Research Article

Biochemical assessment of renal function and its correlation with iron overloading in different variants of thalassemia

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Abstract

Thalassemia is a hereditary disorder characterised by changes in organ function and iron overload resulting from multiple blood transfusions, inefficient erythropoiesis, and increased gastrointestinal iron absorption. This study aimed to compare and correlate renal function tests with blood transfusion frequency, annual blood requirement, and ferritin levels in patients with Thalassemia Major, Intermedia, and Minor. Fifty blood samples were collected from each clinically diagnosed patient with thalassemia major, intermedia, and minor at MGM Hospital in Navi Mumbai for complete blood count estimates, serum ferritin, and renal function tests. The frequency of blood transfusions and the annual blood requirement was recorded. The Pearson correlation coefficient was used to examine the relationship. SPSS Software was used to tabulate and process patient data. A p-value of less than 0.001 was regarded as significant. This study included both male (64) and female (84) participants. The major, intermedia, and minor serum ferritin levels were 2212.5 ug/L, 1643 ug/L, and 53.55 ug/L, respectively. Serum creatinine and sodium levels in thalassemia major (27.41 ± 5.31umol/L, 128.02± 4.56 mmol/L, respectively) and intermedia (36.25 ± 6.19 umol/L, 128.96± 4.39 mmol/L respectively) decreased as the frequency of transfusion and serum ferritin level increased. Uric acid levels (361.64 ± 64.83 umol/L) were higher in patients with thalassemia intermedia. Transfusion therapy, required for Major and Intermedia, increases iron overload, which harms organ function and quality of life. Early detection and genetic counselling are necessary for proper thalassemia management.

Keywords: Blood transfusion, Genetic counselling, Iron-overload, Renal function test, thalassemia

INTRODUCTION

Thalassaemia is an inherited haemoglobin production disorder characterised by a partial or complete failure to synthesise a specific type of globin chain. One or both alleles on chromosome 16 may be disrupted in alpha-thalassaemia, producing some or no alpha globin chains. Defective production in beta-thalassaemia is usually caused by disabling point mutations that result in no (β0) or reduced (β−) beta chain production (Colledge *et al.,* 2010).

The most common type of thalassemia is beta-thalassemia, which is especially prevalent in the Mediterranean region. Thalassemia has been found in nearly every racial group and geographic location on the globe; however, they are most common in a broad belt stretching from Sub-Saharan Africa through the Mediterranean region, the Middle East, and the Arabian Peninsula to the Indian subcontinent, India, and South eastern Asia (Weatherall, 2018). Beta-thalassaemia is classified into three types based on haemoglobin electrophoresis: Major, Intermedia, and Minor. Heterozygotes have thalassaemia minor, a condition characterised by mild anaemia and little or no clinical disability that is only detected when iron therapy for mild microcytic anaemia fails. Homozygotes (thalassaemia major) can either not synthesise haemoglobin A or produce very little; they develop profound hypochromic anaemia after the first 4–6 months of life (Colledge *et al.,* 2010). The hallmark of beta-thalassaemia disease is imbalanced globin chain synthesis, in which α-chain synthesis proceeds at a normal rate, resulting in excess of α-chain in erythrocytes that are unstable and precipitate in bone marrow red cell precursors, resulting in ineffec-

tive erythropoiesis and a group of subsequent patho-physiological mechanisms (Weatherall, 2013; Thein, 2018).

Thus, ß-thalassemia major requires blood transfusions on a regular basis, and every 1 ml of packed red cells adds 1 mg of iron to the body's iron load. Secondary hemosiderosis is caused by increased iron deposition as a result of several life-long transfusions and in-creased iron absorption (Cao *et al.*,1994).

Iron overload can cause serious organ damage by de-positing in the liver, heart, and various other endocrine glands, as well as endocrine organ failure. Cardiotoxici-ty is the most serious and potentially fatal complication of iron overload, requiring chelation therapy (Makis *et al.,* 2016). On the other hand, renal involvement is a rare consequence of beta-thalassemia, with subclinical and insignificant symptoms (Quinn *et al.,* 2011; Malaki *et al.,* 2011; Ali *et al.,* 2008). It includes increased renal blood flow, urine concentration defect, and renal tubular acidosis (Tantawy *et al.,* 2014). The independent im-pact of excess iron load and chronic anaemia could act as risk factor leading to tubular dysfunction in beta-thalassemia patients (Hamed and ElMelegy, 2010). Older patients with alpha thalassemia, beta-thalassemia major, and haemoglobin E/thalassemia have mild impairment of tubular function and decreased glomerular filtration rate (GFR) have been reported (Nickavar *et al.*,2017; Galanello and Origa, 2010). Re-cent advancements in these individuals' medical care have resulted in a higher survival rate and a greater awareness of unexpected consequences (Kohgoy *et al.,* 2008). The aim of the present study was to deter-mine if there was any correlation between iron overload and renal parameters such as serum creatinine, urea, uric acid, sodium, potassium and chloride in different variants of thalassemia.

