KnE Life Sciences
ICO-HELICS
The 1st International Conference on Health, Technology and Life Sciences
Volume 2019

Conference Paper

Hyperferritinemia and Other Factors Related to Glomerular Injury in Beta-Thalassemia Major

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Abstract

Beta-thalassemia major (BTM) is a hereditary hemoglobinopathy, characterized by anemia, increased free radicals and iron overload which can lead to hyperferritinemia, glomerular injury, and kidney dysfunction. This research aimed to analyze the association between hyperferritinemia and urinary podocalyxin (uPCX) as the indicator of glomerular injury in BTM. This retrospective cohort study was conducted on 60 BTM patients aged ≤18 years who came to the pediatrics unit of Dr. Moewardi Hospital Surakarta from April to May 2017. The data were analyzed to determine relative risk and 95%CI for each variable, followed by multivariate analysis with logistic regression. The prevalence of elevated uPCX in BTM patients in this research was 50%. The results showed an association of hyperferritinemia (RR2.51, 95%CI 1.28-4.93, p=0.002), the degree of anemia(RR2.00, 95%CI 1.14-3.52, p=0.01), the number of transfusion (RR 1.75, 95%CI 1.09-2.82, p=0.028), the duration of transfusion (RR 1.83, 95%CI 0.98-3.42, p=0.035), the duration of illness (RR 1.60, 95%CI 0.95-2.72, p=0.071) and the duration of iron chelation therapy (RR 1.58,95%CI 0.79-3.17, p=0.152) with glomerular injury. The multivariate analysis showed that hyperferritinemia and degree of anemia remained significantly associated with glomerular injury (RR 5.46; 95%CI 1.30-23.01; p=0.021 and RR 4.13; 95%CI 1.15-14.85; p=0.030). This study demonstrates a statistically significant association between hyperferritinemia, the degree of anemia, the number of transfusion and the duration of transfusion with kidney injury in BTM patients. Routine monitoring of renal function in BTM is recommended.

Keywords: Beta-thalassemia major, hyperferritinemia, urinary podocalyxin, glomerular injury

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Received: 23 February 2019
Accepted: 6 March 2019
Published: 25 March 2019

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICO-HELICS Conference Committee.

1. Introduction

Beta thalassemia major (BTM) is a genetic disorder characterized by absence (β^0) or decrease (β^+) of β globin chain synthesis of hemoglobin [1]. Thalassemia is the most common genetic disorder, it is estimated that 60000 babies are born with thalassemia every year. In 2001, WHO reported that 7% of the worldwide population was thalassemia

carrier, with the highest incidence was 40% in Asia and 55 million people were South East Asians. In Indonesia, 3-8% people are thalassemia carrier, and in some areas, it reached 10%. It is estimated that annually 3000 newborns are thalassemia carriers [5].

There has not been any medicine that can cure BTM until now. Blood transfusion every 2-4 weeks is the only therapy for anemia in thalassemia patients. This continuous transfusion can result in iron overload in many organs, such as heart, liver, kidney, endocrine organs and others. Organ dysfunction is seen in children over 5 years old receiving transfusion. The accumulation of free iron (*ferrous iron*/Fe^{2+}) can come across Fenton reaction which formed free radicals leading to oxidative stress which will oxidize lipid membrane of the cells (lipid peroxidation) and nucleic acid modification, caused cell death, tissue damage and eventually organ damage [6–8].

The prevalence of kidney disease in BTM patients receiving ≥ 6 units of a *packed red cell* (PRC) in Indonesia is 78.6% [9]. It is reported that albuminuria found in 56% BTM patients [10]. Non-progressive increase of creatinine occurred in 10% BTM patients receiving deferasirox [11].

Albuminuria, defined as urinary albumin to creatinine ratio (UACR), expresses an increased of glomerular endothelial permeability. It is also a general marker of endothelial dysfunction [12, 13]. Urinary albumin to creatinine ratio has a limitation, as it is a repeated examination. This test also influenced by sex, age, urinary tract infection, high protein diet and acute fever [14, 15]. Podocyturia, caused by glomerular podocyte damage, can be assessed using one of the specific markers of podocyte, called podocalyxin. Podocyturia is a subclinical marker that can be detected prior to proteinuria [16]. This is the first study using urinary podocalyxin (uPCX) as a marker for glomerular injury in BTM.

