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Correlation of Serum Ferritin Levels with Liver Iron Overload on Magnetic Resonance Imaging in Thalassemia Major Patients

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ABSTRACT

Blood transfusions are often necessary for people with thalassemia major, which causes iron buildup in critical organs. **Objectives:** To determine the correlation between Serum Ferritin and MRI-based liver iron levels. **Methods:** This cross-sectional analytical study was conducted on 101 beta thalassemia major patients visiting Shahida Islam Medical College and Hospital, Lodhran, in-between July 2024 to December 2024 using a non-probability consecutive sampling technique. Patients of Beta thalassemia major older than 15 years and dependent on blood transfusions were included in the study. Patients having any cardiac disease (valvular or congenital) or with any ongoing infection were excluded from the study. Patients who did not follow up regularly and did not consume chelation were also excluded. The association between serum ferritin concentrations and hepatic iron levels measured by T2 MRI was assessed using Spearman's correlation, with a p-value below 0.050 considered significant. **Results:** In this study of 101 thalassemia major patients, serum ferritin levels showed a significant negative correlation with liver iron overload measured by MRI (r=-0.25, p=0.030). Moderate hepatic iron overload was the most common finding (41.58%). Serum ferritin and AST levels increased significantly with worsening iron burden, while other liver function parameters showed no significant variation across groups. MRI proved more reliable than serum ferritin alone for assessing hepatic iron load. **Conclusions:** This study demonstrated a significant correlation between serum ferritin levels and hepatic iron overload evaluated through T2-weighted MRI in thalassemia major patients.

INTRODUCTION

Thalassemia represents a genetic disorder transmitted in an autosomal recessive pattern, in which hemoglobin formation is significantly impaired [1]. In the majority of patients, especially those with the major type of thalassemia, blood transfusions are frequently required, leading to iron accumulation in multiple organs [2]. The liver, heart, and endocrine systems are the organs where iron builds up the most often. The first organ to be loaded with iron is the liver [3]. In the initial phase of iron accumulation, binding of transferrin takes place with an excess of iron. When transferrin surpasses the capacity to bind iron, then free iron starts appearing and accumulating in organs [4]. Iron, in its free state, is toxic to cells, leading to damage to tissues, causing significant mortality and morbidity. It may result in life-threatening complications, which include arrhythmia, liver fibrosis, heart failure, etc. [5]. Humans lack an active system for eliminating excess iron. Nearly all thalassemia major patients who receive

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regular blood transfusions tend to slowly accumulate toxic amounts of iron over the years and show symptoms by age 10 years or so, sometimes even earlier [6]. By the time they reach early adolescence, they tend to have potentially lethal levels of iron in their body. If left untreated or if treatment is delayed, thalassemia-associated iron toxicity is regarded as the single most common factor causing organ damage [7]. Chelation therapy tends to remain the most effective method for alleviating excess iron overload. Therefore, it is vital to accurately assess the exact quantity of iron overload to manage chelation in beta thalassemia patients receiving transfusions [8]. Adjusting doses of iron chelation is of utmost importance in managing patients' extent of iron overload. Various known methods are available for measuring the overload of iron. Serum ferritin is often used indicator of iron overload, but it is also known to be an acute-phase protein; therefore, multiple factors can influence its levels, such as chelation, vitamin C levels, inflammation, infection, and damage to the liver [9]. On a clinical basis, levels of serum ferritin (SF) reflect the liver stores of iron. Even though a broad correlation exists in-between liver stores of iron and SF, predicting iron overloading from SF levels tends to remain unreliable [10]. In individuals with thalassemia major, serum ferritin is frequently utilized as a surrogate biomarker to measure body iron reserves. It is inexpensive and widely available; however, its reliability can be limited due to fluctuations influenced by inflammation, infection, or liver dysfunction. In contrast, Magnetic Resonance Imaging (MRI), particularly T2 or R2 techniques, is a more precise method of evaluating iron overload since it enables the direct and non-invasive measurement of liver iron content. Studies have shown that while there is often a correlation between serum ferritin and liver iron load measured by MRI, the strength of this association can vary. Thus, MRI serves as a confirmatory method to validate elevated serum ferritin levels and to guide iron chelation therapy more precisely [11]. Liver biopsies were the gold standard for detecting iron excess until recently. However, due to its invasiveness, it was linked with multiple complications [12]. Additionally, iron's distribution within liver cells is uneven, and so it's difficult to attain the best specimen for biopsy [13]. At present, biopsy is increasingly being replaced by Magnetic Resonance Imaging (MRI). A T2 MRI can reliably measure iron concentration in liver cells as well as the heart. It has the benefit of being non-invasive and beneficial for chelation therapy for individual patients [14]. Studies have shown a lack of correlation in-between the iron content of the liver and the iron content of the myocardium. Previously, SF levels were thought to be correlated with iron load in various organs. Recently, studies have demonstrated that other conditions, such as infection, inflammation, and chronic disorders, also influence SF levels [15]. MRI, a reproducible and highly sensitive technique, detects the iron content of tissues. Its ability to enhance tissue's susceptibility towards MR intracellular makes it the recommended choice for measuring excess iron overload. It can help detect increased iron levels even before the occurrence of symptoms [16]. The iron load in thalassemia patients has been evaluated in the literature using techniques such as total iron binding capacity, serum ferritin, serum iron, echocardiography, liver biopsy, and T2 MRI [17]. There has been a mixed association between ferritin levels and iron buildup in organs. However, its levels may be affected by infection, inflammation, or any chronic/malignant condition. As a result, MRI with gradient echo T2 is the gold standard technique for non-invasive iron detection [18].

