β-Thalassemia intermedia: morbidity uncovered

Musallam, K.M.S.; Taher, A.T.

Glomerular Hyperfiltration And Proteinuria In Transfusion-independent Patients With β-thalassemia Intermedia

K.M. Musallam
F.N. Ziyadeh
N.S. Mallat
S. Mallat
F. Jaber
A.A. Mohamed
S. Koussa
A.T. Taher

ABSTRACT

Background: Renal manifestations have been described in β-thalassemia major and were attributed to transfusional iron overload and chelation therapy. Patients with the milder phenotype, β-thalassemia intermedia (TI), remain largely transfusion- and iron chelation-independent while enduring a chronic hemolytic anemia and primary iron overload. Data on renal function in patients with TI are lacking. **Methods:** In this cross-sectional study of 50 TI patients, we evaluated the association of estimated glomerular filtration rate (eGFR) and urinary protein to creatinine (UPr/UCr) ratio with relevant patient, disease, and laboratory indices. **Results:** The median age of patients was 28 years (44% males). The eGFR was >90 ml/min/1.73 m^2 in all patients with a median value of 142.3 ml/min/1.73 m^2. The median UPr/UCr ratio was 213.2 mg/g. There was a negative correlation between age and eGFR, while the UPr/UCr ratio correlated positively with markers of anemia, hemolysis, and iron overload. A total of 24 (48%) patients had evidence of glomerular hyperfiltration while seven (14%) had proteinuria (UPr/UCr ratio >500 mg/g). Patients with proteinuria were characterized by elevated liver iron concentration (>7 mg Fe/g dry weight), non-transferrin-bound iron levels, and nucleated red blood cell counts. **Conclusions:** A considerable proportion of TI patients show evidence of abnormally elevated eGFR, with a

declining trend towards advancing age. The occurrence of proteinuria is associated with anemia, hemolysis, and iron toxicity.

Keywords: anemia; glomerular filtration rate; iron overload; proteinuria thalassemia; tubule cells.

INTRODUCTION

The study of renal manifestations in patients with β-thalassemia has primarily revolved around the potential nephrotoxic effects of iron chelators used to treat transfusional iron overload in β-thalassemia major (TM) patients [1]. Data in transfusion-independent patients with β-thalassemia intermedia (TI) are scarce [2-4]. Three main pathophysiologic mechanisms dominate the disease process in this latter population: chronic anemia and hypoxia, intra- and extra-vascular hemolysis, and primary iron overload due to ineffective erythropoiesis and secondary increase in intestinal iron absorption [5]. In this study we aim to investigate key renal manifestations in a cohort of transfusion-independent patients with TI, and evaluate their association with the underlying disease pathophysiology.

METHODS

This was a cross-sectional study of TI patients attending the Chronic Care Centers in Hazmieh, Lebanon. The current study utilized a completely de-identified dataset. Data were collected as part of now completed clinical studies, and which were approved by the Institutional Review Board. All patients had signed an informed consent form for participating in the original studies in accordance with the Declaration of Helsinki. An age of diagnosis beyond two years, hemoglobin values maintained between 7 and 9 g/dl without

the need for regular transfusional regimen, with or without splenomegaly, were the main criteria to define the TI phenotype on presentation [6]. After excluding patients who were eventually placed on regular transfusion regimens or iron chelation therapy for disease deterioration as well as patients receiving fetal hemoglobin inducers, 50 patients were available for analysis. Some of the patients received occasional transfusions prior to surgery, during infection or pregnancy (see Table 1). By review of genetic records, none of the patients had co-inheritance of α thalassemia [α^{+} ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) or α^{0} ($--^{Med}$ and $--^{SEA}$)] or determinants associated with increased γ chain production [*Xmn*-I +/+ genotype at position –158 of *HβG2*]. Moreover, none of the patients had hemoglobin S, C, E/β or δβ thalassemia. Medical charts were reviewed to retrieve data on demographics (age and sex), splenectomy status, receipt of any occasional transfusions, history of hepatitis B or C or HIV infection, and history of cardiovascular disease.

Laboratory studies were performed for the measurement of serum ferritin level, fetal and total hemoglobin levels, as well as platelet and nucleated red blood cell (NRBC) counts. For liver iron concentration (LIC), direct determination of iron burden was performed with R2 magnetic resonance imaging (MRI) using established methodology, calibrated to mg of iron per g of dry weight in fresh liver biopsy specimens [7]. Measurement of non-transferrin-bound iron (NTBI) was also performed as previously

