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Original Article

Paediatrics Section

Estimation of Microalbuminuria in Children with Beta Thalassaemia Major: A Cross-sectional Study

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ABSTRACT

Introduction: Improvement in the standard of care of Thalassaemia by regular blood transfusion increases life expectancy. Multiple transfusions with concurrent iron overload and chronic anaemia, leading to tissue-level hypoxemia, cause significant renal dysfunction. Microalbuminuria is a sensitive marker of glomerular damage, and studies in thalassemic children have demonstrated variable prevalence rates of microalbuminuria.

Aim: To study the prevalence of microalbuminuria and its association with clinical and laboratory parameters in children with Beta Thalassaemia Major (BTM).

Materials and Methods: A cross-sectional study was conducted from January 2018 to June 2019 at Indira Gandhi Institute of Child Health, Bangaluru, Karnataka,India. A total of 155 children with Beta Thalassaemia Major (BTM) aged 2-18 years, attending the Thalassaemia Day Care Centre, were included in the study. Their demographic details such as age, gender, clinical parameters like frequency of transfusions, type of chelation therapy, height, weight, Body Mass Index (BMI), organomegaly, and laboratory parameters like serum creatinine, ferritin, pretransfusion Hb%, and Urinary Microalbumin Creatinine Ratio (UMCR) were studied as per a predesigned proforma. The association between microalbuminuria with clinical and laboratory parameters was evaluated using the independent sample T-test or Mann-Whitney U test and Chi-square/Fischer's-exact test.

Results: A total of 155 children with BTM were studied. In the present study, out of a total of 155 patients, microalbuminuria was found in 66 (42.6%). A significant increase in the prevalence of microalbuminuria was observed as the age advanced, as the frequency of blood transfusions increased, with low pretranfusion haemoglobin (g%), and with elevated serum ferritin.

Conclusion: In the present study, the prevalence of microalbuminuria was found to be 42.6%. Screening for microalbuminuria is recommended in all children with beta thalassaemia major for the early detection of renal dysfunction, prevention of disease progression, and improvement in the quality of their lives.

Keywords: Blood transfusion, Early biomarker, Haemoglobin, Renal dysfunction, Transfusion-dependent thalassaemia

INTRODUCTION

The Beta Thalassaemia Major (BTM) is an autosomal recessively inherited blood disorder caused by *HBB* gene mutations, resulting in reduced or absent synthesis of specific globin chains and concurrent accumulation of other unpaired globin chains, leading to ineffective erythropoiesis with haemolysis [1]. In 1925, Cooley TB and Lee P described Italian children with growth retardation, hepatosplenomegaly, and severe anaemia as Cooley's anaemia, later known as Thalassaemia [2].

Conventionally, Thalassaemia is treated with transfusions of packed Red Blood Cells (RBC) from a healthy blood donor at regular intervals and chelation therapy to prevent the consequences of anaemia and iron overload, respectively. Improvement in the standard of care by regular blood transfusion and chelation therapy in a day-care centre has increased the life expectancy in children with thalassaemia. Multiple transfusions with concurrent iron overload, chronic anaemia with hypoxemia at the tissue level, and chelator toxicity cause significant renal dysfunction [3]. Urine analysis in clinically asymptomatic children with thalassaemia showed increased urinary excretion of microalbumin, N-acetyl beta-D-glucosaminidase (NAG), calcium, phosphorus, etc., suggesting incipient renal tubulopathy [4]. Microalbuminuria is a sensitive marker of glomerular damage, and studies in children with thalassaemia have demonstrated variable prevalence rates of microalbuminuria [3-6]. Assessment of urinary microalbumin is a non invasive, economical test for the identification of renal dysfunction. Serial monitoring of microalbuminuria helps diagnose an evolving renal pathology [3,5,6].

