Title: Hepatic and renal functions of paediatric patients with thalassaemia

Introduction

Thalassaemia is a disorder of haemoglobin synthesis due to autosomal recessively inherited mutations in globin genes. It is highly prevalent in the tropical belt, which extends from the Mediterranean through the Middle East to the south and South-East Asia(1). Adult haemoglobin is a tetramer of 2 alpha-globin chains and two beta-globin chains. Mutations in the alpha-globin gene in chromosome 16 results in alpha-thalassaemia, and mutation in the beta-globin gene in chromosome 11 results in beta-thalassaemia.

Thalassaemia is the most common hemoglobinopathy among children and adolescents in Sri Lanka. It is considered the most common monogenic disorder in the country, with approximately 60 new cases diagnosed every year (2)(3). The clinical severity of the disease varies and is categorised into two entities; a mild form which requires occasional transfusion (non-transfusion dependent thalassaemia), and a more severe form which requires regular blood transfusion (transfusion-dependent thalassaemia) (4).

The usual clinical course of beta-thalassaemia starts around six months of age when the physiological conversion of fetal haemoglobin to adult haemoglobin occurs. Patients present with features of anaemia like lethargy, poor feeding, failure to thrive, severe pallor, jaundice and hepatosplenomegaly. The diagnosis is confirmed by qualitative and quantitative assessment of haemoglobin by high-performance liquid chromatography. Definitive treatment is bone marrow transplantation which is currently not freely available. The availability of bone marrow transplantation is limited due to cost as well as the limitation of suitable donors (1). Blood transfusion therapy is the main mode of treatment to suppress excessive erythropoiesis and maintain growth. Medical management of these patients includes regular blood transfusion in 2-5 weeks intervals to maintain pretransfusion haemoglobin level between 9-10.5mg/dl and iron chelation to counteract iron overload, which is inevitable (5).

Complications of thalassaemia are multi-factorial. This can be due to disease as well as treatment. Inadequate blood transfusion results in continued extramedullary haematopoiesis. Blood transfusions carry the risk of transfusion-related infections like hepatitis B and C. Also, frequent blood transfusions lead to iron overload. Even in the absence of transfusions, the accelerated rate of erythropoiesis enhances dietary iron absorption from the gut, resulting in iron overload.

Iron overload affects the liver, heart, pancreas and other endocrine organs (6). Monitoring for iron overload is an integral part of the management of thalassaemia.

Justification

Colombo North Teaching Hospital and Kurunegala Teaching Hospital are the two largest thalassaemia centres in Sri Lanka. In this study, we aim to assess the hepatic and renal status of paediatric patients with thalassaemia, which is not evaluated in Sri Lanka. We have observed that most paediatric patients with thalassaemia have an unexplained elevation of hepatic transaminases. Therefore, we hope that this study will provide insight into the hepatic and renal status of paediatric patients with thalassaemia.

Objectives

General objective:

- To describe the hepatic and renal status of paediatric patients with thalassaemia in Sri Lanka

Specific objectives:

- To describe the hepatic status of paediatric patients with thalassaemia in Sri Lanka
- To describe the renal status of paediatric patients with thalassaemia in Sri Lanka
- To determine the factors associated with hepatic transaminitis in patients with thalassaemia in Sri Lanka

Literature review

Hepatic complications of thalassaemia have been evaluated in a few previous studies done in various countries. A study done in Egypt evaluated the effect of iron overload and viral infections on the liver enzymes in thalassaemia. Out of 80 thalassaemia major patients, 50% were anti - HCV positive, and 55% were HCV PCR positive. Patients with high AST and ALT levels had very high serum ferritin compared to normal AST and ALT level patients. Patients who were anti-HCV positive had higher ALT, AST and GTT levels than the hepatitis C negative patients (7).

Another study assessed renal injury in transfusion-dependent thalassaemia patients. The renal injury could be due to iron overload, chronic anaemia or iron chelation therapy. This study

revealed a significant reduction in the glomerular filtration rate (GFR) in patients on deferoxamine. Those patients had normal GFR before the initiation of deferoxamine (8).