This study aimed to assess the association between hyperferritinemia and other factors affecting the glomerular injury in BTM patients, which is characterized by increased urinary podocalyxin (uPCX) due to oxidative stress induced by hyperferritinemia.

2. Methods

This retrospective cohort study was carried out in the Pediatric Department and Clinical Laboratory Department of Moewardi Hospital at Surakarta, Central Java, Indonesia between April and May 2017. The study participants were 60 patients aged ≤18 years old, who were clinically diagnosed with BTM receiving routine transfusion for more than 1 year or >10 units of blood transfusion and the parents agreed their children be enrolled in the study by signing informed consent. The exclusion criteria were the history of

liver and kidney disease other than due to BTM natural clinical course or BTM therapy, infection or acute inflammation and urinary tract infection (UTI).

History taking and review of medical records were performed before blood sampling to obtain patient identity, duration of illness, history of transfusion and chelation therapy. Laboratory data were taken from medical records to find out the history of ferritin level, complete blood count, creatinine and liver transaminases during a previous year before this study.

Urine containers were given to the parents to be filled in the next morning. 10 mL of the urine was taken as a sample. The first 5 ml was used for routine analysis (automated urinalyzer Sysmex UX 2000) and urine creatinine (automated chemistry analyzer Advia-1800). The rest 5 mL was then centrifuged at 1000 g for 15 minutes. The supernatant was taken into aliquot and stored at -80˚C until analysis for uPCX by ELISA (enzyme-linked immunosorbent assay) using human PCX ELISA kit from E-Labscience USA. The result of uPCX was reported as uPCX to urine creatinine ratio.

Ethical clearance was obtained from the biomedical research ethics committee of the Medical Faculty of Universitas Sebelas Maret and Moewardi Hospital at Surakarta. Informed consent was obtained from all parents subjects.

Data were tested for normal distribution using the Kolmogorov–Smirnov. Participants' characteristics were presented using means ± SD or median and min-max for continuous variables and frequencies for categorical variables. Variables normally distributed were compared by independent t-test, variables not normally distributed were compared using Chi-Square and Fisher Exact-test. We applied bivariate and multivariate logistic regression analysis to estimate the effects of hyperferritinemia and other variables on glomerular injury in BTM. The analysis was performed by SPSS statistical software package program version 23, p-value < 0.05 was considered significant.

3. Result

The subjects of this study were comprised of 34 boys (56.7%) and 26 girls (43.3%), the mean age was 10.73±4,22 years old and the average age of receiving transfusion was 84.37±47.97 months. Pretransfusion hemoglobin level was 7.92±0.68mg/dL. All subjects have eGFR of more than 110 mL/min/1.73m^2.

Thirty-four subjects (56.7%) had a ferritin level of ≥2500 ng/mL in one year prior to the study. Median [minimum–maximum] of the ferritin level, the duration of illness, the amount of transfusion in the previous year, the duration of chelating therapy and

uPCX level were 2257.10 (713.40-8988.20) ng/mL; 69.5 (11.0-211.0) months; 24.0 (12.0-42.0) units/year; 48.0 (0.0-198.0) months and 123.0 (5.76-3019.13) ng/mmol urine creatinine, respectively. Based on the uPCX level (≥126 ng/mmol urine creatinine and <126 ng/mmol urine creatinine), the subjects were categorized into two groups, i.e with and without glomerular injury, each of them comprised of 30 subjects (50%).

Comparison between groups with and without glomerular injury showed significant difference in frequency of hyperferritinemia episode in previous year, mean of serum ferritin, duration of transfusion, number of transfusion, duration of chelation therapy, and an average of hemoglobin level prior to transfusion (p=0.002; p=0.000; p=0.013; p=0.004; p=0.023 and p=0.002, respectively), age and duration of illness did not differ significantly between two groups (p=0.430 and p=0123, respectively). Demographic and biochemistry characteristics of the subjects have been summarized in Table 1.