Bakr A et al., in their review, found that the prevalence of renal dysfunction in BTM has increased due to better management by regular blood transfusion and chelation therapy [7]. Doddamani P et al., suggested that Urinary Microalbumin Creatinine Ratio (UCMR) should be included in the routine follow-up of these patients [3]. Bekhit OE et al., in their study, found that glomerular and tubular dysfunction exist in children with BTM. Urinary NAG excretion can be a reliable index of tubular toxicity and a possible predictor of proteinuria, which can be recommended for the evaluation of renal dysfunction in these patients [4]. In a study by Hameed EA et al., it was found that glomerular and tubular dysfunction is confirmed in patients with BTM, which could be attributed to oxidative stress and Deferoxamine (DFO) therapy [8]. Yassin N et al., found that microalbuminuria was correlated with age and recommended inclusion of microalbuminuria in the routine follow-up of patients with BTM [9]. Datta V et al., in their study, found that the mean serum creatinine levels did not show a significant difference; however, microalbuminuria would help in the early detection of patients with renal dysfunction and prompt initiation of therapy [10].

Studies have been conducted on renal dysfunction in children with BTM [3,4,8-10] and found variable results. Hence, the present study was undertaken to estimate the prevalence of renal dysfunction using microalbuminuria as an early marker of renal injury in children with BTM and to evaluate its association with clinical and laboratory parameters.

MATERIALS AND METHODS

The present cross-sectional study was conducted over one and a half years from January 2018 to June 2019 at Indira Gandhi Institute of Child Health, Bengaluru, Karnataka, India. The procedures followed were in accordance with the ethical standards of the Institution and with the Helsinki Declaration of 1975, which was

revised in 2000. The protocol was approved by the Institutional Ethics Committee at a meeting held on 23.11.2017 (vide IEC No: IGICH/ACA/EC/07/2017-18).

Inclusion criteria: All 155 BTM children between the age group of 2-18 years, registered under the Thalassaemia Day Care Centre for blood transfusion at Indira Gandhi Institute of Child Health, Bengaluru, Karnataka, India, within the study duration, who were willing to give written informed assent and whose caregivers were willing to give written informed consent were included in the study.

The diagnosis of BTM was based on the standard criteria, including transfusion-dependent anaemia, massive splenomegaly, growth retardation, bone malformations, and peculiar facies in untreated individuals, like bossing of the skull, overgrowth of the maxillary region, with the gradual appearance of a Mongoloid face [4]. Examination of the peripheral blood smear preparation in such subjects revealed severe microcytic hypochromic anaemia, marked anisocytosis, and fragmented nucleated RBCs. Variant haemoglobin analysis by high-pressure liquid chromatography showed predominantly foetal haemoglobin (HbF>50% and HbA2 <4%) [4].

Exclusion criteria: Patients with other haemoglobinopathies, such as sickle cell anaemia, thalassaemia intermedia, thalassaemia minor, sickle-beta thalassaemia, any other associated haemolytic disorder (e.g., glucose 6-phosphate dehydrogenase deficiency), those with documented acute or chronic infection, including those with urinary tract infection at the time of sampling, patients on diuretic therapy, antiepileptic drugs, and those with primary renal disease and diabetes mellitus, were excluded from the study.

Study Procedure

Data collection: A predesigned proforma was used to record the relevant clinical details like age, gender, frequency of transfusions, and type of chelation therapy. Anthropometric measurements like height, weight, and BMI were recorded according to the World Health Organisation (WHO) and Indian Academy of Paediatrics (IAP) growth charts, depending on the age of the children [11,12]. Detailed examination findings were recorded, including organomegaly. A random midstream urine sample (10 mL) was collected from all the children in a sterile container without preservative and assayed for urinary microalbumin and creatinine. UMCR was calculated by dividing urinary microalbumin (µg/dL) by urinary creatinine (mg/dL) [13]. A 2 cc blood sample was collected in a gel tube (yellow cap) for serum creatinine estimation and serum ferritin. Another 2 cc blood sample was collected in an Ethylene Diamine Tetra Acetate (EDTA) tube for pretransfusion Hb%. Samples were preserved at -20°C until further processing [14]. The samples were processed using instruments and methods of estimation, and the cut-off range is shown in [Table/Fig-1] [13,15-20].