TRegular blood transfusions in patients with beta thalassemia major lead to progressive iron accumulation in vital organs, particularly the liver, resulting in serious complications if not monitored properly. Although serum ferritin is widely used as a convenient and inexpensive biomarker to estimate body iron stores, its reliability is limited because it can be influenced by inflammation, infection, and liver dysfunction. Magnetic Resonance Imaging (MRI), especially T2-weighted techniques, provides a more accurate and non-invasive assessment of hepatic iron concentration. However, limited local studies have evaluated the correlation between serum ferritin levels and MRI-based liver iron overload in thalassemia patients, highlighting the need for further investigation. This study aimed to determine the correlation between biochemical markers of iron overload and MRI-based liver iron levels.

METHODS

This cross-sectional study was conducted on 101 beta thalassemia major patients at Shahida Islam Medical College and Hospital, Lodhran, from July to December 2024, with approval from the Institutional Review Board (IRB No. SIMC/ET.C./00031/24). A total of 140 patients were initially recruited through a non-probability consecutive sampling technique from the hospital's wards and outpatient department. Patients older than 15 years with a confirmed diagnosis of beta thalassemia major requiring blood transfusions were included, while those with cardiac disease (valvular or congenital), ongoing infections, irregular follow-up, or poor compliance with chelation therapy were excluded. The required sample size was calculated using the OpenEpi online tool with the formula $n = [DEFF \times Np(1-p)] / [(d^2/Z^2 1-\alpha/2 \times (N-1) + p(1-p)]$. Based on a hypothesized prevalence of 7% in Pakistan, with a 95% confidence level, 5% margin of error, a population size of 1,000,000, and a design effect of 1, the minimum sample size was estimated at 101 participants. After obtaining

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informed consent, data were collected on a structured proforma, including baseline demographics, biochemical measurements, and MRI findings. Liver enzymes (ALT, AST, and SGPT) were measured using commercial colorimetric kits (Randox, Crumlin, UK). Hemoglobin levels were estimated by the cyanmethemoglobin method, considered the gold standard: 20 µL of blood was mixed with Drabkin's reagent (potassium ferricyanide and potassium cyanide), allowed to react at room temperature for 5 minutes, and absorbance was measured at 540 nm using a spectrophotometer against a standard calibration curve. Ferritin levels were quantified with a human ELISA kit (EH300RB, Thermo Fisher, Lithuania), and optical density was measured at 450 nm using a Stat Fax 2100 reader (USA). Hepatic iron status was evaluated by magnetic resonance imaging on a 1.5 Tesla Siemens scanner (Germany). T2 relaxation times were processed with CMR Tools (London, UK), and patients were classified as normal (T2 > 6.3 ms), mildly overloaded (2.8–6.3 ms), moderately overloaded (1.4–2.8 ms), or severely overloaded (<1.4 ms) [19]. Data management and statistical testing were performed with SPSS version 23.0. The Shapiro–Wilk test analyzed variables distribution. Relationships between MRI-derived iron levels and biochemical markers (ALT, AST, SGPT, and serum ferritin) were assessed using Spearman's rank correlation, and group differences across iron overload categories were compared using the Kruskal–Wallis test. A p-value below 0.050 was considered statistically significant.

