Journal of Hematology & Thromboembolic Diseases

Gokce et al., J Hematol Thrombo Dis 2014, 2:6
http://dx.doi.org/10.4172/2329-8790.1000173

Research Article Open Access

Insidious Renal Damage in Patients with Thalassemia Major: Is it More Serious than Appreciated?

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Rec date: Oct 17, 2014, **Acc date:** Nov 22, 2014, **Pub date:** Nov 28, 2014

Abstract

Aim: To investigate early renal injury in thalassemia major patients by using novel serum markers and demonstrate the factors leading to renal injury.

Material and Methods: Seventy-one thalassemia major patients (37 males) who were on regular transfusion and chelation programme with deferasirox have been enrolled in this study. Thirty-five healthy children of the same age served as a control group. Serum urea-creatinine, electrolytes, cystatin C and glomerular filtration rate were all noted in our cohort and compared with the control group. Proteinuria was also investigated by calculating UPr/UCr ratio.

Results: The median serum creatinine level was 0.4 mg/dL (range; 0.2-0.76 mg/dL) and the median glomerular filtration rate level was 192 mL/min/1.73 m^2 (range; 106-308 mL/min/1.73 m^2) in patients. More than half of the patients had glomerular hyperfiltration (n=48, 67.6%). Hyperfiltration was prominent in thalassemia group (66.2% vs. 19.4%) (p=0.0001). Mean serum cystatin C levels were found to be statistically elevated in patient group comparing with controls (p=0.0001). Serum cystatin C level was above >1 mg/L in 46 patients (64.7%) whereas only three in controls (p=0.0001). Sex, age, hemoglobin level and cardiac T2* MRI results were not related to the elevated cystatin C levels. On the other hand, liver iron burden and serum ferritin levels were noted to be statistically correlated to elevated cystatin C level (p=0.024 and p=0.04). Deferasirox dose below 25 mg/kg/day was also found in relation with elevated cystatin C levels.

Conclusion: Renal injury is multifactorial, insidious but progressive in thalassemia patients. High index of suspicion and routine use of novel markers are required for detection of renal injury during the follow up of thalassemia patients.

Keywords: Cystatin C; Deferasirox; Renal İnjury; Thalassemia Major

Introduction

The survival of the patients with β-thalassemia major has improved recently due to both effective oral chelating agents and novel imaging modalities pointing visceral iron burden earlier. Extending patients' survival, organ dysfunctions have been significant issue for hematologists since 2000s. Little attention has been paid to renal injury in patients with thalassemia major (TM) although a large number of studies have been performed on various organ complications [1-4].

Regarding renal injury, several factors might play role in patients with TM. Chronic hypoxia, ongoing hemolysis, iron overload owing to transfusions and possible nephrotoxicity of chelators are well-known reasons of the deterioration in renal functions [5].

In the present study, we aimed both to evaluate renal functions of our TM patients and evaluate the factors leading to renal injury. We also thought that a better understanding and early awareness of the renal dysfunction in TM patients could enable the development of novel strategies to prevent progression of the disease.

Material and Methods

Seventy-one TM patients (37 males) who were on regular transfusion and chelation programme with deferasirox (DFX) have been enrolled in this study. Demographic data of the patients including age, sex, duration and volume of transfusion as well as the dose of DFX were all recorded retrospectively. Regarding the dosage of DFX, we grouped the patients as Group 1 who were on DFX therapy with a dosage below 25 mg/kg/day (20-25 mg/kg/day). On the other hand; the patients who were on a dose above 25 mg/kg/day (25-40 mg/kg/day) constitued Group 2. Thirty-five healthy children of the same age and sex served as a control group.

An overnight fasting blood was obtained for hematological and biochemical tests including hemoglobin, ferritin, cystatin C (Cys-C) and serum urea-creatinine and electrolytes. Serum Cys-C was analyzed by rate nephelometry (N Latex Cystatin C, N Protein Standart UY, Dade-Behring). Normal values are between 0.53 - 0.95 mg/L, according to manufacturer.

A timed urine excretion of protein can be estimated by the ratio of urine protein to creatinine concentrations in a first morning voided specimen. Ratios (mg/mg)<0.5 in children <2 years and <0.2 in older

children are normal. A ratio >2 suggests nephrotic range of proteinuria [6].