described [8]. NTBI is a low-molecular-weight form of iron that is detected in conditions of iron overload when transferrin becomes fully saturated and is unable to bind excess iron. Moreover, serum creatinine (SCr) levels were obtained. We estimated glomerular filtration rate (eGFR) using the isotope dilution mass spectrometry (IDMS)-traceable Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [9]: eGFR (ml/min/1.73 m^2) = 141 X $\min(SCr/\kappa,1)^{\alpha}$ X $\max(SCr/\kappa,1)^{-1.209}$ X 0.993^{Age} X 1.018 (if female) X 1.159 (if black) [Where SCr is in mg/dl, κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1]. For children <15 years (6 patients, median height 150.5 cm), we used the IDMS-traceable Bedside Schwartz equation [10-11]: eGFR (ml/min/1.73 m^2) = 0.413 x Height in cm/SCr. Urinary protein (UPr) and urinary creatinine (UCr) levels were also obtained to calculate the UPr/UCr ratio. UPr was measured using a turbidimetric method (COBAS, Roche Diagnostics GmbH, Mannheim, Germany) with the normal range for the assay being <140 mg/l. Accordingly, the normal UPr/UCr ratio was determined as <200 mg/g.

Statistical analysis

Descriptive statistics are presented as medians (interquartile range [IQR]), and percentages. Comparisons were made using the Mann

Whitney U test for continuous variables and the Fisher's exact test for categorical variables. Bivariate correlations were evaluated using the Spearman's correlation coefficient (r_s). Logistic regression analysis was used to determine the odds ratio (95% confidence interval [CI]) of developing outcomes according to categorized study variables. Receiver operating characteristic (ROC) curve analysis was used to determine the maximum sum of specificity and sensitivity and establish thresholds that discriminate occurrence of outcomes for continuous study variables. All p-values are two-sided with the level of significance set at 0.05.

RESULTS

Patients characteristics are described in Table 1. The median age of the 50 patients in this study was 28 (IQR: 15) years (range, 8-63 years), with 22 patients (44%) being males. The median SCr level was 0.5 (IQR: 0.2) mg/dl (range, 0.2-0.9 mg/dl) and the median eGFR was 142.3 (IQR: 24.4) ml/min/1.73 m^2 (range, 90.6-301.5 ml/min/1.73 m^2) (Figure 1). None of the patients had eGFR less than 90 ml/min/1.73 m^2. The median UPr/UCr ratio was 213.2 (IQR: 225.8) mg/g (range, 93.3-1538.9 mg/g).

A total of 24 (48%) patients had evidence of glomerular hyperfiltration relying on previously described definitions in a similarly aged, non-diabetic population (>149 and >134 ml/min/1.73

m^2 for women and men, respectively [12]). There was a significant and negative correlation between age and eGFR (Table 2A and Figure 2).

A total of 30 (60%) patients had abnormal UPr/UCr ratio (≥200 mg/g), and seven (14%) patients had a UPr/UCr ratio >500 mg/g indicating proteinuria. The UPr/UCr ratio correlated positively with serum ferritin level, NTBI level, LIC, and NRBC count; yet correlated negatively with hemoglobin level (Table 2A) (Figure 3). Splenectomized patients had a higher median UPr/UCr ratio compared with nonsplenectomized patients (Table 2B). The median eGFR and UPr/UCr ratio were comparable between both sexes and in patients with a history of occasional transfusion, pulmonary hypertension, or thromboembolic disease compared with those without (Table 2B).

The median age was significantly lower in patients with glomerular hyperfiltration than in patients without glomerular hyperfiltration (p=0.002) (Table 3). Each 1-year increase in age was associated with a 0.90 (95% CI: 0.84 to 0.97) decreased odds of having glomerular hyperfiltration. The decline in eGFR with age followed the linear formula: eGFR (ml/min/1.73 m^2) = (-1.600 x Age in years) + 189.593 (see Figure 2).

Four of the seven patients with proteinuria (UPr/UCr ratio >500 mg/g) also had glomerular hyperfiltration (Figure 4). Patients with proteinuria had a higher median LIC compared with patients with normal UPr/UCr ratio (p=0.041) (Table 4) as well as those with an abnormal UPr/UCr ratio of ≥200 to 500 mg/g (p=0.047). On ROC curve analysis, a LIC threshold of >7 mg Fe/g dry weight discriminated patients who had from those who did not have proteinuria (area under the curve: 0.76, p=0.030, sensitivity 100%, specificity 51.2%). Patients with proteinuria also had higher median NTBI levels and NRBC counts compared with patients with normal UPr/UCr ratio (Table 4).

DISCUSSION

A spectrum of renal involvement has been described in some hemoglobinopathies, most typically in sickle cell disease. Sickle cell anemia is a vasoocclusive entity that has been implicated in causing tubulomedullary lesions and dysfunction, glomerulopathy with proteinuria, and progressive kidney failure that leads to end-stage renal disease (ESRD) [13-14]. Thalassemias have not been associated with well documented renal effects.

