Assessment of Renal Dysfunction in Adult Patients with Transfusion Dependent β Thalassemia Using Urinary Retinol Binding Protein

Abstract

Background: Individuals with transfusion-dependent thalassemia (TDT) have been found to experience renal impairment, which can range from mild to severe. This trial aimed to assess renal dysfunction in adult individuals with TDT using urinary retinol binding protein (RBP).

Methods: Fifty adults with a history of TDT diagnosed using high performance liquid chromatography (HPLC) as children (Group I) and fifty healthy adults serving as controls (Group II) participated in the research.

Results: The TDT (Group I) group had significantly higher levels of urinary retinol binding protein/creatinine ratio (RBP/Cr), urinary calcium/creatinine ratio (Ca/Cr), urinary uric acid/creatinine ratio (UA/Cr), and albumin creatinine ratio (ACR) compared to the control (Group II) group. The urinary RBP/Cr ratio was positively correlated with the urinary Ca/Cr ratio, the urinary UA/Cr ratio, and ACR, and negatively correlated with estimated glomerular filtration rate (eGFR). The urinary RBP/Cr ratio significantly predicted the urinary Ca/Cr ratio and urinary UA/Cr ratio, with odds ratios of 1.544 and 4.590, respectively, $P < 0.05$.

Conclusions: One way to identify tubular dysfunction in TDT patients at an early stage is to evaluate the urinary RBP/Cr ratio.

Keywords: Transfusion Dependent Beta Thalassemia, Renal Dysfunction, Urinary Retinal Binding Protein.

Introduction:

Reduced or non-existent production of β-globin, an essential component of adult hemoglobin, leads to an imbalance throughout globin chains making process, resulting in β-thalassemia (β-TM) syndromes, the most common hereditary monogenic illnesses worldwide [1].

As a result, β-TM causes a decrease in Hb production and an increase in α-globin [2]. Transfusion-dependent beta-thalassemia (TDT) major, non-transfusion-dependent beta-thalassemia (NTDT) intermedia or thalassemia minor are all part of the clinical and hematological spectrum that encompasses β-TM illness, which can range from moderate to clinically overt disorders [3].

The thalassemia epidemic mostly affects the African continent, Southeast Asia, and the Mediterranean [4]. Thalassemia is a major public health concern in developing nations, but it has also spread to industrialized nations due to migration from countries with a high prevalence of the disease [5].

Multiple forms of renal illness, like proteinuria, glomerular dysfunction, renal tubular acidosis, and tubular damage as well, are common in β-TM patients and often lead to morbidity. To improve prognosis, decrease the occurrence of end-stage renal disease and death, and prevent or reverse declines in kidney function, early diagnosis of individuals at risk for renal impairment is critical [6, 7].

The liver manufactures RBP, a protein with a relatively low molecular weight. Transporting vitamin A is its primary role. An indicator of proximal tubular dysfunction is RBP [8].

This study sets out to use urinary RBP as a tool for assessing renal impairment in adults suffering from transfusion-dependent β thalassemia (TDT)

Methods:

This case-control study included 50 adults who were diagnosed with TDT as children utilizing high-performance liquid chromatography (HPLC). At the time of initial diagnosis, these patients met the following criteria: they were less than two years old, had a mean hemoglobin level of 6-7g/dl, Hb F>50%, and HbA2 < 4% [9, 10] and 50 healthy individuals as control. The research took place from February 2023 to January 2024 with the blessing of the Tanta University Hospitals Ethical Committee in Tanta, Egypt (approval code: 36264MS78/2/23). Each participant gave their written, informed consent obtained from parents or legal guardians of any participant age of 18.

patients with other causes of hemoglobinopathies, systemic diseases affecting kidneys (diabetes mellitus, hypertension, cardiovascular disease and liver disease), acute systemic infection (including urinary tract infection), malignancies, pregnancy and lactating women were excluded.

Participants were split into two groups: one that received TDT and another that served as a control group consisting of healthy adults.