MATERIALS AND METHODS

From January to July 2021, a descriptive cross-sectional study was conducted in the Department of Biochemistry and Paediatrics at MGM Medical College in Navi Mumbai. According to the inclusion criteria, 198 cases (148 in the study group and 50 in the control group) were enrolled. All participants signed a written informed consent form. The Institutional ethical commit-tee approved the project.

All participants provided a detailed clinical history, which included demographic information, anthropomet-ric measurements, frequency of blood transfusion, and annual blood requirement. A 5ml blood sample was taken from each participant and transferred to an EDTA and plain vacutainer for haematological parameters like Complete blood count (CBC) and biochemical analysis like serum ferritin, renal function test and electrolytes.

Complete blood count analysis was performed in the laboratory, and biochemical parameters included a re-nal function test with electrolytes and ferritin.

Complete blood count was performed using a cell counter Sysmex XN-1000, Renal function test and elec-trolytes were performed using Beckman coulter AU480 and serum ferritin was estimated on Cobas e 411.

SPSS version 25 was used for statistical analysis, and $P < 0.001$ was considered significant.

RESULTS

Total 198 participants were enrolled, out of which 148 were thalassaemic having 64 (43.24%) male and 84 (56.75%) female participants. Amongst thalassaemic subjects, 50 (33.78%) were thalassemia major, 48 (32.43%) thalassemia intermedia and 50 (33.78%) tha-lassemia minor, respectively. Amongst the enrolled subjects, it was noticed that percentage of female sub-jects suffering from thalassemia was higher than that of males.

On comparing the haematological parameters of three variants (thalassemia major, Intermedia and Minor) , the study found that red blood cell and haemoglobin levels before transfusion were lower in thalassaemic major and intermedia than in thalassaemic minor as expected as shown in Table 1.

On comparing biochemical parameters between the thalassaemic variants, it was found that ferritin levels in

Table 1. Showing comparison of haematological parameters in the study and control group of thalassemia

Haematological parameters	Mean± SD			
	Major (n=50)	Intermedia (n=48)	Minor (n=50)	Control (n=50)
RBCs (10^{12}/L)	3.31 ± 0.12[*]	3.70 ± 0.17[*]	4.90 ± 0.41	4.98 ± 0.39
Pre-haemoglobin (mmol/L)	4.47 ± 0.95[*]	4.67 ± 0.83[*]	5.94 ± 0.38[*]	9.27 ± 0.78
MCV (Fl)	77.69 ± 4.38[*]	77.60 ± 5.62[*]	59.76 ± 4.27[*]	88.15 ± 4.45
MCH (pg./cell)	26.54 ± 1.86[*]	26.34 ± 2.20[*]	20.56 ± 1.77[*]	28.81 ± 1.22
MCHC (g/dl)	34.20 ± 1.04	34.04 ± 1.29	25.87 ± 1.41[*]	33.73 ± 1.27
Ferritin (ug/L)	2212.5 (979.4)[*]	1643 (312.7)[*]	53.55 (17.44)	61.15 (9.03)

*p<0.001 patients versus controls

Table 2. Showing comparison of biochemical parameters in the study and control group of thalassemia.

Parameters	Mean± SD			
	Major (n=50)	Intermedia (n=48)	Minor (n=50)	Control (n=50)
Creatinine(umol/L)	27.41 ± 5.31[*]	36.25 ± 6.19[*]	67.2 ± 8.84	56.59 ± 5.31
Urea (mmol/L)	9.01 ± 1.73[*]	7.85 ± 1.74	7.45 ± 0.81	7.09 ± 1.29
Uric acid (umol/L)	236.14 ± 28.55	361.64 ± 64.83[*]	256.36 ± 39.85	297.4 ± 2.38
Sodium (mmol/L)	128.02± 4.56[*]	128.96± 4.39[*]	141.34 ± 3.79	137.9 ± 3.45
Potassium (mmol/L)	2.85 ± 0.23[*]	3.04 ± 0.11[*]	4.39 ± 0.33	4.21 ± 0.44
Chloride (mmol/L)	97.84 ± 2.60	98.94 ± 2.36	100.7 ± 1.47	99.9 ± 1.31

[*]$p < 0.001$ patients versus controls

thalassemia major were higher [2212.5 (979.4) ug/L] than that of thalassaemic intermedia [1643 (312.7) ug/L]. The Ferritin levels in Thalassemia minor subjects were significantly lower with p- value of <0.001 level of significance in comparison with thalassemia major and intermedia.