Our study demonstrates that renal abnormalities are common in transfusion-independent patients with TI who also never received iron chelation therapy. A considerable proportion of patients showed

elevated eGFR and probable evidence of glomerular hyperfiltration and some patients had glomerular permeability abnormalities or substantial renal tubular cell damage manifesting as overt proteinuria.

There was a positive correlation between UPr/UCr ratio and elevated iron overload indices, with increasing LIC as a risk factor for the occurrence of proteinuria. This finding is in agreement with studies that attributed increased urinary excretion of several markers of proximal tubular damage to iron overload in TM patients [1, 15-16]. Reversal of these tubular defects following iron chelation therapy has also been documented [16-17]. Preliminary studies show that rats subjected to chronic iron-loading develop iron deposits that are clearly evident in glomeruli, proximal tubules, and interstitium together with signs of significant glomerulosclerosis, tubular atrophy, and interstitial fibrosis [18]. Autopsy series of patients with TM have also shown hemosiderin deposits in both the terminal portion of proximal tubules and in distal tubules [19]. Iron accumulation can result in the production of reactive oxygen species and subsequent cellular injury [20-22]. The mechanism of injury is mediated by mitochondrial stress as evident from increased cytochrome c efflux, lactate deyhdrogenase release, and reduction in adenosine triphosphate [23-24]. Oxidants also increase the glomerular basement membrane damage by increasing its susceptibility to proteolytic damage and decreasing the synthesis of

glomerular proteoglycans which are essential to its integrity [25]. The UrPr/UCr ratio also correlated negatively with total hemoglobin level in our study, which is in agreement with studies showing a good correlation between the severity of anemia and markers of tubular abnormalities in TM patients [26]. Chronic anemia and hypoxia are also associated with oxidative stress, lipid peroxidation, and functional abnormalities in tubular cells [27-28].

Although most patients had high eGFR, levels decreased with advancing age. The average decline in eGFR in the patients appeared to be greater than that expected due to physiological aging in the normal adult population [29]. The causes of high GFR and the decreased levels with increasing age are unclear. Creatinine clearance and GFR are increased in children with TM [2]. Anemia may reduce systemic vascular resistance, leading to a hyperdynamic circulation that can increase the renal plasma flow and GFR [30]. This, however, may eventually lead to stretching of the glomerular capillary wall and subsequent endothelial and epithelial injury together with transudation of macromolecules into the mesangium leading to dysfunction [31]. In the long-term, these changes may cause a progressive decline in GFR. Moreover, chronic hypoxia of tubular cells with increased metabolic demand may cause apoptosis or epithelial-mesenchymal transdifferentiation leading to the development of tubulointerstitial injury and consequent glomerular sclerosis and kidney fibrosis [1, 32]. Moreover, tubular cell damage

from heavy iron-overload allows the injured cells to release cytokines and growth factors into the interstitium that can cause tubulointerstitial scarring and glomerular sclerosis, leading to further decrease in GFR [33].

The question thus arises as to whether TI patients are at increased risk for worsening proteinuria and progressive loss of GFR over a period of years, similar to the experience with sickle cell disease [13-14]. In this regard it is noteworthy that some patients with TI develop ESRD requiring dialysis. In the total cohort of 120 patients with TI registered at the Chronic Care Center in Lebanon, we encountered six patients on chronic hemodialysis, two of whom had been proteinuric when kidney biopsy showed mesangial cell proliferation and evidence for focal/segmental glomerulosclerosis (unpublished data).

How splenectomy contributes to renal abnormalities in TI patients is unclear. Splenectomy has been associated with several complications in TI patients, especially thrombotic disease [34-35] attributed to an increase in the number of hemolyzed red blood cells with thrombogenic potential [36]. A positive correlation between NRBC counts and UPr/UCr ratio was evident in our study, with an increased likelihood of developing proteinuria at higher NRBC counts. The mechanisms of renal injury due to these prothrombotic RBCs merits further evaluation, although an association between

hemolysis and renal abnormalities in patients with sickle cell anemia has been established [13, 37-38]. Moreover, it has been suggested that the intact spleen may be a reservoir of excess iron and may have a possible scavenging effect on iron free fractions including NTBI, thus protecting from iron-related end-organ damage [8, 39]. High NTBI levels were associated with the occurrence of proteinuria in our cohort.

Early detection and treatment of chronic anemia/hypoxia and iron overload in TI patients may be useful to prevent progression of kidney disease. This is in line with recent evidence suggesting the benefits of regular transfusion and iron chelation therapy in TI patients [5]. Iron chelation therapy, however, has also been linked to nonprogressive increases in SCr levels, especially with the use of the novel iron chelator defersairox [1]. It may be questionable whether this would be considered an adverse event in TI patients or rather a useful defense mechanism against the potential damage of persistent glomerular hyperfiltration. Attempts to introduce screening tools that identify which patients are at highest risk for subsequent renal damage should also be considered. The BOLD MRI, a non-invasive technique that detects changes in oxygenation level, can be considered for such purpose [40].