Estimated glomerular filtration rate (eGFR) was calculated by Schwartz formula for children under the age of 18 years as follow: (height x k)/Cr in which k 0.44 for children under 2 years and 0.55 for children over 2 years [7]. In young adults, eGFR was calculated by the equation proposed by the Modification of Diet in Renal Disease Study Group [8]. The mean GFR level for the patients between 2 years and adolescence was accepted as 127 mL/min/1.73 m^2 whereas the levels above 165 mL/min/1.73 m^2 were thought as hyperfiltration [9]. Cys-C based eGFR was calculated by using Bökenkamp equation which is [GFR (mL/min/1.73 m^2) = 137/serum cystatin C-20.4] [10,11]. Renal dysfunction was defined as eGFR less than < 90 ml/min/1.73 m^2 and/or abnormally elevated serum Cys-C.

The iron overload was analyzed for each patient based on median ferritin level of the last 6 months together with liver and heart T2* MR imaging analyses. The patients were classified as high risk in terms of cardiac complications if their $T2^*$ MRI values were <10 ms, medium risk if they were between 10-20 ms, and low risk if they were > 20 ms [12]. The liver iron burden (LIC) was determined by $R2^*$ MRI using well-known methodologies, calibrated to mg/g iron by dry weight fresh liver biopsy specimens [13].

The written informed consent was taken from all participants or their legal caregivers. The local ethical committe approved the study. All data were analyzed using the NCSS 2007 Statistical Software statistical package.

Results

The median age of the 71 patients in this study was 12 years (range; 4 - 27 years), with 37 males (52.1%). The median serum creatinine level was 0.4 mg/dL (range; 0.2-0.76 mg/dL) and the median GFR level was 192 mL/min/1.73 m^2 (range; 106 - 308 mL/min/1.73 m^2). Fortunately, none of our patients had an GFR level below <90 mL/min/1.73 m^2. On the other hand, more than half of the patients had glomerular hyperfiltration (n=48, 67.6%).

Estimated GFR levels were statistically higher in patients than control group (p=0.0001). Hyperfiltration (eGFR >165 mL/min/1.73 m^2) was prominent in TM group (66.2% vs 19.4%)(p=0.0001). On the other hand, Cys-C based GFR levels were lower in patient group (p=0.0001). However, there was no correlation between GFR levels and age, sex, DFX dosage, ferritin and cardiac iron level (p >0.05). But; liver iron burden was found to be statistically higher in the patients with elevated GFR levels (p=0.02).

Mean serum Cys-C levels were found to be statistically elevated in TM patients comparing with controls (p=0.0001). Serum Cys-C level was above >1 mg/L in 46 patients (64.7%) whereas only three in controls (p=0.0001). Sex, age, hemoglobin level and cardiac $T2^*$ MRI results were not related to the elevated Cys-C levels. Liver iron burden (>7 mg/g iron by dry weight liver) and serum ferritin levels (> 1000 ng/mL) were noted to be statistically correlated to elevated Cys-C level (p=0.024 and p=0.04). DFX dose below 25 mg/kg/day was also found in relation with elevated Cys-C levels. Cys-C based GFR levels were statistically lower in the patients with Cys-C level above 1 mg/mL (p=0.0001).

Although no overt proteinuria was detected in our patients, 34 (47.8%) patients had an abnormal UPr/UCr ratio (>200 mg/g), and 7 (9.8%) had a UPr/UCr ratio above > 500 mg/g indicating proteinuria. It was noted that these seven patients had mild proteinuria (300-1000 mg/24 hours). Five of them had also glomerular hyperfiltration.

Discussion

TM-associated nephropathy has recently been a growing matter of concern because renal failure starts to affect most aging TM patients. Renal injury once begins, the pattern of deterioration is insidious but progressive at the same time. The pathophysiology of renal injury in TM patients seems to be multifactorial, attributed to various factors such as chronic anemia, oxidative stress due to ongoing hemolysis, excessive free iron, renal iron deposition and chelating agents [5,14]. Early awareness and intervention would probably decrease the morbidity and mortality.

Regarding pathophysiology, glomerular hyperfiltration, if untreated, can lead to streching of the glomerular capillary wall due to increase in glomerular pressure, resulting in endothelial and epithelial injury together with transudation of macromolecules into mesangium. Renin-angiotensin-aldosteron system seems to contribute to the intrarenal pressure [15]. This might preceede glomerulosclerosis and progressive tubulo-interstitial injury. Glomerular hyperfiltration in patients with TM has been underlined in several studies [16,17]. A previous cross-sectional study demonstrated increased glomerular filtration rate and impaired renal function in 20.8% and 7.8% of the TM patients, respectively [18]. In our study, glomerular hyperfiltration was noted in 47 of the patients who have a median pretransfusion hemoglobin level of 8.6 g/dL (range; 7.6-10.5 g/dL). Although it was not statistically important, this result underlines the importance of hypertransfusion approach in prevention of organ damage besides its role in inhibition of extramedullary hematopoiesis. Chronic anemia is thought to cause a decrease in systemic vascular resistance and subsequent increase in renal plasma flow which might play a role in glomerular hyperfiltration, triggering the renal injury [19].