In transfusion-dependent thalassaemia, tubular dysfunction may be mild to severe. Initially, it can be undetectable. One study evaluated the best marker for the identification of renal dysfunction in thalassaemia and concluded that urine neutrophil gelatinase-associated lipocalin (UrNAGAL) to creatinine ratio was the most accurate early detection of renal dysfunction (9). However, testing of UrNAGAL is not widely available.

Another study done on 100 patients with beta-thalassaemia major found a strong association between serum ferritin and AST and ALT levels. Hepatitis C antibody positive patients had a higher level of AST and ALT levels compared to hepatitis C negative patients. The authors concluded that liver involvement in beta-thalassaemia major is due to iron overload and chronic hepatitis (10).

There is another study which was published in *Beni- Suef university journal of basic science and applied sciences*. They assessed the non-invasive assessment and risk factors for liver fibrosis in paediatric patients with beta-thalassaemia. It used transient elastography to assess liver fibrosis in patients with beta thalassaemia major. It identified hepatitis C virus positivity, frequent blood transfusion, age and regular chelation as risk factors for liver fibrosis (11).

Another study that assessed the pathogenic factors leading to renal injury revealed that the urinary malondialdehyde to creatinine ratio (UMDA/Cr) and the tubular phosphate reabsorption (TRP) were statistically different in children with thalassaemia and normal children. High serum levels of potassium, phosphorus and uric acid in the patient group were believed to be due to the rapid erythrocyte turnover. The presence of high urine protein to creatinine, urinary N-acetyl-D-glucosaminidase to creatinine (UNAG/Cr) and UMDA/Cr ratios shows that in these patients with proximal renal tubular damage may be secondary to oxidative lipid peroxidation mediated by the iron overload (12).

There was another study with the objective of evaluating renal tubular and glomerular function in paediatric patients with beta-thalassaemia and correlating the renal findings to iron overload. The study population was 37 patients with beta thalassaemia major and beta thalassaemia intermedia. The Control group was 12 children without abnormalities in iron metabolism or a renal disorder.

The study reported no difference between blood urea, nitrogen, serum creatinine, creatinine clearance, electrolytes, fractional excretion of sodium and potassium and tubular phosphorus reabsorption and uric acid. But uric acid excretion was significantly higher in thalassaemia patients. UNAG to creatinine ratio was high in thalassaemia patients, which was directly related to the amount of transfused iron but not to the ferritin value. The study concluded that renal tubular functions were impaired in children with beta-thalassaemia major and intermedia. However, the authors could not explain the cause of the reported abnormalities (13).

In another study, they have assessed tubular dysfunction in paediatrics patients with thalassemia major.Study population was 140 with beta thalassemia major patients and healthy children.Urine was taken to assess urinary sodium,Potassium,calcium,protein,uric acid,creatinine,urine Osmolality,urinary N-acetyl-beta-D glucosaminidase (UNAG) activity.Blood were taken for full blood count,blood urea and nitrogen,Fasting blood sugar,serum creatinine,serum electrolytes,serum ferritin.Blood were taken before transfusion.Study population mean age is 11.2years(7-16 years).Study results, had normal creatinine and GFR,mean UNG 17.8 IU/L (normal 0.15-11.5IU/L), control group had normal level.(3.2 IU/L).82 patients had high UNAG,58 had high ferritin (62.4%),13 (15.9%) had hypercalciuria,9 (6.4%) had proteinuria (mean age 12y and Upr/Ucr > 0.2),69 (49.3%) out of 140 patients and 45 (65.2%) having UNAG had uricosuria,10(7%) had microscopic hematuria,10(7%) (mean age 13.5 had uricosuria and diabetes mellitus.Conclusion was tubular dysfunction is a relatively common complications of beta thalassemia major patients.UNAG and it's index best to detect renal tubular dysfunction.They have recommended to monitor UCa/Ucr,UUA/Ucr,urinalysis in beta thalassemia patients (14)

Methods

A cross-sectional descriptive study will be conducted at the two largest thalassaemia centres in Sri Lanka: Kurunegala and Ragama. These two thalassaemia centres care for over half of children with thalassaemia in Sri Lanka. The study will be conducted from January to March 2023.