One limitation of our study is estimation rather than direct measurement of GFR. The currently available formulas for

estimation of GFR from SCr values in children have not been validated for high GFR values. A 12-year-old boy in this report had extremely high eGFR (~300 ml/min/1.73 m^2); when we excluded the patient from further analyses results remained the same (data not shown). In adults, however, the CKD-EPI equation used herein offers a better precision of GFR measurement at higher levels than the Modification of Diet in Renal Disease (MDRD) equation. In all cases, when we conducted the same analyses using SCr instead of eGFR (while defining glomerular hyperfiltration as a SCr value of <0.4 mg/dl), results remained essentially unchanged (data not shown).

In conclusion, renal abnormalities may be a common finding in patients with TI. Close monitoring and thorough follow-up of at-risk patients are recommended. Nonetheless, longitudinal studies are necessary to confirm whether these renal abnormalities may have long-term effects and to truly establish the deleterious effects of TI on the nephron.

Transparency Declarations: no relevant conflicts of interest to disclose. This study did not receive external funding.

Authors Contributions: Conception and design: KMM, FNZ, ATT. Data collection: FJ, AAM, SK. Statistical Analysis: KMM. Data interpretation: KMM, FNZ, NSM, SM, ATT. Manuscript

drafting: KMM and NM. Manuscript editing and review for intellectual content: FNZ, SM, ATT. Manuscript approval of submission: All authors.

REFERENCES

1. Ponticelli C, Musallam KM, Cianciulli P, Cappellini MD. Renal complications in transfusion-dependent beta thalassaemia. *Blood Rev* 2010;24:239-244.
2. Quinn CT, Johnson VL, Kim HY, Trachtenberg F, Vogiatzi MG, Kwiatkowski JL *et al.* Renal dysfunction in patients with thalassaemia. *Br J Haematol* 2011;153:111-117.
3. Smolkin V, Halevy R, Levin C, Mines M, Sakran W, Ilia K *et al.* Renal function in children with beta-thalassemia major and thalassemia intermedia. *Pediatr Nephrol* 2008;23:1847-1851.
4. Ali D, Mehran K, Moghaddam AG. Comparative evaluation of renal findings in Beta-thalassemia major and intermedia. *Saudi J Kidney Dis Transpl* 2008;19:206-209.
5. Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of beta thalassaemia intermedia. *Br J Haematol* 2011;152:512-523.
6. Camaschella C, Cappellini MD. Thalassemia intermedia. *Haematologica* 1995;80:58-68.
7. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK *et al.* Noninvasive measurement and imaging

of liver iron concentrations using proton magnetic resonance. *Blood* 2005;105:855-861.

8. Taher A, Musallam KM, El Rassi F, Duca L, Inati A, Koussa S *et al.* Levels of non-transferrin-bound iron as an index of iron overload in patients with thalassaemia intermedia. *Br J Haematol* 2009;146:569-572.

9. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604-612.

10. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA *et al.* New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009;20:629-637.

11. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol* 2009;4:1832-1843.

12. Ficociello LH, Perkins BA, Roshan B, Weinberg JM, Aschengrau A, Warram JH *et al.* Renal hyperfiltration and the development of microalbuminuria in type 1 diabetes. *Diabetes Care* 2009;32:889-893.

13. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 2010;376:2018-2031.

14. Guasch A, Navarrete J, Nass K, Zayas CF. Glomerular involvement in adults with sickle cell hemoglobinopathies: Prevalence and clinical correlates of progressive renal failure. *J Am Soc Nephrol* 2006;17:2228-2235.

15. Koliakos G, Papachristou F, Koussi A, Perifanis V, Tsatra I, Souliou E *et al.* Urine biochemical markers of early renal dysfunction are associated with iron overload in beta-thalassaemia. *Clin Lab Haematol* 2003;25:105-109.

16. Michelakakis H, Dimitriou E, Georgakis H, Karabatsos F, Fragodimitri C, Saraphidou J *et al.* Iron overload and urinary lysosomal enzyme levels in beta-thalassaemia major. *Eur J Pediatr* 1997;156:602-604.

17. Sadeghi-Bojd S, Hashemi M, Karimi M. Renal tubular function in patients with beta-thalassaemia major in Zahedan, southeast Iran. *Singapore Med J* 2008;49:410-412.

18. Zhou XJ, Laszik Z, Wang XQ, Silva FG, Vaziri ND. Association of renal injury with increased oxygen free radical activity and altered nitric oxide metabolism in chronic experimental hemosiderosis. *Lab Invest* 2000;80:1905-1914.

19. Landing BH, Gonick HC, Nadorra RL, Hyman CB, Wells TR, Villarreal-Engelhardt G *et al.* Renal lesions and clinical findings in thalassemia major and other chronic anemias with hemosiderosis. *Pediatr Pathol* 1989;9:479-500.