Name of the test	Method	Normal range	References
Serum creatinine (mg/dL)	Modified Jaffe's/ AU 480 Beckman Coulter	Males:0.7-1.3 Females: 0.6-1.1	[15,16]
Urine creatinine (mg/dL)	Modified Jaffe's/ AU 480 Beckman Coulter	Males: 20-320 mg/ dL Females 275	[15,16]
Serum ferritin (ng/mL)	CLIA/Cobas 6000. Roche Diagnostics Pvt. Ltd.	Males: 24 to 336 ng/mL; Females: 11 to 307	[17,18]
Hb% (g/dL)	Mindray Haematology Analyser BC 5100	Males:13-17 Females: 12-15 g/dL	[19]
Hb separation by High Performance Liquid Chromatography (HPLC)	VARIANT™ II Haemoglobin Testing System. BioRad Laboratories.	HbA2:1.7%-3.2% β-thalassaemia carriers: 4.0% and 7%. HbA2: borderline: 3.2%-3.8%. HbF:<1.5% of total haemoglobin.	[20]
Urinary Microalbumin Creatinine Ratio (UMCR) (µg/mg)	Immunoturbidimetric method Modified Jaffe's/ AU 480 Beckman Coulter	0-29 creatinine	[13]

[Table/Fig-1]: Showing the details of parameters, method of estimation, normal range and references [13,15-20].
CLIA: Clinical laboratory improvement amendments

STATISTICAL ANALYSIS

The data were entered using Microsoft Excel version 2010 and analysed using R software version 3.6.1. The data were divided into two groups: those with microalbuminuria and those without microalbuminuria. All categorical data were presented as frequency and percentages. All continuous data were summarised using the mean, Standard Deviation (±SD), or median, Interquartile Ranges (IQR) based on the data distribution. All clinical and laboratory parameters were compared using the Chi-square or Fisher's exact test for categorical variables. All continuous measurements were assessed using independent sample t-test or Mann-Whitney U test based on the normal distribution assumption. The Shapiro-wilk test was used to test for normality. Risk factors for microalbuminuria were studied using step-wise multivariate logistic regression analysis for all the above-mentioned parameters. The Odds Ratio (OR) associated with a given factor was an estimate of the risk for microalbuminuria when the factor was present relative to that when the factor was absent; 95% Confidence Intervals (CI) were used as a measure of the statistical precision of each OR. For all comparisons, the p-value was considered significant at the 5% level of significance.

RESULTS

A total of 155 BTM subjects aged 2-18 years, attending the Thalassaemia day care centre, were included in the study. A total of 66 (42.6%) children were found to have microalbuminuria, and 89 (57.4%) did not have microalbuminuria. Microalbuminuria was present in the majority. A total of 35 (22.58%) children were between 5-10 years of age group, followed by 14 (9.03%) children between 10-15 years of age. There were 44 (28.39%) males and 22 (14.19%) females with a M:F ratio of 2:1 in the microalbuminuria-present group. In the microalbuminuria-absent group, there were 47 (30.32%) males and 42 (27.10%) females with a M:F ratio of 1.11:1. The number of females in the microalbuminuria group was less than that of the microalbuminuria-absent group [Table/Fig-2].

In the present study, the median frequency of blood transfusion in children with microalbuminuria was 103.5, and in children without microalbuminuria was 66 (p-value <0.001), suggesting a positive association of microalbuminuria with the frequency of blood transfusion [Table/Fig-3]. The mean pretransfusion haemoglobin% in children with microalbuminuria was 8.53±1.05, whereas in those without microalbuminuria was 9.83±1.3 (p-value <0.001), suggesting a negative association of microalbuminuria with pretransfusion haemoglobin%. The median level of serum ferritin in children with microalbuminuria was 4100 ng/mL, whereas in those without microalbuminuria was 1800 ng/mL, with a significant p-value <0.001 as shown in Table/Figure-4, suggesting a positive association of microalbuminuria with serum ferritin level. The median urinary microalbumin level was 1695 µg/mL in patients with microalbuminuria and 780 µg/mL in those without microalbuminuria [Table/Fig-4].