Laboratory testing, a physical examination, and a comprehensive medical history were all performed on the subjects, including a complete blood count (CBC), serum creatinine (Cr), calcium (Ca), phosphorus (Po4), uric acid (UA), blood urea, C-reactive protein (CRP), lactate dehydrogenase (LDH), and serum ferritin. Additionally, they were tested for urinary calcium/creatinine (Ca/Cr), urinary uric acid/creatinine (UA/Cr), albumin Creatinine (ACR), and urinary RBP4/Cr ratios, as well as estimated glomerular filtration rate (eGFR).

Sampling and processing:

8 ml of peripheral blood was taken under strict aseptic conditions into one ethylene diamine tetra acetic acid (EDTA) tube for CBC and one serum tube with clot activator. The serum tube was then centrifuged at 3000 g for 15 minutes. We ensured that the EDTA and serum samples

were sent to the lab for analysis no later than 2 hours after collection. CBC was performed on an electronic automatic analyzer (ERMA Inc. Poland, catalogue No. 3459.9020). A Diestro electrochemical detector with an ISE calibrating package from Diestro, Argentina (catalogue number IN0100) was used for determining serum Ca. KONELAB PRIME 60i was used to detect urea, Cr, UA, LDH, CRP and serum inorganic phosphorus using reagents from Thermo Fisher Scientific Oy-Finland (Catalogue numbers: 981304, 981811, TR24321, EEA013, 981933 and TR30026, respectively). Serum ferritin was measured using automated chemistry analyzer (Cobas 6000) using ROCHE Diagnostic kits (REF: 04885317190). The CKD-EPI equation, when simplified, is: GFR = 141 × min (Scr/κ, 1) α × max (Scr/κ, 1) ^-1.209 × 0.993^age × 1.018 (for females) or 1.159 (for Black individuals), where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, and α is -0.329 for females and -0.411 for males. "Min" represents the smaller of Scr/κ or 1, while "max" denotes the larger. For urine analysis, 30 ml morning samples were collected in sterile containers and centrifuged at 1000×g for 20 minutes to separate particles. 15 ml was used immediately for albumin creatinine ratio (ACR), urinary UA/Cr ratio, and urinary Ca/Cr ratio. The other half was aliquoted and stored at – 20o C until used for determination of (URBP4/Cr ratio). Diestro electrochemical detector with ISE calibrating package from Diestro, Argentina (catalogue number IN0100) was used for determining urinary ionized Ca. KONELAB PRIME 60i was used to detect urinary Cr, UA and microalbumin using reagents from Thermo Fisher Scientific Oy-Finland (Catalogue numbers: 981811, TR24321, and 72331657 respectively). Cut off value for ACR is 30-300 Alb/Cr (mg/g)[11], (UCa/Cr) 15 mg Ca /mg Cr and for Urinary UA / Cr ratio is 1.5 mg UA/mg create [12]

Measurement of Urinary retinol binding protein4/ creatinine ratio:

Using enzyme-linked immunosorbent assay kit from develop (catalogue no: DL-RBP4-Hu):

This kit quantifies RBP4 in plasma, human serum, and urine utilizing a sandwich enzyme immunoassay. A microtiter plate has an RBP4-specific antibody already applied to it. A biotin-conjugated antibody that is specific to RBP4 is added to the plate after standards or samples. Following this, Avidin which has been coupled to Horseradish Peroxidase (HRP) is administered and left to incubate. Spectrophotometric measurements are taken at 450 nm ± 10 nm after the addition of TMB substrate to the wells containing RBP4, biotin-conjugated antibody, and enzyme-conjugated Avidin. This color change is then prevented using sulfuric acid.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA. The data distribution was checked for normality using the Shapiro-Wilks test and histograms. Two groups were compared using an unpaired Student's t-test for quantitative parametric variables, which were given as means and standard deviations (SD). With quantitative non-parametric data, the Mann Whitney U test was used to determine the median and interquartile range (IQR

). For qualitative variables, the Chi-square test was used for analysis, and the results were given as percentages and frequencies. Spears' rho test was used to determine if there was a relationship between the variables. To determine what indicators could be used to forecast renal failure, binary logistic regression was employed. For statistical purposes, a p-value of less than 0.05 was deemed significant.