Bivariate analysis between hyperferritinemia and other factors affecting glomerular injury were presented in Table 2. Hyperferritinemia (serum ferritin ≥2500 ng/mL) was a risk factor of glomerular injury in BTM patients with a relative risk (RR) of 2.51 (95% CI 1.28-4.93; p=0.002).

Relative risk and 95%CI of other variables affecting glomerular injury such as severity of anemia, amount of transfusion per year, duration of transfusion, duration of illness and duration of chelation therapy were 2.00 (95%CI 1.14-3.52; p= 0.01); 1.75 (95%CI: 1.09-2.82; p= 0.028); 1.83 (95%CI: 0.98-3.42; p= 0.035); 1.60 (95%CI: 0.95-2.72; p= 0.071) and 1.58 (95%CI: 0.9-3.17; p= 0.152, respectively).

Multivariate analysis was demonstrated with adjustment on the severity of anemia (Table 3, model 1), both variables showed significant association with a glomerular injury with RR of 5.68 (95% CI 1.72–18.71; p=0.004) and RR of 4.00 (95% CI 1.24–12.93; p=0.021), respectively. With adjustment on duration of transfusion (model 2), amount of transfusion and duration of transfusion (model 3), amount of transfusion, duration of transfusion and duration of illness (model 4), amount of transfusion, duration of transfusion, duration of illness and duration of chelation (model 5), hyperferritinemia and severity of anemia still demonstrated significant association with glomerular injury. The strength of association between hyperferritinemia and severity of anemia with a glomerular injury after adjustment with other variables were RR 5.46 (95% CI: 1.30–23.01; p=0.021) and 4.13 (95% CI: 1.15–14.85; p=0.030), respectively. Multivariate analysis between hyperferritinemia and other factors affecting glomerular injury were presented in Table 3.

Table 1: Demographic and biochemistry characteristics of the subjects.

	Total (n=60)	without glomerular injury/ (n=30)	with glomerular injury / (n=30)	p
Age (years)	10.73 ± 4.22[a]	10.29 ± 4.24[a]	11.16 ± 4.22[a]	0.43
Sex (%)[c]				0.602
Boys	26 (43.3)	14 (46.67)	12 (40.00)	
Girls	34 (56.7)	16 (53.33)	18 (60.00)	
Chelating agent (%)[c]				0.056
Deferoxamine	2 (3.3)	0 (0)	2 (6.67)	
Deferiprone	49 (81.7)	28 (93.33)	21 (70.00)	
Deferasirox	9 (15)	2 (6.67)	7 (23.33)	
Splenectomy (%)[c]				1
Yes	5 (8.3)	3 (10)	2 (6.67)	
No	55 (91.7)	27 (90)	28 (93.33)	
Family history (%)[c]				1
Yes	18 (30)	9 (30)	9 (30)	
No	42 (70)	21 (70)	21 (70)	
Hyperferritinemia (%)[c]				0.002*
Yes	34 (56.7)	11 (36.67)	23 (76.67)	
No	26 (43.3)	19 (63.33)	7 (23.33)	
Median of serum ferritin (ng/mL)	2257.1 (713.40-8988.20)[b]	1682.8 (765.00-5373.20)[b]	3156.1 (713.40-8988.20)[b]	0.000*
Duration of illness (months)	69.5 (110-211.0)[b]	70.17 ± 44.75[a]	89.27 ± 49.60[a]	0.123
Duration of transfusion (months)	84.37 ± 47.97[a]	69.27 ± 41.80[a]	99.47 ± 49.65[a]	0.013*
Unit of transfusion (unit/year)	24.0 (12.0-42.0)[b]	24.0 (12.0-36.0)[b]	24.0 (12.0-42.0)[b]	0.004*
Duration of chelation (months)	48.0 (0.0-198.0)[b]	48.70 ± 42.00[a]	75.70 ± 47.10 [a]	0.023*
Average of Hb (mg/dL)	7.92 ± 0.68[a]	8.19 ± 0.53[a]	7.66 ± 0.72[a]	
uPCX (ng/mmol urine creatinine)	123.0 (5.76-3019.13)[b]	67.27 ± 37.99[a]	431.28 ± 587.70[a]	0.002*

[a] data normally distributed (mean ± SD), independent t-test

[b] data not normally distributed [median (minimum-maximum)] categorical data [frequency (%)], Chi-square or Fisher-Exact test

* p significantif < 0.05.