Step-wise multivariate logistic regression analysis was done to predict the risk factors for microalbuminuria. Age (OR of 1.16; 95% CI 1.07 to 1.27; p-value=0.0002); frequency of transfusions (OR of 1.98; 95% CI: 1.29 to 3.06; p-value=0.002); height (cm) (OR of 1.04; 95% CI: 1.02 to 1.05; p-value=0.0002); weight (kg) (OR of 1.06; 95% CI: 1.02 to 1.11; p-value=0.0035); pretransfusion Hb% (OR- 0.20, 95% CI: 0.08 to 0.52; p-value <0.001) and serum ferritin (OR- 8.63; 95% CI: 4.3-17.33; p-value <0.001); Urine Creatinine (OR of 0.98; 95% CI: 0.97 to 0.997; p-value=0.02) were associated with an increased risk of microalbuminuria, which was statistically significant [Table/Fig-5-12].

Age in years	Microalbuminuria present			Microalbuminuria absent			Grand total	p-value#
	Males	Females	Total	Males	Females	Total		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
2-5	7 (4.52%)	2 (1.29%)	9 (5.81%)	16 (10.32%)	18 (11.61%)	34 (21.94%)	43 (27.74 %)	0.005
5-10	23 (14.84%)	12 (7.74%)	35 (22.58%)	22 (14.19%)	16 (10.32%)	38 (24.52%)	73 (47.1%)	
10-15	9 (5.81%)	5 (3.23%)	14 (9.03%)	8 (5.16%)	5 (3.23%)	13 (8.39%)	27 (17.42%)	
15-18	5 (3.23%)	3 (1.94%)	8 (5.16%)	1 (0.65%)	3 (1.94%)	4 (2.58%)	12 (7.74%)	
Total	44 (28.39%)	22 (14.19%)	66 (42.6%)	47 (30.32%)	42 (27.10%)	89 (57.42%)	155 (100%)	

[Table/Fig-2]: Age and gender-wise prevalence of microalbuminuria in the study subjects.
#Chi-square test

Clinical parameters		Microalbuminuria present 66 (42.6%)	Microalbuminuria absent 89 (57.4%)	p-value
No. of transfusions	Median	103.5	66	<0.001#
	IQR	72.25;158.75	46;108	
	Minimum	9	6	
	Maximum	456	189	
Chelation therapy				
Nil	n (%)	2 (3.03%)	3 (3.37%)	<0.001*
Deferasirox	n (%)	46 (69.70%)	79 (88.76%)	
Deferasirox+ Deferiprone	n (%)	18 (27.27%)	7 (7.87%)	
Weight (Kg)	Mean±SD	22.38±8.67	18.17 ± 7.88	0.002#
Weight for age (kg)				
Under weight	n (%)	11 (16.67%)	18 (20.22%)	0.01*
Normal	n (%)	55 (83.33%)	71 (79.78%)	
Height (cm)	Mean±SD	119.71±18.81	106.19±21.47	0.002#
Height for age (cm)				
Stunted	n (%)	27 (40.91%)	33 (37.07%)	0.01*
Normal	n (%)	39 (59.09%)	56 (62.92%)	
Body Mass Index (BMI)				
Normal	n (%)	49 (74.24%)	54 (60.67%)	0.02*
Under weight	n (%)	7 (10.61%)	16 (17.98%)	
Overweight	n (%)	9 (13.64%)	18 (20.22%)	
Obese	n (%)	1 (1.52%)	1 (1.12%)	
Organomegaly				
No Organomegaly	n (%)	38 (57.58%)	59 (66.29%)	0.03*
Liver/Spleen	No. (%)	6 (9.09%)	9 (10.11%)	
Spleen	No. (%)	8 (12.12%)	6 (6.74%)	
Liver+Spleen	No. (%)	14 (21.21%)	15 (16.85%)	