RESULTS

There were 101 individuals with beta thalassemia major in the research. Participants' median age was 21 years (IQR: 19–26 years). Among them, 63 (62.38%) were male and 38 (37.62%) were female. Diabetes mellitus was present in 24 (23.76%) patients. The median serum ferritin level was 3550 ng/ml (IQR: 2275–5950). Hepatic iron overload, assessed by MRI, was found to be moderate in 42 (41.58%) patients, while 26 (25.74%) each had normal or mild overload, and 7 (6.93%) had severe overload. The median ALT, AST, and ALP values for liver function were 42 U/L (IQR: 21–69), 39 U/L (IQR: 25–52), and 328 U/L (IQR: 200–495), respectively. The median levels of direct and total bilirubin were 0.32 mg/dL (IQR: 0.23–0.45) and 2.3 mg/dL (IQR: 1.7–3.8), respectively (Table 1).

Table 1: Baseline Demographics of Patients Included in the Study (N=257)

Variables		Median (IQR) / Frequency (%)	p-Value
Age (Years)		21 (19-26)	–
Gender	Male	63 (62.38 %)	0.180
	Female	38 (37.62 %)	
Diabetic status		24 (23.76 %)	–
Serum Ferritin (ng/ml)		3550 (2275-5950)	–
Hepatic Iron Load (T2 MRI)	Normal	26 (25.74 %)	<0.002
	Mild Overload	26 (25.74 %)	
	Moderate Overload	42 (41.58 %)	
	Severe Overload	07 (6.93 %)	
Liver Function Tests	ALT (u/L)	42 (21-69)	<0.001
	AST (u/L)	39 (25-52)	
	ALP (u/L)	328 (200-495)	
	Total Bilirubin (mg/dl)	2.3 (1.7-3.8)	
	Direct Bilirubin (mg/dl)	0.32 (0.23-0.45)	

*Chi-square and Kruskal-Wallis test applied

Across different grades of hepatic iron overload, T2' MRI values showed a marked decline with rising iron accumulation (p<0.001). Gender distribution and diabetes status did not differ significantly among the groups (p=0.210 and p=0.320, respectively). Hemoglobin levels tended to decrease with higher iron load, although the change was not statistically significant (p=0.080). Serum ferritin levels increased significantly with worsening iron overload, rising from 2275 ± 1825 ng/ml in the normal group to 5950 ± 3150 ng/ml in the severe group (p<0.001). Among liver enzymes, AST showed a significant elevation (p=0.02), while ALT, ALP, total bilirubin, and direct bilirubin remained statistically unchanged across the groups (Table 2).

Table 2: Comparison of Various Variables with Different Iron Deposition Levels on T2 Hepatic MRI (N=101)

Variables		Hepatic Iron Load				
		Normal (n=26)	Mild (n=26)	Moderate (n=42)	Severe (n=7)	p-Value
T2' MRI		8.2 (6.8-14.2)	3.8 (3.4-5.0)	2.3 (1.7-2.5)	1.1 (0.8-1.3)	<0.001*
Gender	Male	14 (13.86 %)	22 (21.78 %)	22 (21.78 %)	05 (4.95 %)	0.210
	Female	12 (11.88 %)	04 (3.96 %)	20 (19.8 %)	02 (1.98 %)	
Diabetes	Yes	03 (2.97 %)	06 (5.94 %)	08 (7.92 %)	07 (6.93 %)	0.320
	No	23 (22.77 %)	20 (19.8 %)	34 (33.66 %)	0	
Hemoglobin (mg/dl)		10.1 ± 1.1	9.8 ± 0.9	9.7 ± 1.0	8.9 ± 0.9	0.080
Serum Ferritin (ng/ml)		2275 ± 1825	2870 ± 1275	4250 ± 2875	5950 ± 3150	<0.001*
ALT (u/L)		25 (21-38)	31 (26-40)	36 (29-46)	42 (25-69)	0.080
AST (u/L)		28.5 (25.2 -29.5)	32 (28.4 -36.5)	39.5 (31.2-44.5)	42.5 (38.5 -52)	0.020
ALP (u/L)		270 (200 -325)	385 (252 -430)	327 (280-410)	426 (325 -495)	0.310
Total Bilirubin (mg/dl)		1.7 (1.4 -2.0)	2.8 (2.1 -3.1)	2.6 (3.5-4.1)	3.0 (2.9 -3.6)	0.220
Direct Bilirubin (mg/dl)		0.28 (0.2 -0.32)	0.32 (0.26 -0.35)	0.39 (0.24 -0.43)	0.34 (0.33 -0.45	0.380

*Chi-square and Kruskal-Wallis test applied

A correlation analysis revealed that serum ferritin levels were inversely related to hepatic iron load on MRI, showing statistical significance (r=–0.25, p=0.030) (Figure 1).