All paediatric patients aged 1-16 years attending Kurunegala and Ragama thalassaemia centres during the study period will be recruited into the study. The diagnosis of thalassaemia is based on haemoglobin subtype quantification using high-performance liquid chromatography done prior to the commencement of transfusions. Parents of the participants were briefed about the study,

and informed written consent from guardians and assent from children over 12 years will be obtained before recruiting into the study.

Data will be collected using a data collection form by interviewing parents, perusal of clinical records and physical examination of the subjects. A trained data collector will interview patients and their parents to gather data on basic demographics, types of thalassaemia, transfusion status, age at diagnosis, age at 1st transfusion, annual transfusion requirements, frequency of transfusion, iron chelation, age at commencing of iron chelation, last three serum ferritin value and last three pre-transfusion haemoglobin value. Next, physical examinations will be performed by trained doctors to measure anthropometric parameters and liver and spleen sizes. Weights will be measured using a calibrated beam balance, while height measurements will be done using a stadiometer.

Next, data on the biochemical and radiological tests done as a part of the routine care of these patients will be gathered from patient records. Specifically, we will gather data on hepatic transaminase, liver profile, hepatitis screening, renal function tests, abdominal ultrasonography, and liver MRI.

Data will be entered into an SPSS database and analysed using IBM SPSS Statistic 22.0. Categorical variables will be presented as frequency and percentages, whereas continuous variables will be presented as mean and standard deviation. Factors associated with hepatic transaminitis will be analysed using the Chi-square test and binary logistic regression. The cut-off for statistical significance is set at $p<0.05$.

Ethical approval for the study will be obtained from the Ethics review committee of the Sri Lanka College of Paediatricians. Data will be collected only after obtaining informed written consent from the guardians of study participants. None of the personal data will be recorded. Administrative approval to carry out the study will be obtained from the Directors and Consultants in charge of the Thalassaemia Units of Ragama and Kurunegala Teaching Hospitals. Collected data will be kept confidential on a password-protected personal computer. Data will be kept only for the minimum duration required.

The results of the study will be presented at a future scientific session /congress and will be published in a suitable journal.

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Annexure 01: Data collection form

1. Serial number: ……..
2. Date: ……
3. Date of birth: …….
4. Age: …….
5. Sex: ……..
6. Weight: ……kg
7. Height: ……..cm
8. Thalassemia center: …….
9. Type of thalassemia: …….
10. Transfusion status: TDT/ NTDT
11. Age at diagnosis: ……..
12. Age at 1st transfusion: ……..
13. Annual transfusion requirement: …… (last 12 months)
14. Frequency of transfusion: ……
15. Iron chelations

	Age at commencing.	dose
Deferoxamine.	……:.:…..:…	…….
Deferasirox.	…….	…….

16. Last 3 serum ferritin.

	Date.	Value.
1	……..	…….:……
2	…….,	……
3	…….,	……

17. Last 3 free transfusion hemoglobin

	Date.	Value
1	……..	……
2	…….	……
3	…….	……..:…….

18. Liver Size (below costal margin): ……cm

19. Spleen Size: ……,, cm

20. Splenectomy: done or not done

21. USS Finding

Liver echogenicity: ……..	Liver Size:……cm
Spleen Size: ……..cm	Ascites: ……
Portal Hypertension: ……	Other remarks: ……

22. Hepatic functions

AST: …….	ALT: ……,,	Albumin:……	Globulin: ……
PT/INR:……	Gamma GT: ……	Total bilirubin:…	Direct: ……
Indirect: …….	ALP: ……..		

23. Renal functions

UFR: ……	Serum creatinine:……	Blood urea: ……..
K+: ……..…	Na+: ……	UPCR: ……..

24. Fibro scan performed. (Yes/No)

Findings if performed……

25. Liver Biopsy performed. (Yes/No)

Results if performed……