As shown in Table 2, the review of the result of biochemical parameters showed that levels of creatinine and electrolytes such as sodium, and potassium were found to be significantly lower with p- value of <0.001 in Major and Intermedia as compared to thalassemia minor and control. Uric acid levels were found to be higher in thalassemia Intermedia in comparison with thalassemia major and Minor. Other renal function tests, such as urea and electrolytes, such as chloride, were found to be normal in transfusion required thalassemia. The correlation between renal function, electrolytes and ferritin is shown in Figs. 1,2,3,4,5,6.

The study also conducted correlation analysis using Pearson's coefficient of ferritin and creatinine in two subgroups (thalassemia Major and Intermedia) of the study group. It was found to be significant negatively correlated in all three variants with r = -0.7041 (p = < 0.0001) and r = -0.7231 (p = < 0.0001) for Major and Intermedia. Fig. 1 and 2 shows the correlation between ferritin and creatinine in the Major and Intermedia variant, respectively.

The electrolytes, such as sodium and potassium, with ferritin levels were also correlated. Serum ferritin levels were significant negatively correlated with sodium levels in Major, Intermedia with r = -0.7137 (p = < 0.0001), r = -0.9188 (p = < 0.0001), respectively. Fig. 3 and 4 show the correlation between ferritin and sodium in Major and Intermedia variants. Serum ferritin levels were significant negatively correlated with potassium levels in with r = -0.4936 (p = 0.0002), r = -0.3768 (p = 0.007) for Major and Intermedia, respectively. Fig. 5 and 6 show the correlation between ferritin and potassium levels in Major and Intermedia variants.

DISCUSSION

Thalassemia is the most common inherited anaemia in which transfusions are required regularly to support survival and physiologic functioning. Individuals with thalassemia are preconditioned to complications from blood transfusions, such as iron overload (Borgna-Pignatti *et al.,* 2004; Kwiatkowski *et al.,* 2012; Cunningham *et al.,* 2004). As discussed by Nickavar *et al.,* 2017, Proteinuria affected about 4% of patients, and protein excretion was normal in most of the three variants, namely Major, Intermedia, and Minor. However, it was significantly lower in patients with thalassemia minor, which could be explained by the severity of pathophysiologic mechanisms being less severe in this group of patients. They also showed eGFR increased insignificantly. The severity of anaemia, frequency of blood transfusion, and iron load all influence tubular
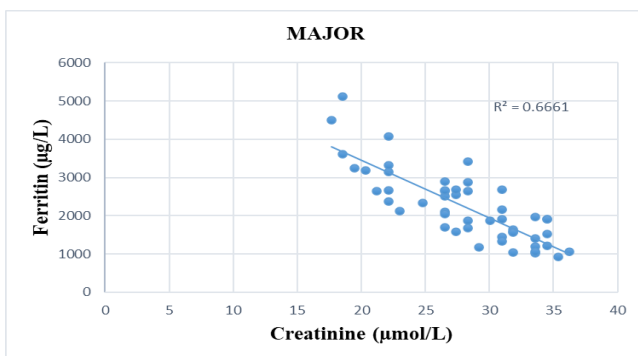

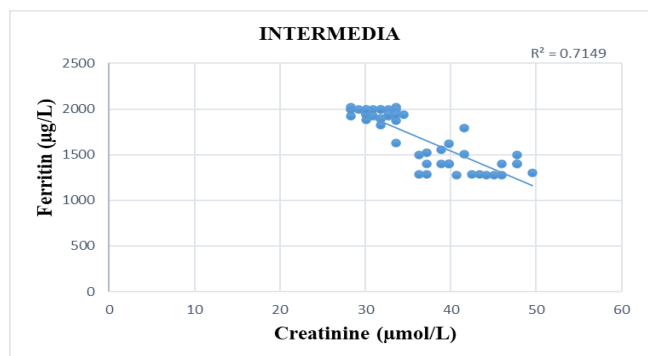**Fig. 1**. *Correlation between ferritin and creatinine levels in Thalassemia Major*