Results:

Age and gender were insignificantly different between both groups. **Table 1**

Urinary UA / Cr ratio, ACR, urinary RBP / Cr ratio, Platelets, WBCs, CRP, LDH, serum ferritin were significantly higher in group I when than group II ($P < 0.05$). Hb and eGFR were significantly lower in group I than group II ($P < 0.05$). **Table 2**

Urinary RBP / Cr ratio was positively correlated with urinary Ca / Cr ratio, urinary UA / Cr ratio, ACR, CRP, LDH, WBCs, serum calcium, and serum ferritin. Urinary RBP / Cr ratio was negatively correlated with Hb, and eGFR .**Table 3**

By comparing the median of urinary RBP / Cr ratio based on renal dysfunction factors there was a significant difference between urinary Ca/Cr ratio and urinary UA / Cr ratio groups as regards urinary RBP / Cr ratio ($p < 0.001$). **Table 4**

This binary logistic regression for urinary RBP / Cr ratio in predicting urinary Ca/ Cr ratio, urinary UA / Cr ratio showed urinary RBP / Cr ratio significantly predict urinary Ca/ Cr ratio, urinary UA / Cr ratio ($P < 0.05$) with an odds ratio 1.544, 4.590 respectively. **Table 5**

Discussion

Approximately 9–10% of Egyptians are carriers of the prevalent autosomal recessive genetic condition thalassemia, which affects around 1,000 children per year [13].

β-TM is a hereditary condition marked by inefficient erythropoiesis and different degrees of impaired hemoglobin beta-chain synthesis [14].

In the current study, the TDT group showed significantly lower Hb and significantly higher platelets, and WBCs when compared with the control group. In agreement with our result, Mohammad and Al-Doski. [15] showed that Hb was substantially lower in the homozygous β-TM major group when compared with the control group. Disagreeing with our findings, Sadeghi et al. [16] observed that total leukocytic count was insignificantly different between patients and controls.

According to our results, the TDT group showed substantially higher serum UA than the control group. In contrast to our findings, Sadeghi et al. [16] noted that plasma concentrations of UA were insignificantly different between patients and controls.

In the present study, the TDT group showed significantly higher urinary UA / Cr ratio and ACR than control group. Contrary to our findings, Youssry et al. [17] noted that UA/ Cr ratio was

insignificantly different between patients and controls. Supporting our findings, Uzun et al. [18] informed that The TM group had a higher urinary UA/Cr ratio than the control group.

Compared to the control group, we found that their CRP, LDH, and serum ferritin levels were much higher. This aligns with the findings of Saad et al. [17] who reported that Compared to the control group, the sick group had considerably higher levels of LDH and ferritin. Confirming our result, Deraz et al. [19] showed that in the β-TM major group, serum ferritin levels were noticeably greater than in the control group.

Based on our findings, the TDT group outperformed the control group in terms of the urine RBP/urinary Cr ratio. In the same line, Bilir et al. [20] illustrated that the urinary RBP / Cr ratio was significantly higher in patients than the control group.

For this investigation, urinary RBP/Cr ratio was positively correlated with urinary Ca /Cr ratio, urinary UA /Cr ratio, ACR, WBCs, CRP, LDH, and serum ferritin, and negatively correlated with Hb, eGFR, and serum Ca.

That is in line with what we found, Youssry et al. [17] showed a positive correlation between urinary RBP/Cr ratio and ACR and serum ferritin. It was consistent with Uzun et al. [18] demonstrated that the ferritin, RBP, and ACR levels were positively correlated.