4. Discussion

This study involved 60 patients with BTM, in which glomerular injury based on a uPCX level of ≥126 ng/mmol urine creatinine occurred in 50% subjects. This demonstrated that half of the BTM patients suffered from glomerular injury. Kidney damage in BTM is caused by multifactorial, such as iron overload, severe chronic anemia, chronic hypoxia

Table 2: Bivariate analysis between hyperferritinemia and other variables affecting glomerular injury in BTM.

Variable	With glomerular injury	Without glomerular injury	RR (95% CI)	p
Hyperferritinemia ≥ 2500 ng/mL < 2500 ng/mL	 23 7	 11 19	2.51 (1.28-4.93)	0.002*
Average of Hb < 8 g/dL ≥ 8 g/dL	 20 10	 10 20	2.00 (1.14-3.52)	0.010*
Amount of transfusion ≥ 25 unit/year < 25 unit/year	 14 16	 6 24	1.75 (1.09-2.82)	0.028*
Duration of transfusion ≥ 60 months < 60 months	 22 8	 14 16	1.83 (0.98-3.42)	0.035*
Duration of illness ≥ 72 months < 72 months	 18 12	 11 19	1.60 (0.95-2.72)	0.071
Duration of chelation therapy ≥ 28 months < 28 months	 24 6	 19 11	1.58 (0.79-3.17)	0.152
*p significant if< 0,05				

and nephrotoxicity effect of iron chelation therapy. Those processes result in free radicals formation, causing oxidative stress which will oxidize lipid of the cell membrane (lipid peroxidation) and protein compound of the cell leading to cell damage and cell death, which eventually ends with organ damage, including kidney [17, 18].

Glomerular hypoxia can result in increased activation of protein kinase-C (PKC), intracellular calcium, decreased nitric oxide, increased of endothelin and increased of transforming growth factor-β1 (TGF-β1) which activate fibroblast, changing extracellular matrix metabolism in glomerular cells and fibrogenesis [19, 20].

Glomerular filtration barrier consists of 3 layers, i.e endothelial cells covered by glycoprotein with negative charges, glomerular basement membrane (GBM) in the middle, and epithelial cell named podocyte attached on GBM [21]. Podocyte is a visceral epithelial cell of the kidney, together with GBM and endothelial cell, will ensure the selective permeability of the glomerular filtration barrier. One of the protein compounds of the apical membrane domain located in the luminal side of the urinary space is podocalyxin(PCX). Loss of glomerular visceral cells was associated with glomerular sclerosis and loss of kidney function [22, 23]. Perturbation on filtration slit or podocyte injury can trigger massive proteinuria. Podocalyxin, as expressed on the apical membrane of podocyte, was shed directly into urine as a result of podocyte injury, thus urinary

Table 3: Multivariate analysis among hyperferritinemia and other factors affecting glomerular injury.