20. Kassab-Chekir A, Laradi S, Ferchichi S, Haj Khelil A, Feki M, Amri F *et al.* Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. *Clin Chim Acta* 2003;338:79-86.

21. King SM, Donangelo CM, Knutson MD, Walter PB, Ames BN, Viteri FE *et al.* Daily supplementation with iron increases lipid

peroxidation in young women with low iron stores. *Exp Biol Med (Maywood)* 2008;233:701-707.

22. Kokoszko A, Dabrowski J, Lewinski A, Karbownik-Lewinska M. Protective effects of GH and IGF-I against iron-induced lipid peroxidation in vivo. *Exp Toxicol Pathol* 2008;60:453-458.

23. Zager RA, Johnson AC, Hanson SY. Parenteral iron nephrotoxicity: potential mechanisms and consequences. *Kidney Int* 2004;66:144-156.

24. Zager RA, Johnson AC, Hanson SY. Proximal tubular cytochrome c efflux: determinant, and potential marker, of mitochondrial injury. *Kidney Int* 2004;65:2123-2134.

25. Shah SV. Oxidants and iron in chronic kidney disease. *Kidney Int Suppl* 2004:S50-55.

26. Sumboonnanonda A, Malasit P, Tanphaichitr VS, Ong-ajyooth S, Sunthornchart S, Pattanakitsakul S *et al.* Renal tubular function in beta-thalassemia. *Pediatr Nephrol* 1998;12:280-283.

27. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. *Curr Mol Med* 2008;8:609-619.

28. Nagababu E, Gulyani S, Earley CJ, Cutler RG, Mattson MP, Rifkind JM. Iron-deficiency anaemia enhances red blood cell oxidative stress. *Free Radic Res* 2008;42:824-829.

29. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc* 1985;33:278-285.

30. Davis LE, Hohimer AR. Hemodynamics and organ blood flow in fetal sheep subjected to chronic anemia. *Am J Physiol* 1991;261:R1542-1548.

31. Lafferty HM, Anderson S, Brenner BM. Anemia: a potent modulator of renal hemodynamics in models of progressive renal disease. *Am J Kidney Dis* 1991;17:2-7.

32. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol* 2006;17:17-25.

33. Alfrey AC. Role of iron and oxygen radicals in the progression of chronic renal failure. *Am J Kidney Dis* 1994;23:183-187.

34. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S *et al.* Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. *Blood* 2010;115:1886-1892.

35. Taher AT, Musallam KM, Karimi M, El-Beshlawy A, Belhoul K, Daar S *et al.* Splenectomy and thrombosis: the case of thalassemia intermedia. *J Thromb Haemost* 2010;8:2152-2158.

36. Cappellini MD, Motta I, Musallam KM, Taher AT. Redefining thalassemia as a hypercoagulable state. *Ann N Y Acad Sci* 2010;1202:231-236.

37. Haymann JP, Stankovic K, Levy P, Avellino V, Tharaux PL, Letavernier E *et al.* Glomerular hyperfiltration in adult sickle cell

anemia: a frequent hemolysis associated feature. *Clin J Am Soc Nephrol* 2010;5:756-761.

38. Schein A, Enriquez C, Coates TD, Wood JC. Magnetic resonance detection of kidney iron deposition in sickle cell disease: a marker of chronic hemolysis. *J Magn Reson Imaging* 2008;28:698-704.

39. Tavazzi D, Duca L, Graziadei G, Comino A, Fiorelli G, Cappellini MD. Membrane-bound iron contributes to oxidative damage of beta-thalassaemia intermedia erythrocytes. *Br J Haematol* 2001;112:48-50.

40. Inoue T, Kozawa E, Okada H, Inukai K, Watanabe S, Kikuta T *et al.* Noninvasive evaluation of kidney hypoxia and fibrosis using magnetic resonance imaging. *J Am Soc Nephrol* 2011;22:1429-1434.

Table 1. Characteristics of the 50 patients.

Parameter	**Value**
Age in years, median (IQR: range)	28 (15: 8-63)
Male, n (%)	22 (44)
Splenectomized, n (%)	39 (78)
Occasional transfusions, n (%)*	15 (30)
Hepatitis B or C or HIV, n (%)	0 (0)
Systemic hypertension, n (%)	0 (0)
Pulmonary hypertension, n (%)	28 (56)
Thromboembolic disease, n (%)	14 (28)
Heart failure, n (%)	0 (0)
Diabetes mellitus, n (%)	0 (0)
Laboratory measurements	
Hemoglobin level in g/dl, median (IQR: range)	8.2 (2.4: 4.9-13.1)
Fetal hemoglobin level in %, median (IQR: range)	33.8 (58.9: 8.7-100.0)
Platelet count $x10^9$/l, median (IQR: range)	762.5 (655.0: 135.0-1733.0)
NRBC count x $10^3/mm^3$, median (IQR: range)	313.0 (441.8: 0.0-1622.0)
Serum ferritin level in µg/l, median (IQR: range)	835.5 (1022.1: 18.0-3157.5)
LIC in mg Fe/g dry weight, median (IQR: range)	8.1 (10.6: 0.6-32.1)
NTBI level in µmol/l, median (IQR: range)	3.2 (5.5: -3.7-10)

*Prior to surgery, during infection or pregnancy.
NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Table 2. Bivariate correlations between study parameters, eGFR, and UPr/UCr ratio.