With odds ratios of 1.544 and 4.590, respectively, the urine RBP/Cr ratio and the urinary Ca/Cr ratio were found to be significant predictors of renal dysfunction variables in a binary logistic regression analysis. In the same line, Rezk et al. [21] illustrated that urinary RBP / Cr ratio served as a reliable predictor of end-stage renal disease. A study by Ratajczyk et al. [21] supports our results and highlights the significant role of urinary RBP4 as a biomarker for predicting renal tubular damage.

The study's sample size was limited, and it only included data from one center, which are limitations.

Conclusions:

The TDT group had a considerably greater urinary RBP / Cr ratio than the control group and correlated with glomerular (ACR and eGFR) and tubular (urinary Ca/cr and urinary UA/cr ratios) renal function. Urinary RBP / Cr was a significant predictor for tubular dysfunction and could distinguish between tubular dysfunction and normal tubular function in TDT patients at an early stage.

List of abbreviations:

TDT**:** transfusion-dependent thalassemia

HPLC: high performance liquid chromatography

RBP/Cr: retinol binding protein/creatinine ratio

Ca/Cr: calcium/creatinine ratio

UA/Cr: urinary uric acid/creatinine ratio

ACR: albumin creatinine ratio

eGFR: estimated glomerular filtration rate

β-TM: beta-thalassemia

NTDT: non-transfusion dependent beta-thalassemia

CBC: complete blood count

Cr: creatinine

Ca: calcium

Po4: phosphorus

UA: uric acid

CRP: C-reactive protein

EDTA: ethylene diamine tetra acetic acid

HRP: Horseradish Peroxidase

SD: standard deviations

IQR: interquartile range

Statements and Declarations:

Ethics approval and Consent to participate:

The research took place from February 2023 to January 2024 approved by the Tanta University Hospitals Ethical Committee in Tanta, Egypt (approval code: 36264MS78/2/23). Each participant gave their written, informed consent was obtained from parents or legal guardians of any participant under age of 18 .

Consent to publish:

All authors give their consent for publication in the journal.

Data Materials and/or Code availability:

Data is available on reasonable requests from the corresponding author.

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Table 1: Demographic data in studied groups

		Group I(n=50)	Group II(n=50)	Test	P
Age (years)		25.62±5.43	25.86±6.26	0.205	0.838[(a)]
Sex	**Male**	23(46.0%)	27(54.0%)	0.640	0.424[(b)]
	Female	27(54.0%)	23(46.0%)		

Data are presented as mean ± SD or frequency (%), (a): independent-sample T test, (b): chi-square Test.

Table2: Laboratory investigations in studied groups

Serum Ca (mg/dL)	9.35±0.69	9.27±0.56	0.570	0.570[(a)]
Serum phosphorus (mg/dL)	3.8±0.28	3.76±0.22	0.825	0.411[(a)]
Serum UA (mg/dL)	5.39±0.69	5.09±0.66	2.149	**0.034*[(a)]**
Blood urea (mg/dL)	28.18±10.76	27.6±9.68	0.283	0.778[(a)]
Urinary ca/creatinine ratio (mg ca /mg Cr)	0.12(0.097– 0.16)	0.12(0.09– 0.16)	945.5	0.806[(b)]
Urinary UA /creatinine ratio (mg UA/mg Cr)	1.4±0.78	0.84±0.20	4.813	**<0.001*[(a)]**
ACR (mg albumin/g Cr)	24(14-32.75)	14(10- 21)	500.5	**<0.001*[(b)]**
eGFR (ml/min/1.73m^2)	100.1±7.04	103.84±6.33	2.792	**0.006*[(a)]**
CRP (mg/L)	13.04±7.22	3.6±1.35	9.077	**<0.001*[(a)]**
LDH (U/L)	511.16±132.59	179.44±40.98	16.901	**<0.001*[(a)]**
Serum ferritin (ng/mL)	925 (704.25–712.0)	64 (45-81)	0.001	**<0.001*[(b)]**
Urinary RBP /urinary Cr ratio (mg RBP/g Cr)	1.55(0.8–5.68)	0.4(0.24–0.71)	249.0	**<0.001*[(b)]**