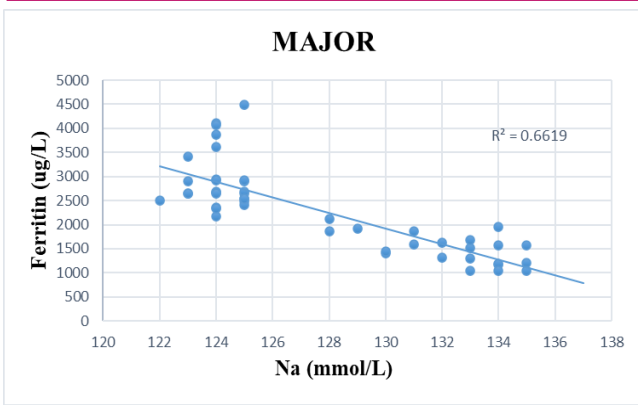**Fig. 2.** *Correlation between ferritin and creatinine Levels in Thalassemia Intermedia*

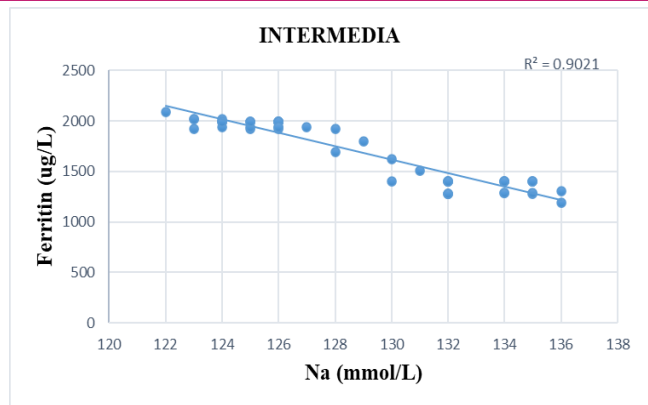**Fig. 3.** *Correlation Between Ferritin and Sodium Levels in Thalassemia Major*

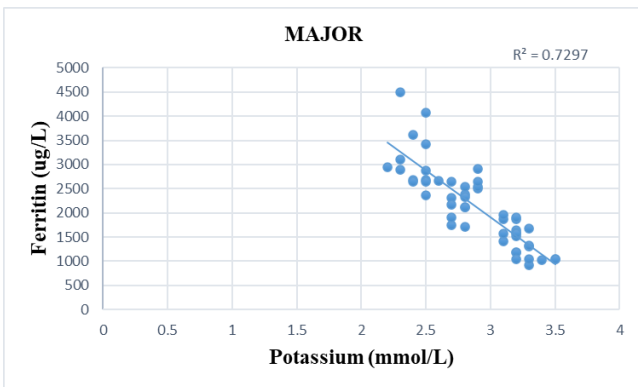**Fig. 4.** *Correlation between ferritin and sodium levels in Thalassemia Intermedia*

Fig. 5. *Correlation Between Ferritin and Potassium Levels in Thalassemia Major*
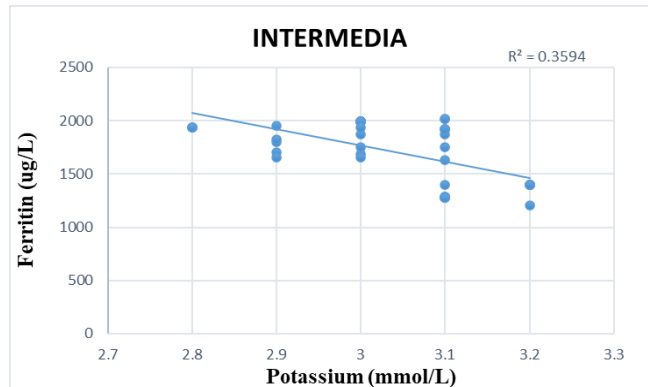