(A)

	Age (years)	Hemoglobin level (g/dl)	Fetal hemoglobin level (%)	Platelet count ($x10^9$/l)	NRBC count ($x10^3$/l)	Serum ferritin level (µg/l)	LIC (mg Fe/g dry weight)	NTBI (µmol/l)
eGFR (ml/min/1.73 m^2)								
Spearman's correlation coefficient	-0.705	0.082	-0.040	0.217	0.199	-0.260	-0.100	0.132
p-value	<0.001	0.571	0.787	0.131	0.166	0.069	0.491	0.361
UPr/UCr ratio (mg/g)								
Spearman's correlation coefficient	0.073	-0.252	0.190	0.241	0.237	0.282	0.357	0.444
p-value	0.614	0.008	0.195	0.092	0.048	0.048	0.011	0.001

eGFR = estimated glomerular filtration rate; UPr/UCr = urinary protein/creatinine ratio; NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron.

(B)

	Sex		Splenectomized		Occasionally transfused		PHT		VTE	
	Male	**Female**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
eGFR (ml/min/1.73 m^2)										
Median	142.4	142.0	142.3	134.5	140.8	142.3	141.0	150.5	133.5	145.4
(IQR)	(28.9)	(23.0)	(23.0)	(32.9)	(32.0)	(27.5)	(20.5)	(28.9)	(28.9)	(27.9)
p-value	0.769		0.393		0.597		0.287		0.054	
UPr/UCr ratio (mg/g)										
Median	208.3	223.4	240.2	158.8	240.2	211.6	189.6	240.2	220.8	213.2
(IQR)	(277.9)	(199.2)	(276.6)	(78.0)	(635.4)	(180.9)	(223.103)	(240.0)	(319.2)	(199.7)
p-value	0.953		0.011		0.204		0.113		0.940	

eGFR = estimated glomerular filtration rate; UPr/UCr = urinary protein/creatinine ratio; PHT = pulmonary hypertension; VTE = venous thromboembolism; IQR = interquartile range.

Table 3. Comparison of study parameters between patients with and without glomerular hyperfiltration.

Parameter	No glomerular hyperfiltration n = 26	Glomerular hyperfiltration* n = 24	p-value
Age in years, median (IQR)	31.5 (13.8)	18.5 (14.8)	0.002
Male, n (%)	9 (34.6)	13 (54.2)	0.254
Splenectomized, n (%)	20 (76.9)	19 (79.2)	1.000
Occasional transfusions, n (%)	9 (34.6)	6 (25)	0.545
Pulmonary hypertension, n (%)	16 (61.5)	12 (50)	0.569
Thromboembolic disease, n (%)	12 (46.2)	2 (8.3)	0.051
Hemoglobin level in g/dl, median (IQR)	8.3 (2.4)	8.2 (2.7)	0.634
Fetal hemoglobin level in %, median (IQR)	37 (57)	33.1 (59.2)	0.710
Platelet count $x10^9$/l, median (IQR)	722 (539)	872.5 (658.8)	0.281
NRBC count x $10^3/mm^3$, median (IQR)	309.0 (409.5)	313.0 (501.5)	0.690
Serum ferritin level in µg/l, median (IQR)	1145 (1155)	659.5 (684.5)	0.071
LIC in mg Fe/g dry weight, median (IQR)	9.9 (13.5)	7 (9)	0.332
NTBI level in µmol/l, median (IQR)	3.0 (6.4)	3.6 (6.0)	0.698

*eGFR >149 and >134 ml/min/1.73 m^2 for women and men, respectively.

NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Table 4. Comparison of study parameters between patients with and without proteinuria.