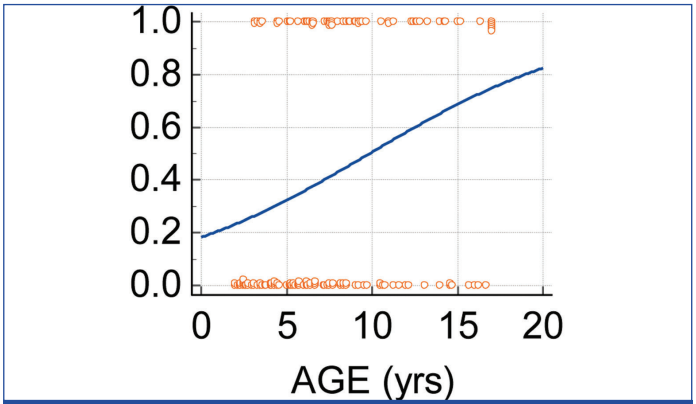
[Table/Fig-3]: Clinical parameters of study subjects with Beta Thalassaemia Major.

Laboratory parameters		Microalbuminuria present 66 (42.6%)	Microalbuminuria absent 89 (57.4%)	p-value
Pretransfusion Hb%	Mean±SD	8.53±1.05	9.83±1.3	<0.001*
Serum creatinine (mg/dL)	Mean±SD	0.36±0.13	0.33±0.12	0.14#
Absolute microalbumin levels (µg/mL)	Median	1695	780	<0.001*
	IQR	1205;2345	510,1240	
	Minimum	350	200	
	Maximum	15550	2200	
Urine creatinine (mg/dL)	Mean±SD	38.85±25.43	46.91±27.24	0.02*
Urine microalbumin creatinine ratio (µg/mg creatinine)	Mean±SD	59.60±39.78	20.48±5.69	<0.001*
Serum ferritin (ng/mL)	Median	4100	1800	<0.001*
	IQR	2825; 5400	1100; 2500	
	Minimum	1700	240	
	Maximum	7000	6717	

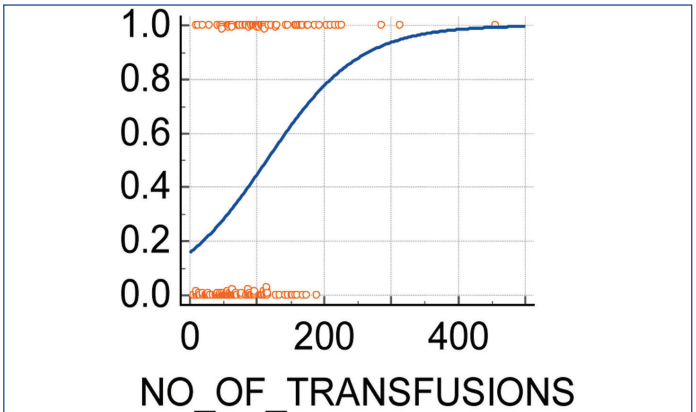
[Table/Fig-4]: Laboratory parameters of study subjects with BTM.
Independent Sample t-test.*Mann-Whitney U Test#. All the laboratory parameters were found to be significant with a p-value < 0.05 except serum creatinine which was not significant with a p-value >0.05.

Parameters	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Age (years)	1.16	1.07 to 1.27	0.0002
Frequency of transfusions	1.98	1.29 to 3.06	0.002
Height (cm)	1.04	1.02 to 1.05	0.0002
Weight (kg)	1.06	1.02 to 1.11	0.0035
Pretransfusion Hb%	0.20	0.08 to 0.52	<0.001)
Serum ferritin	8.63	4.3-17.33	<0.001
Urine creatinine	0.98	0.97 to 0.997	0.02