It is well-known that serum creatinine is alone an unreliable indicator of renal functions because muscle mass, protein intake and hepatic disease affects its serum levels. Cys-C is an endogenous, non-glycosylated basic protease inhibitor which has been an ideal marker of renal functions. Its production is not affected by age, gender and body mass index and correlates better than creatinine with measured GFR [20]. On the other hand, Papassotiriou et al have reported that Cys-C is a poor biomarker of renal function and raised in up to 60% of the patients taking DFX [21]. On the contrary, elevated Cys-C levels were found to be related with lower DFX doses in our study. Besides among patients with hyperfiltration, 31 (64.5%) had elevated levels of Cys-C. That's why elevated Cys-C levels should be thought as an early sign of renal injury in TM patients like in patients with diabetes mellitus type 1 [22]. Moderate to severe liver iron load was also found to be associated with both elevated Cys-C levels and glomerular hyperfiltration in our group. The same relation was not shown with cardiac iron burden. This finding might point a speculation that renal iron deposition begins with or just after hepatic iron deposition and before cardiac deposition, but still it should be kept in mind that several factors may play roles in renal side effects besides iron overload.

Fortunately, no nephrotic proteinuria was detected in our group, probably due to relatively younger age of our cohort comparing with the previous studies [23]. Nevertheless, mild proteinuria was observed in 7 of our patients, 5 of them had also glomerular hyperfiltration.

On the other hand, it is known that most commonly used oral chelator, DFX, has nephrotoxic side effects. Acute kidney injury has been reported in the post-marketing surveillance of DFX [24,25]. Hyperchloraemic metabolic acidosis induced by DFX – that resolved after withdrawing – has also been reported previously [26]. In a multicentre randomized phase 3 trial, mild, dose dependent increases in serum creatinine were observed in 38% of patients receiving DFX at doses of 20-30 mg/kg/day. These increases were usually within normal range, and they never exceeded twice the upper limit of normal [27]. It was also reported that decreases in GFR levels in pediatric TM patients taking DFX with a dosage 24.8 ± 9.6 mg/kg per day [28]. In our cohort, all the patients were on DFX therapy. We could not demonstrate a relation between DFX dose and GFR level. But, lower DFX doses (<25 mg/kg/day) were found to be statistically related to elevated Cys-C levels (p=0.003). This might be explained by relative depletion of iron rather than nephrotoxic effect of DFX. It is well-known that iron is involved in a large number of processes which are critical for cellular energy production via mitochondrial electron transport. So, its depletion can cause damage in renal tubular and glomerular cells, yielding renal failure [29,30]. A routine renal assessment before and during DFX therapy for all TM patients and special attention and closer monitoring are required for TM patients with lower iron burden.

Current evidence supports the benefit of renin-angiotensin-aldosteron system inhibition even in the advanced phases of renal failure. In a meta-analysis of 1860 non-diabetic patients, it was shown that angiotensin-converting enzyme (ACE) inhibition significantly reduced risk of creatinine doubling or kidney failure [31]. In patients with transfusion dependent hemoglobinopathies, it has also been shown that ACE inhibition decreased the proteinuria [32]. On the other hand, Karvounis HI et al showed that ACE inhibitors also improved the left ventricular systolic and diastolic functions in TM patients [33]. Using ACE inhibition, TM patients may provide benefit in terms of both renal and cardiac functions at the same time.

In conclusion, we thought that serum Cys-C level could be an earlier parameter than creatinine pointing renal damage and associated with glomerular hyperfiltration. Besides, liver iron deposition should alert us about renal injury earlier. The early identification of patients at high risk of developing renal failure is of paramount importance, because it might provide specific approaches aiming to decrease or delay risk of end-stage renal failure. On the other hand, increased attention to the kidneys in TM patients will indeed improve long-term health outcomes by reducing both kidney and cardiovascular disease.

Acknowledgement

The authors declared that there is no conflict of interest.

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