Parameter	UPr/UCr <200 mg/g n = 20	UPr/UCr ≥200-500 mg/g n = 23	p-value*	UPr/UCr >500 mg/g n = 7	p-value*
Age in years, median (IQR)	25.5 (14.8)	29 (16)	0.435	25 (12)	0.912
Male, n (%)	8 (40)	11 (47.8)	0.760	3 (42.9)	1.000
Splenectomized, n (%)	12 (60)	20 (87)	0.078	7 (100)	0.068
Occasional transfusions, n (%)	5 (25)	5 (21.7)	1.000	5 (71.4)	0.065
Pulmonary hypertension, n (%)	15 (75)	10 (43.5)	0.063	3 (42.9)	0.175
Thromboembolic disease, n (%)	6 (30)	6 (26.1)	1.000	2 (28.6)	1.000
Hemoglobin level in g/dl, median (IQR)	8.6 (2.0)	8.1 (2.3)	0.262	7.7 (1.8)	0.361
Fetal hemoglobin level in %, median (IQR)	28.4 (36.6)	34.5 (57.0)	0.225	48 (50.1)	0.167
Platelet count $x10^9$/l, median (IQR)	690.5 (699.5)	851 (513)	0.197	856 (509.5)	0.086
NRBC count x $10^3/mm^3$, median (IQR)	165.5 (390.0)	334 (467)	0.071	525 (285.5)	0.027
Serum ferritin level in µg/l, median (IQR)	760.5 (1088.8)	863.5 (946.0)	0.450	1590 (1046.5)	0.121
LIC in mg Fe/g dry weight, median (IQR)	4.7 (9.4)	8 (8)	0.205	14.1 (5.4)	0.041
NTBI level in µmol/l, median (IQR)	0.7 (6.6)	4.0 (4.1)	0.005	4.9 (2.8)	0.013

*Compared with the UPr/UCr <200 mg/g group.

UPr/UCr = urinary protein/creatinine ratio; NRBC = nucleated red blood cell; LIC = liver iron concentration; NTBI = non-transferrin-bound iron; IQR = interquartile range.

Figure 1. Scatter plot of serum creatinine (SCr) and estimated glomerular filtration rate (eGFR).

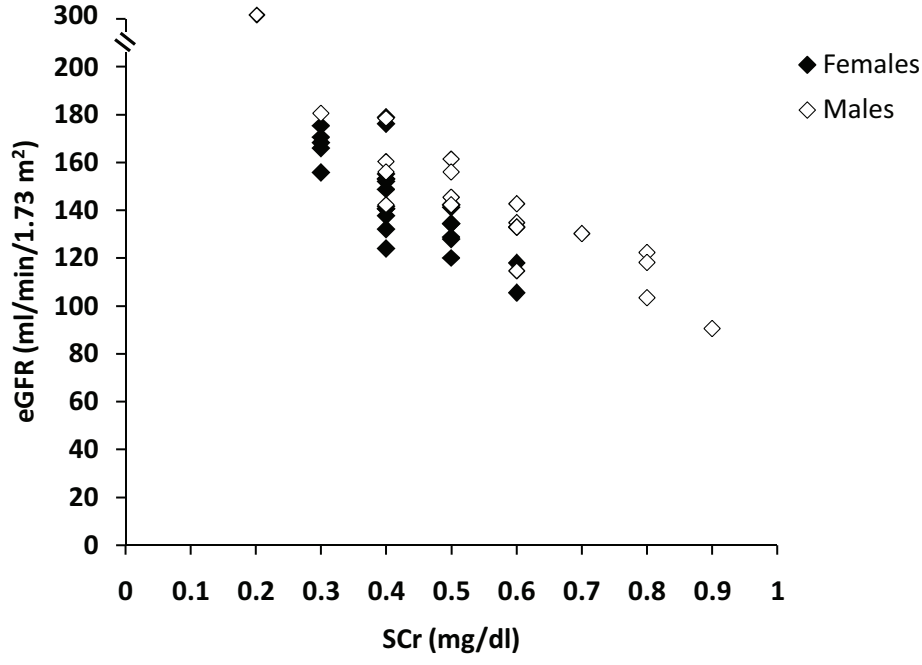

Figure 2. Linear regression analysis of age and estimated glomerular filtration rate (eGFR).

