = -5.95$	–	< 0.001

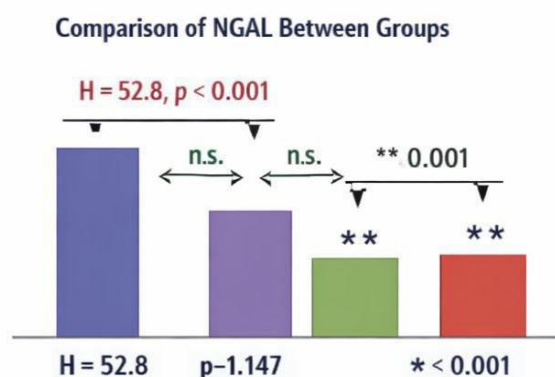


Figure3: Comparison of NGAL Levels Between Groups.

For NGAL by gender, no statistically significant differences in NGAL levels were observed between males and females across any of the studied groups ($p > 0.05$). (Table4, Fig.4).

Table4: NGAL Levels According to Gender for Each Group

Group	Gender	n	Mean $\pm$ SD	Median (IQR) (Male vs Female)	P value
Without infection	Female	17	163.92 $\pm$ 17.85	168.98 (152.42 – 175.40)	0.62
	Male	13	161.75 $\pm$ 19.10	167.98 (151.42 – 176.58)	
Infection	Female	16	153.64 $\pm$ 16.90	152.66 (142.11 – 162.88)	0.48
	Male	14	160.68 $\pm$ 18.20	160.25 (147.25 – 168.55)	
Control	Female	14	67.89 $\pm$ 26.40	71.34 (53.80 – 87.80)	0.71
	Male	16	64.72 $\pm$ 30.50	68.98 (32.65 – 90.34)	

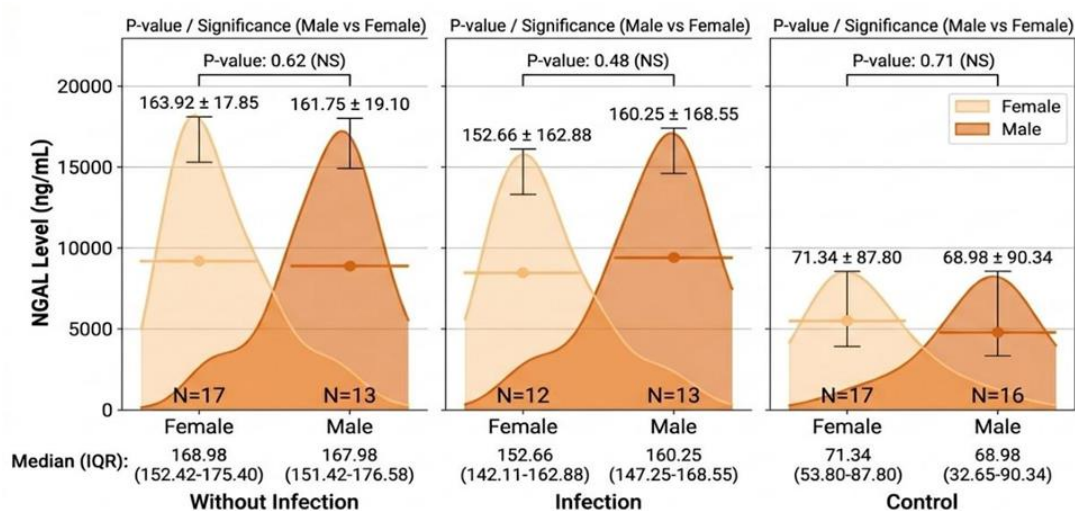


Figure4: NGAL Levels by Gender

Table5, Fig.5. The table showed that for iron chelation therapy, a significant association with UTI status was also observed ($P = 0.04$). Patients receiving deferasirox had a slightly lower risk of UTI compared to those receiving deferoxamine, with an OR of 0.84 and a 95% CI of 0.26–2.71, indicating that the effect of iron chelation therapy is less pronounced than that of NGAL. a significant association between NGAL levels and urinary tract infection

(UTI) status ($P = 0.002$). Patients with elevated NGAL levels (>159.537 ng/mL) were more likely to develop a UTI than those with lower levels, with an odds ratio (OR) of 1.71 and a 95% CI of 0.62–4.75. This suggests that elevated NGAL is associated with an increased risk of UTI.