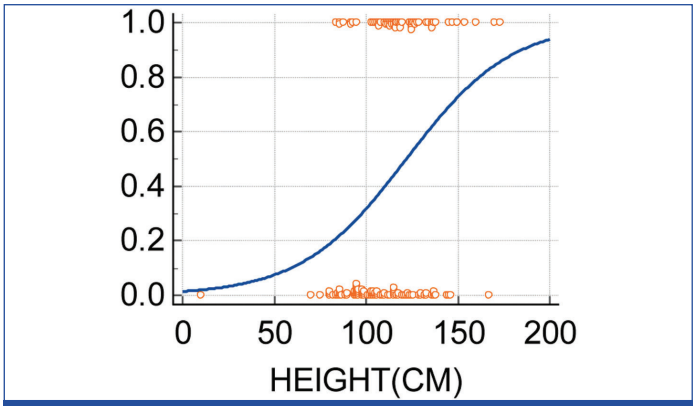
[Table/Fig-5]: Showing step-wise multivariate logistic regression analysis to predict the risk factors for microalbuminuria.



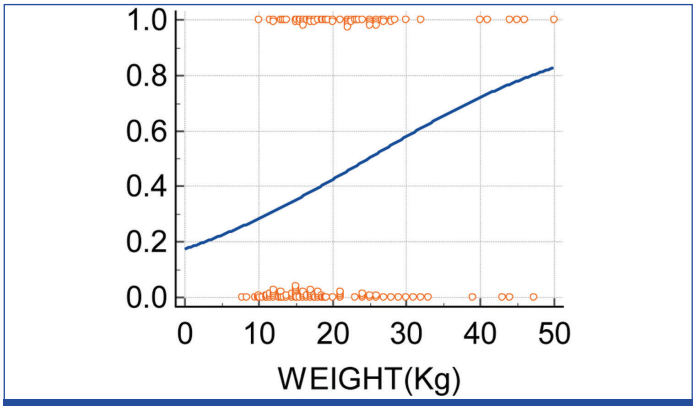
[Table/Fig-6]: Showing logistic regression analysis for age of children with thalassaemia.
As age increases the risk of microalbuminuria increases



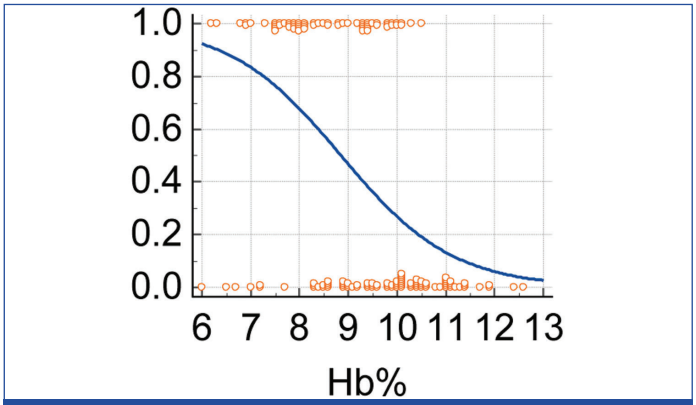
[Table/Fig-7]: Showing logistic regression analysis for frequency of transfusions.
As the frequency of transfusions increases the risk of microalbuminuria increases.



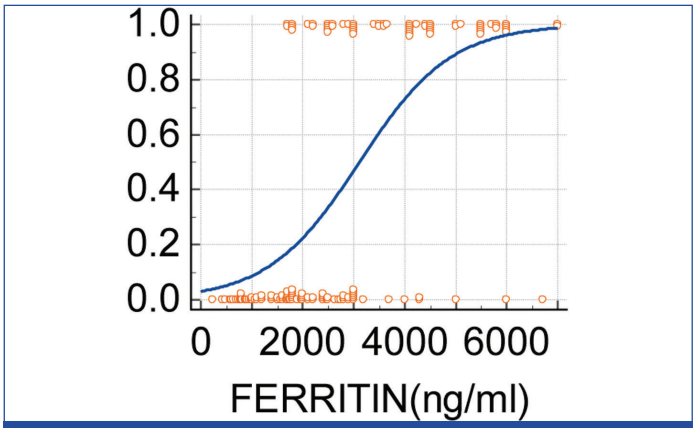
[Table/Fig-8]: Showing logistic regression analysis for height (cm).
As the height (cm) increases the risk of microalbuminuria increases