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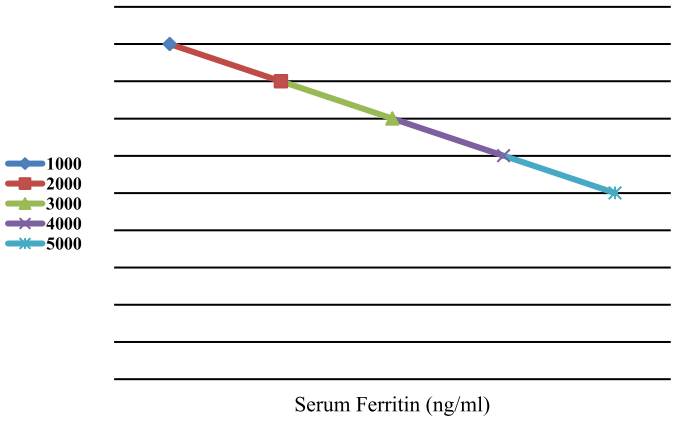

Figure 1: Correlation of Serum Ferritin with Liver Iron Load [Spearman Correlation Applied](r=-0.25, p=0.030)

DISCUSSION

The study demonstrated a strong association between serum ferritin (SF) levels and hepatic iron accumulation in patients with beta thalassemia major, as measured by T2 MRI. Significant iron overload was observed in seven patients (6.93%). Patients with severe hepatic iron deposition exhibited higher AST and ALT levels compared to those with normal, mild, or moderate iron overload. However, a statistically significant association was observed only with AST levels. T2 MRI was preferred for iron quantification because it is non-invasive, safe, rapid, and reproducible, providing an indirect measure inversely correlated with intracellular iron deposition [20]. Consequently, T2 MRI is regarded as the gold standard for diagnosing and monitoring iron status in thalassemia major patients [21]. Consistent with our findings, Voskaridou et al. reported a substantial correlation between heart and liver T2 values and SF levels [22], while Fischer *et al.* observed similar results. Unlike our study, their research included cardiac iron assessment; due to financial constraints, cardiac T2 MRI was not evaluated in our study [23]. Other studies also reported significant correlations between SF levels and liver T2 MRI but found no significant association with cardiac T2 values [24]. Although SF is commonly used to estimate body iron stores in beta thalassemia major, its reliability is limited by co-existing clinical conditions such as inflammation, liver disease, or infection, which can alter SF levels [25]. Liver diseases are frequent in thalassemia major and are typically reflected by elevated AST and ALT levels. Our findings are in line with previous studies, including Mohammad *et al.*, who reported a significant positive correlation between ALT and SF levels [26]. In contrast, current study observed a significant correlation only with AST. Despite evaluating both SF levels and hepatic MRI for iron load, this study has limitations, including a small sample size, single-center design, and cross-sectional nature, which restricted patient follow-up and assessment of chelation therapy effectiveness. Future multi-center, longitudinal studies are warranted to validate these findings. Integration of MRI-based T2* assessments into routine clinical protocols could provide a reliable, non-invasive estimate of hepatic iron concentration, overcoming the limitations of SF measurements influenced by inflammation or liver dysfunction. Wider adoption of these imaging techniques may allow early detection of iron overload, timely optimization of chelation therapy, and reduced risk of iron-related complications. Establishing population-specific reference ranges and cost-effective imaging protocols could further enhance the feasibility of MRI-guided monitoring, particularly in resource-limited clinical settings.

This study has several limitations that should be considered while interpreting the findings. The research was conducted at a single center with a relatively small sample size, which may limit the generalizability of the results to the wider thalassemia population. In addition, the cross-sectional design did not allow long-term follow-up to evaluate changes in iron overload or response to chelation therapy. Future multicenter longitudinal studies with larger cohorts are recommended to further validate the relationship between serum ferritin and MRI-based iron assessment and to optimize monitoring strategies for thalassemia patients.

CONCLUSIONS

This study demonstrated a statistically significant correlation between serum ferritin levels and hepatic iron overload, as assessed by T2-weighted MRI, in patients with thalassemia major. Although serum ferritin remains a convenient and accessible biomarker for monitoring iron status, its variability can limit its accuracy in reflecting true liver iron overload. MRI-based assessment offers a more precise evaluation of hepatic iron overload and should be integrated into routine monitoring protocols, particularly in patients receiving regular transfusions.

Authors' Contribution

Conceptualization: MYK
Methodology: NM
Formal analysis: SA, NN
Writing and Drafting: SA, NN, KA, SI
Review and Editing: SA, NN, KA, SI, NM, MYK

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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