Table5: Association of NGAL Levels, Iron Chelation Therapy, And UTI Status with Odds Ratio And 95% CI

Variable	Category	Patients with UTIs (n=30)	Patients without UTIs (n=30)	OR (95% CI)	P value
Iron Chelation Therapy	Deferasirox	22 (73.3%)	23 (76.7%)	0.84 (0.26–2.71)	0.04
	Deferoxamine	8 (26.7%)	7 (23.3%)	—	—
NGAL Level	> 159.537	17 (56.7%)	13 (43.3%)	1.71 (0.62–4.75)	0.002
	≤ 159.537	13 (43.3%)	17 (56.7%)	—	—

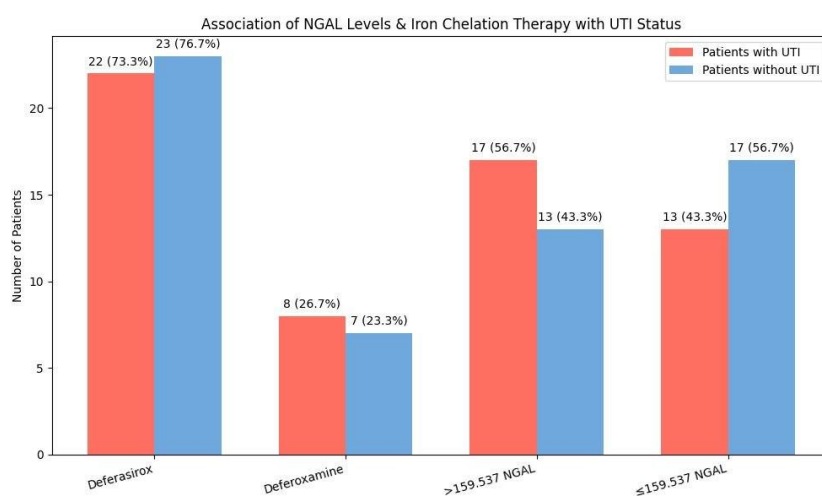


Figure5: Association of NGAL Levels and Iron Chelation Therapy with UTI Status

4. Discussion

There is not much research on how often persons with β -thalassemia major have kidney difficulties. But kidney difficulties are the fourth most common health condition in persons with β -thalassemia, after problems with the endocrine system, heart, and liver. Recent research indicated that 1.8% of individuals with TDT experienced renal complications. Renal injury occurs due to the advancement of glomerular and tubular damage. It is very important to find glomerular and tubular dysfunctions early on in order to stop kidney diseases or get kidney function back to normal. (Demosthenous et al., 2019). proposed that urine NGAL levels might serve as a dependable marker of renal impairment in individuals with b-TM and that Urinary NGAL levels may serve as a biomarker for the screening of renal impairment, hence identifying persons predisposed to future decreased kidney function. (El-Hawy et al., 2023). Other studies detect serum NGAL to identify Diabetic Kidney Disease (DKD), a microvascular consequence of diabetes and a key factor leading to end-stage renal disease, as shown by the NGAL test. It significantly increases in the serum of patients within a few hours after ischemia-reperfusion injury (Maryam et al., 2025). Neutrophil gelatinase-associated lipocalin, an iron-transporting protein generated from human neutrophils, plays a crucial role in the innate immune response to bacterial infection. Bacteria need iron to grow and metabolize while they are within a host. It has been shown that activated neutrophils in the diseased host release NGAL, which prevents bacteria from taking up iron by using environmental iron and slowing their growth. (Jassim et al., 2026). Our analysis revealed a statistically significant difference in urinary NGAL levels between

cases and controls, with a median of 167.27 ng/ml for cases without UTI, for cases who had UTI, 156.98 ng/ml and compared with controls, 74.84 ng/ml for controls, with a p-value < 0.001. Others' research statistically significant cases and controls regarding uNGA level as median was 498.0 ng/ml for cases compared with controls, 423.3 ng/ml, with a P value of 0.034 (Fouad et al., 2019). Corroborating the findings of Karaman et al. (2019), who indicated that uNGAL levels were markedly elevated in the b-TM group compared to the control group ($p = 0.019$). Moreover, individuals with b-thalassemia major develop acute renal failure and Fanconi syndrome as a result of using chelating medications. In this study, iron chelation was used in this trial, given to all B-TM patients (both with and without infection) on deferasirox (73.3% and 76.7%, respectively). The same as in another study, use deferasirox at a rate of 87.5% (El-Hawy et al., 2023). In our study, age was a statistically significant factor for thalassemia with UTI and thalassemia without UTI, 0.001 and 0.002, respectively. Unlike another study, thalassemic patients. There was no significant difference between groups in terms of age and sex ($P > 0.05$). (Karaman et al., 2019). There is no substantial difference between males and females in the cases and control groups; the p-value was higher than <0.05 . comparable with (Şen et al., 2015), no significant difference by gender between cases and controls, the p- value <0.05 .

Conclusion

The process mostly impacts tubule cells as renal inflammation progresses. This can be the result of oxidative stress's harm on multiple levels. Substances detected in urine NGAL point to problems with reabsorption. When there is an overabundance of NAG, the Tubules have more severe issues. Understanding the pathophysiology of tubulopathies in people with β -TM and being able to diagnose them without invasive procedures would increase the likelihood that doctors can recognize the start of early kidney impairment and combat it with innovative therapeutic options during the early stages of the disease.

Conflicts of Interest

The authors declare no conflict of interests

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