[Table/Fig-9]: Showing logistic regression analysis for weight (kg).
As the weight (kg) increases the risk of microalbuminuria increases



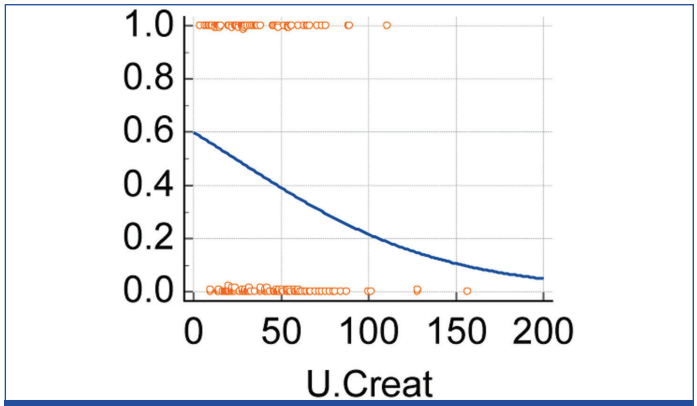
[Table/Fig-10]: Showing logistic regression analysis for Hb%.
As Hb% increases the risk of microalbuminuria decreases



[Table/Fig-11]: Showing logistic regression analysis for serum ferritin.
As ferritin increases the risk of microalbuminuria increases

DISCUSSION

The management of thalassaemia has greatly improved in the past few years. People with β-thalassaemia requiring chronic transfusions are now living longer and have a better quality of life than before.



[Table/Fig-12]: Showing logistic regression analysis for urine creatinine.
As Urine creatinine increases the risk of microalbuminuria decreases

Due to the prevention of the toxic effects of iron on the heart and liver by the usage of appropriate chelation, such complications have reduced. Previously unexplored complications of thalassaemia, like renal dysfunction, are more evident now. However, literature on renal dysfunction in thalassaemia is limited.

The UMCR is considered to be one of the early indicators of kidney disease and a predictor of the outcome. Hence, the present study measured UMCR and looked for the prevalence of microalbuminuria in BTM patients and the association of microalbuminuria with clinical and laboratory parameters.

The present study included 155 children with BTM from a single centre. The prevalence of microalbuminuria in the present study was 42.6%, which is similar to other studies like Tantawy AA et al., (29%) and Hamwi D and Alquobaili F (30%), indicating significant evidence of renal injury in BTM children with no clinical features of renal disease [5,21]. The present study is the largest paediatric study conducted in recent years. All the studies compared were prospective studies from a single centre.

In the present study, as the age increases, the prevalence of microalbuminuria also increases, which was similar to the studies conducted by Doddamani P et al., and Hamwi D and Alquobaili F in which a positive correlation was found between microalbuminuria and the age of the child and the duration of blood transfusion [3,21]. However, Hamwi D and Alquobaili F found no significant correlation of microalbuminuria with age [21]. These discordant results could be explained by the difference in age in the study group.

The mean pretransfusion Hb% of studies published by Hashemizadeh H and Noori R, Pemde H et al., Pemde HK et al., Najafipour F et al., were 8.5±1.5 gm/dL, 9.2±0.97 gm/dL, 9.7±0.4 gm/dL, respectively, which were similar to 9.2±1.36 gm/dL observed in the current study [22-24]. The Thalassaemia International Federation recommends a moderate transfusion regimen to maintain the average pretransfusion haemoglobin level between 9-10.5 gm/dL [25].

