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 thalassaemia is beta-thalassaemia, which is especially prevalent in the Mediterranean region. Thalassaemia 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-
Biochemistry and Paediatrics at MGM Medical College sectional study was conducted in the Department of From January to July 2021, a descriptive cross-sectional study was conducted in the Department of MATERIALS AND METHODS
variants of thalassemia. uric acid, sodium, potassium and chloride in different kidney parameters such as serum creatinine, urea, and renal function test. It includes increased renal blood flow, urine concentration defect, and renal tubular acidosis (Tantawy et al., 2014). The independent impact 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). Recent advancements in these individuals' medical care have resulted in a higher survival rate and a greater awareness of unexpected consequences (Kohgyoy et al., 2008). The aim of the present study was to determine 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 committee approved the project.
All participants provided a detailed clinical history, which included demographic information, anthropometric measurements, frequency of blood transfusion, and annual blood requirement. A 5ml blood sample was taken from each participant and transferred to an EDTA 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 renal function test with electrolytes and ferritin. Complete blood count was performed using a cell counter Sysmex XN-1000, Renal function test and electrolytes 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%) thalassemia minor, respectively. Amongst the enrolled subjects, it was noticed that percentage of female subjects 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>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Mean± SD</th>
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<tbody>
<tr>
<td></td>
<td>Major (n=50)</td>
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<tr>
<td>RBCs (10(^{12})/L)</td>
<td>3.31 ± 0.12(^*)</td>
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<tr>
<td>Pre-haemoglobin (mmol/L)</td>
<td>4.47 ± 0.95(^*)</td>
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<tr>
<td>MCV (Fl)</td>
<td>77.69 ± 4.38(^*)</td>
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<tr>
<td>MCH (pg./cell)</td>
<td>26.54 ± 1.86(^*)</td>
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<tr>
<td>MCHC (g/dl)</td>
<td>34.20 ± 1.04</td>
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<tr>
<td>Ferritin (ug/L)</td>
<td>2212.5 (979.4)(^*)</td>
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\(^*\)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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Major (n=50)</th>
<th>Intermedia (n=48)</th>
<th>Minor (n=50)</th>
<th>Control (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (umol/L)</td>
<td>27.41 ± 5.31</td>
<td>36.25 ± 6.19</td>
<td>67.2 ± 8.64</td>
<td>56.59 ± 5.31</td>
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<tr>
<td>Urea (mmol/L)</td>
<td>9.01 ± 1.73</td>
<td>7.85 ± 1.74</td>
<td>7.45 ± 0.81</td>
<td>7.09 ± 1.29</td>
</tr>
<tr>
<td>Uric acid (umol/L)</td>
<td>236.14 ± 28.55</td>
<td>361.64 ± 64.83</td>
<td>256.36 ± 39.85</td>
<td>297.4 ± 2.38</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>128.02 ± 4.56</td>
<td>128.96 ± 4.39</td>
<td>141.34 ± 3.79</td>
<td>137.9 ± 3.45</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>2.85 ± 0.23</td>
<td>3.04 ± 0.11</td>
<td>4.39 ± 0.33</td>
<td>4.21 ± 0.44</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>97.84 ± 2.60</td>
<td>98.94 ± 2.36</td>
<td>100.7 ± 1.47</td>
<td>99.9 ± 1.31</td>
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</table>

*p<0.001 patients versus controls*
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 commonly 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 β-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 β-thalassemia variants and may be related to iron overload. 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 overload.

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

REFERENCES