Fig. 6. *Correlation Between Ferritin and Potassium Levels in Thalassemia Intermedia*

and glomerular dysfunction in thalassemia patients (Uzun *et al.,* 2015). Sadeghi et al., 2021 found evidence of renal tubular disorder in patients with Thalassemia Minor, with at least one parameter of renal tubular dysfunction reported in 45% of patients.Therefore, the correlation was undertaken between RFTs, electrolytes, and serum ferritin levels in different variants of thalassemia patients who received regular blood transfusions and were poorly chelated. Mutations that affect the status of at least one globin gene are the main cause of thalassemia. The ß the-globin gene has been shown to contain roughly 200-point mutations and a few deletions on chromosome 11 (Musallam *et al.,* 2012). The body's iron deposits are normally kept between 200 and 1500 mg in persons, with a normal iron deposition of 5 mg/kg in women and 13 mg/kg in males (Baig *et al.,* 2006). Despite the fact that every unit of blood contains nearly 200 mg of iron, thalassemia patients' iron levels gradually and eventually rise as a result of continuous blood transfusions (Mariani *et al.*, 2009). The critical values of serum ferritin vary from 1000-3000 g/L in different studies to determine the toxic level of iron, and the standard values of serum ferritin levels have a very wide range in males (10-220 g/L) and females (10-85 g/L) in normal conditions (Hershko, 2010). Because iron levels and serum ferritin have a positive correlation, serum ferritin concentration is com-

monly used to calculate iron overload in thalassemia patients (Angulo *et al.,* 2008).

Transfused blood contains iron, which is rarely excreted by the body. On a typical blood transfusion routine, patients with beta-thalassemia accumulate iron at a rate of 0.3–0.6 mg/kg of body weight per day (Cappellini *et al.,* 2014; Standards for the clinical care of children and adults with thalassaemia in the UK, 2016). Similarly, in the present study, the average serum ferritin levels of Major, Intermedia, and Minor patients were 2212.5 (979.4), 1643 (312.7), and 53.55 (17.44), indicating iron overload in regularly transfused patients. Furthermore, it was discovered that creatinine, sodium, and potassium levels were lower in patients who were transfused on a regular basis, which could be due to an increase in the glomerular filtration rate (Tortora and Derrickson, 2009).Thalassemia increases creatinine clearance and glomerular filtration rate. Anaemia may lower systemic vascular resistance, resulting in hyperdynamic circulation that raises renal plasma flow and GFR (Davis and Hohimer, 1991). These changes can eventually cause glomerular capillary wall stretching and subsequent endothelial and epithelial injury, as well as macromolecule transudation into the mesangium, which is associated with glomerular dysfunction (Lafferty *et al.,* 1991). In the long run, such changes may result in a progressive decrease in GFR. Furthermore, chronic hypoxia

causes apoptosis or epithelial-mesenchymal transition in tubular cells with increased metabolic demand, resulting in tubulointerstitial injury and subsequent glomerulosclerosis and kidney fibrosis (Nangaku, 2006; Ponticelli *et al.,* 2010). Whereas uric acid levels were higher only in Intermedia, which was expected given the increased cellular turnover caused by ineffective erythropoiesis and the use of hydroxyurea (Samuels and Howe, 1964). According to studies comparing blood biochemistry and urinalysis in ß-thalassemia major and thalassemia intermedia patients, Ali et al. (2008) recently reported that hyperuricemia and microscopic haematuria are more common in thalassemia intermedia than in thalassemia major.

Renal parameters and electrolytes such as creatinine, sodium, and potassium levels were found to be significantly negatively correlated with p-value <0.001 in transfusion-required variants such as Thalassemia Major and Intermedia. According to the findings of this study, iron overload may be the cause of the renal manifestations of thalassemia; however, other factors, such as iron chelation therapy and oxidative damage, may also be involved.

Beta-thalassemia patients frequently have renal tubular abnormalities. The degree of anaemia correlated with the severity of the damage, with the least severe patients receiving a hyper transfusion and iron chelation therapy, implying that the damage was caused by anaemia and increased oxidation caused by excess iron deposits (Porter *et al.,* 2011). In patients with iron overload, the Fenton reaction increases free radical production. These free radicals accumulate in the liver, heart, and other organs, causing havoc and causing extensive tissue damage (Sumboonnanonda *et al.,* 2003).

Conclusion

The findings suggest that renal tubular function is impaired in beta-thalassemia variants and may be related to iron overloading. Early detection of patients at high risk of developing renal failure is critical because it may allow taking specific measures to delay the progression of renal damage and thus reduce the incidence of end-stage renal failure and mortality. The study's main limitations were the small number of patients in each group and the lack of more specific tests for the evaluation of tubular dysfunction. Following that, more specific tests such as ultrasound could be added as a confirmatory test for kidney functioning to investigate the relationship between iron overloading.

Conflict of interest
The authors declare that they have no conflict of interest.

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