Data are presented as mean ± SD or median (IQR). *Significant P value <0.05. Hb: haemoglobin, WBCs: white blood cells, Ca: calcium, UA: uric acid, ACR: albumin creatinine ratio, eGFR: estimation glomerular filtration rate, CRP: C-reactive protein, LDH: lactate dehydrogenase, RBP: retinol binding protein, Scr: serum creatinine, (a): Independent-Sample T Test, (b): Mann Whitney U test.

Table 3: Correlations between urinary RBP /Cr ratio and other parameters

	Urinary RBP / Cr ratio(mg/g)	
	r_s	P
Age (years)	-0.142	0.185
Sex	0.043	0.690
Splenectomy	0.022	0.882
Hb (g/dl)	-0.580	**<0.001***
WBCs (10^9/L)	0.226	**0.033***
Platelets (10^9/L)	0.125	0.243
Scr (mg/dL)	-0.01	0.920
Blood urea (mg/dL)	-0.048	0.634
Serum Ca (mg/dL)	-0.248	**0.019***
Serum phosphorus(mg/dL)	0.025	0.819
Serum UA (mg/dL)	0.195	0.067
Urinary Ca/ Cr ratio (mg Ca /mg Cr)	0.284	**0.007***
Urinary UA/ Cr ratio (mg UA/mg Cr)	0.697	**<0.001***
ACR (mg albumin/g Cr)	0.458	**<0.001***
CRP (mg/dL)	0.361	**<0.001***
LDH (U/L)	0.546	**<0.001***
Serum ferritin (ng/mL)	0.576	**<0.001***
eGFR (mL/min/1.73m^2)	-0.236	**0.026***

*Significant P value <0.05. rs: spearman correlation, Hb: hemoglobin, WBCs: white blood cells, Ca: calcium, UA: uric acid, ACR: albumin/creatinine ratio, CRP: C-reactive protein, LDH: lactate dehydrogenase, eGFR: estimated glomerular filtration rate, RBP: retinol binding protein, Scr: serum creatinine.

Table 4: Comparing the median urinary RBP / Cr ratio based on renal dysfunction factors in patients with thalassemia

		N=50		Test	P
Renal dysfunction factors					
ACR (mg albumin /g Cr)	**≤ 30**	13(26.0%)	2.29(0.46–9.66)	184.5	0.215[(b)]
	>30	37(74.0%)	1.43(0.87–5.22)		
Urinary Ca/ Cr ratio (mg ca /mg Cr)	**≤ 0.15**	36(72.0%)	1.14(0.56–2.64)	184.5	**<0.001***[(b)]
	>0.15	14(28.0%)	7.18(2.07–9.62)		
Urinary UA/ Cr ratio (mg UA/mg Cr)	**≤ 1.5**	27(54.0%)	0.92(0.5–1.29)	9.5	**<0.001***[(b)]
	>1.5	23(46.0%)	5.87(2.43–7.5)		

Data is presented as frequency (%) or median (IQR). *Significant P value <0.05. ACR: albumin/creatinine ratio, ca: calcium, UA: uric acid, RBP: retinol binding protein, Cr:Creatinine

Table 5: Binary logistic regression for prediction of renal dysfunction factors by urinary RBP/Cr ratio

	Wald	P	OR	95% C.I for OR	
				Lower	Upper
Urinary Ca/ Cr ratio (mg ca /mg Cr)					
Urinary RBP / Cr ratio	11.890	**<0.001***	1.544	1.206	1.976
Urinary UA / Cr ratio (mg UA/mg Cr)					
Urinary RBP / Cr ratio	6.626	**0.010***	4.590	1.438	14.644

*Significant P value <0.05. OR: Odds ratio, C.I: confidence interval, UA: uric acid, RBP: retinol binding protein, Cr: creatinine.