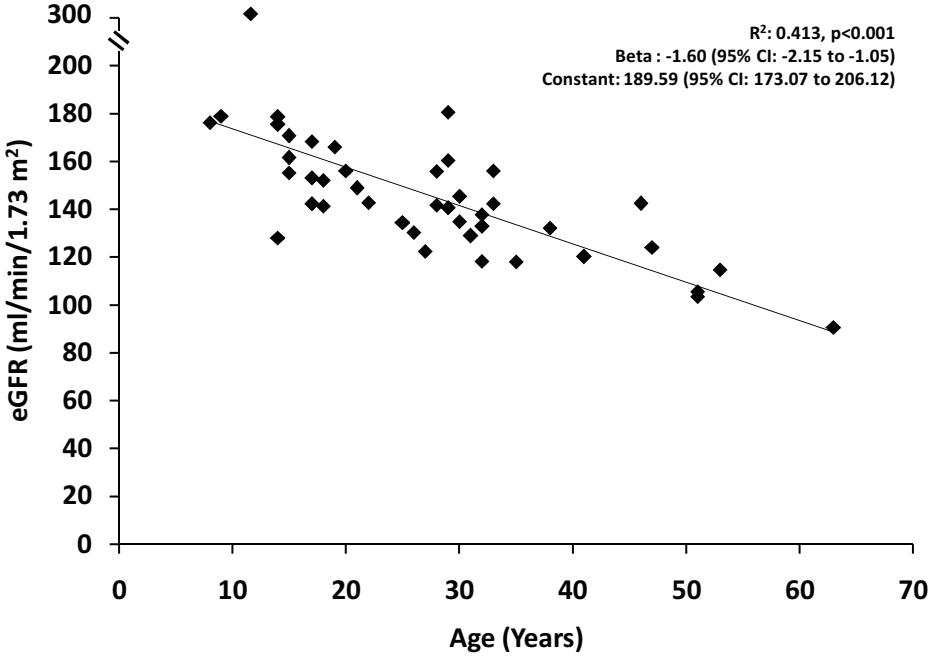

Figure 3. Correlation between urinary protein/urinary creatinine (UPr/UCr) ratio and (A) liver iron concentration (LIC), (B) non-transferrin-bound iron (NTBI), (C) nucleated red blood cell (NRBC) count, and (D) total hemoglobin (Hb) level.

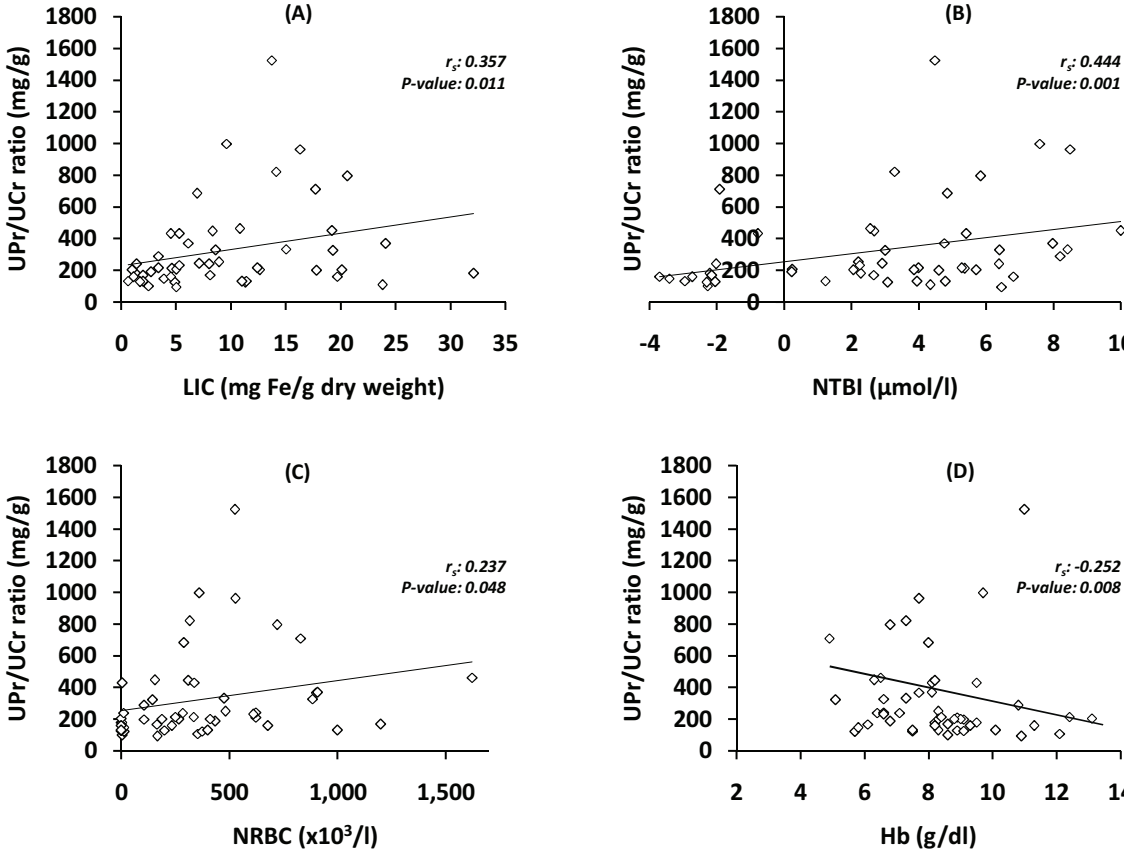

Figure 4. Scatter plot of estimated glomerular filtration rate (eGFR) and urinary protein/urinary creatinine (UPr/UCr) ratio. Hollow circles indicate patients with glomerular hyperfiltration which was defined as >149 ml/min/1.73 m2 for females and >134 ml/min/1.73 m2 for males.