	RR	95%CI	p
Model 1			
Hyperferritinemia	5.68	1.72 – 18.71	0.004*
Severity of anemia	4	1.24 – 12.93	0.021*
Model 2			
Hyperferritinemia	5.01	1.49 – 16.83	0.009*
Severity of anemia	3.63	1.10 – 11.96	0.034*
Duration of transfusion	1.99	0.59 – 6.70	0.266
Model 3			
Hyperferritinemia	4.01	1.12 – 14.41	0.033*
Severity of anemia	3.73	1.12 – 12.50	0.033*
Amount of transfusion	1.98	0.50 – 7.87	0.334
Duration of transfusion	1,85	0.54 – 6.34	0.331
Model 4			
Hyperferritinemia	4.87	1.21 – 19.50	0.026*
Severity of anemia	4.29	1.20 – 15.33	0.025*
Amount of transfusion	1.98	0.93 – 7.97	0.335
Duration of transfusion	2.85	0.56 – 14.37	0.206
Duration of illness	2.85	0.36 – 11.74	0.414
Model 5			
Hyperferritinemia	5.46	1.30 – 23.01	0.021*
Severity of anemia	4.13	1.15 – 14.85	0.030*
Duration of chelation	1.78	0.36 – 8.84	0.481
Amount of transfusion	1.96	0.49 – 7.89	0.341
Duration of transfusion	2.6	0.51 – 13.11	0.248
Duration of illness	2.55	0.41 – 15.83	0.314
* p significant if <0.05			

podocalyxin poses as a subclinical marker of glomerular damage prior to albuminuria [24].

Hara et al reported a significant increase in uPCX in diabetic patients with normoalbuminuria [25]. Mohamed et al obtained a significant positive correlation between uPCX and albuminuria. Urinary PCX was a risk factor for microalbuminuria and diabetic nephropathy [25, 26].

Blood transfusion along with iron chelation either single or combination increases the survival rate of BTM patients. This raises the manifestation of various organ dysfunction as complications of BTM due to iron overload, including kidney dysfunction. There are many observations on BTM complications on various organs, but very little monitoring in kidney complication. Prevalence of kidney dysfunction in this study is nearly similar

to that of Quinn et al [27] and Wirawan et al [9] which are 59% and 78.6%, respectively. Lower prevalence (18.5%) was reported by Lai et al [28]. This difference is due to the site of the studies, the age of subjects, therapeutic protocol, the amount of transfusion, dosage and adherence to iron chelation therapy and the determination method of kidney dysfunction.

In this study, there was a significant difference of the median of serum ferritin level between two groups, however, a single measurement of serum ferritin cannot be used as an independent predictor of kidney dysfunction, neither glomerular or tubular. Serum ferritin is unable to represent the severity of tissue hemosiderosis. It is believed that kidney damage results from iron overload, but this can not be assessed with a single measurement of serum ferritin [27, 28].

More than half of our subjects (56.7%) experienced a history of hyperferritinemia with serum level of ≥2500 ng/mL. Priyantiningsih [29] reported in 6 months monitoring after DFX therapy, ferritin level found remained high. This study found that a history of hyperferritinemia (serum ferritin of ≥2500 ng/mL) has a significant association with a glomerular injury with RR=2.51; 95% 1.28-4.93; p=0.002).

Severe and recurrent anemia can result in tissue hypoxemia and increased oxidative stress. Recurrent anemia also causes decreased vascular resistance, increased renal blood flow and hyperfiltration, as showed in this study, all subjects have eGFR of more than 110 mL/min/1.73m^2. These changes precipitate glomerular capillary wall stretching, endothelial and epithelial damage, along with transudation of a macromolecule into mesangium. Prolonged hyperfiltration promotes the impaired function of the glomerulus [27, 28, 30]. This study found that the severity of anemia is an independent risk factor for glomerular injury.

Hemoglobin level should be maintained for a range of 9.5-10.5 g/dl in order to maintain growth and development in normal range until the age of 10-11, however over that age, a complication may occur due to iron overload and chronic anemia. Gokce et al [31] found that BTM patients with glomerular hyperfiltration had a median hemoglobin level of 8.6 g/dL (7,6-10,5 g/dL). This hemoglobin range cannot be extrapolated in all health facilities as it depends on individual health center protocol and availability of local resources.

5. Conclusion

This study exhibits the association between hyperferritinemia with a glomerular injury in BTM patients. In addition to hyperferritinemia, the severity of anemia is also an independent risk factor. Routine monitoring of renal function in BTM patients is highly recommended.

Acknowledgments

We would like to thank the research institution that has supported the research activities to be carried out properly.

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