The mean ferritin level of the current study was 2835±1628 ng/mL, which was comparable to similar studies with mean ferritin levels of 2183±525 ng/mL done by Hashemizadeh H et al., 3138±1499 ng/mL done by Pemde H et al., and 2888±948 ng/mL done by Najafipour F et al., respectively [22-24]. In the present study, the prevalence of microalbuminuria was positively associated with serum ferritin level. Studies conducted by Hamwi D and Alquobaili F and Ziyadeh FN et al., also showed a positive association between microalbuminuria and serum ferritin level, explaining the toxic effects of unchelated iron on renal glomeruli and tubules [21,26].

Serum creatinine levels in the present study were within the normal range with a mean of 0.36±0.13 mg/dL, which was comparable to the results expressed by other studies, namely Doddamani P et al., with 0.7±0.11 mg/dL, Adly AA et al., with 0.17±0.06 mg/dL, Tantawy AA et al., with 0.8±0.1 mg/dL, and Hamwi D and

Alquobaili F with 0.521±0.009 mg/dL, respectively [3,5,6,21]. Serum creatinine is known to be an unreliable indicator of changes in kidney function as it is affected by factors unrelated to renal function, such as muscle mass, protein intake, inflammatory illness, and hepatic disease. Additionally, creatinine is partially secreted by renal tubules and frequently overestimates Glomerular Filtration Rate (GFR) [27].

The following parameters were not assessed in the present study but can be assessed in future studies. Serum cystatin C has been demonstrated to be superior to creatinine in the evaluation of minor reduction in GFR in some studies [28,29]. Cystatin C, a 122 amino acid non glycosylated low molecular weight (13 kDa) age and gender-independent cysteine protease inhibitor, is considered to be a housekeeping gene, which is transcribed at a relatively constant level and is expressed in all nucleated cells. It is freely filtered in the renal glomeruli and totally reabsorbed and metabolised in the proximal tubule. Thus, the serum concentration of Cystatin C is mainly determined by GFR [30].

Other early markers of renal dysfunction include β2 Microglobulin (β2MG), a low molecular weight protein which, under normal circumstances, is freely filtered at the glomerulus but almost totally reabsorbed by renal tubules [31]. Elevation of β2MG in urine is a sensitive and reliable early marker of tubular dysfunction [32,33].

N-acetyl-β-D-glucosaminidase (NAG) [34-36], a high molecular weight (140 KDa) lysosomal enzyme which plays a role in the breakdown of glycoproteins in proximal renal tubular cells, is considered mainly as a marker of renal tubular function. NAG is secreted by tubular epithelium. Its measurement has been undertaken in a variety of diseases associated with renal injury, such as thalassaemia major [8,37-40], glomerulonephritis [41], lupus [42], diabetes [43], and rheumatoid arthritis [44]. The urinary NAG levels are increased before the increase in serum creatinine, urea, and microalbuminuria [39-41]. Early identification of subjects at high-risk of developing renal failure is of great importance, as it may allow specific measures to delay the progression of renal damage and thus reduce the incidence of end-stage renal failure and mortality [34-36].

Limitation(s)

In the present study, authors were not able to estimate Cystatin C, β2MG, or NAG due to logistic reasons. Chelating agents are known to cause nephrotoxicity, particularly deferasirox, which is also known to cause microalbuminuria. Hence, patients on deferasirox need to be followed up to look for nephrotoxicity. Rising serum creatinine levels are an indication of nephrotoxicity, which was not assessed in present study.

CONCLUSION(S)

Over 42.6% of children with BTM on chronic transfusion therapy were found to have microalbuminuria. Advancing age, increased frequency of transfusion, low pretransfusion haemoglobin %, and raised serum ferritin were the four risk factors associated with significant microalbuminuria. Hence, interventional strategies aiming at the maintenance of normal haemoglobin and serum ferritin levels are recommended to prevent further renal damage. Further studies are needed to differentiate between microalbuminuria due to the progression of BTM or due to chelating agents. Urinary microalbumin assay, a novel non interventional and economical test, can be included in the screening protocol of children with β-thalassaemia for early detection of renal disease. Other novel biomarkers like cystatin C, β2-Microglobulin, and NAG can also be recommended to detect asymptomatic renal dysfunction.

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