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Dysregulation of Glucose Homeostasis in Transfusion-Dependent Thalassaemia Patients With Iron Overload as Risk Factor of Endocrinopathies: A Comparative Study

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Author`s Contribution

All authors contributed significantly to this work, participating in conception, study design, data acquisition, analysis, and interpretation. They were involved in drafting, revising, and critically reviewing the article, ultimately giving their approval for the version to be published

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ABSTRACT

Objective: To provide insights into potential endocrine complications associated with elevated SF levels in patients with BTM.

Methodology: This cross-sectional study, conducted at the Thalassaemia Center of Pakistan Institute of Medical Sciences (PIMS), Islamabad, investigated the association between plasma glucose and serum ferritin (SF) levels in BTM patients. Demographic variables, number of annual blood transfusions, and age at transfusion-dependent thalassaemia (TDT) diagnosis were recorded. Ferritin levels were categorized using a cutoff of 2500 ng/mL, and glucometabolic status was assessed via an oral glucose tolerance test (OGTT). Statistical analysis was performed using IBM SPSS Statistics, Version 27.

Results: The study included 118 participants divided into two groups based on serum ferritin (SF) levels (>2500 ng/mL and <2500 ng/mL). Patients with SF levels >2500 ng/mL showed a higher percentage of impaired glucose tolerance (75.0%) compared to those with SF levels <2500 ng/mL (54.8%), with a small percentage having diabetes mellitus (7.1%). Linear regression revealed a significant predictive relationship between SF levels and blood glucose levels ($F(1,116) = 21.4$, $p < 0.05$, $R^2 = .156$). Additionally, a chi-square test showed significant results for SF levels and 2-hour OGTT ($\chi^2(2, N=118) = 13.097$, $p = 0.001$).

Conclusion: Our study confirms the link between high serum ferritin levels and elevated risk of impaired glucose tolerance and diabetes mellitus. Specifically, serum ferritin levels above 2500 ng/mL indicate a heightened risk of endocrinopathies, particularly impaired glucose tolerance. These findings stress the importance of regular serum ferritin monitoring in thalassaemia patients undergoing blood transfusions to detect and manage glucose metabolism disorders promptly.

Keywords: Beta Thalassaemia, Ferritins, Glucose intolerance, Iron overload, Diabetes.

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Introduction

Beta thalassaemia major, an autosomal recessive disorder, manifests as defects in beta chain synthesis, presenting either in homozygous (Beta thalassaemia major, BTM) or heterozygous (thalassaemia minor) states. Globally, beta thalassaemia affects approximately 80 million carriers, with 23,000 babies born with thalassaemia major annually, predominantly in low-middle income countries.[1] In Pakistan, with an estimated 9.8 million carriers, BTM is a significant public health concern, with around 50,000 registered patients and 5,000-9,000 new cases annually, placing Pakistan among the countries with the highest transfusion-dependent thalassemia (TDT) prevalence.[2] BTM necessitates lifelong blood transfusions for sustaining hemoglobin levels leading to secondary iron overload and deposition.[3,4] Iron overload in TDT arises due to the inability of the human body to naturally

eliminate excess iron from transfused blood. Each unit of packed red blood cells contains elemental iron, and in TDT, frequent transfusions result in the accumulation of labile iron in plasma. This iron binds to transferrin for transport, saturating it and leading to the formation of non-transferrin-bound iron (NTBI), which is readily taken up by various organs including the liver, heart, and endocrine glands.[5] The excessive iron deposition in these organs contributes to cellular dysfunction through the production of reactive oxygen species, leading to apoptosis and necrosis. The process is mediated by transferrin receptors (TfR1 and TfR2), with TfR1 being more widespread and having a higher affinity for iron. Despite elevated iron levels, TfR1 is downregulated while TfR2 remains active in the liver, perpetuating iron accumulation. This pathophysiological process results in clinical complications such as cardiac siderosis, hepatic dysfunction, and endocrine abnormalities in TDT patients.[6]

In today's medical landscape, the outlook for individuals diagnosed with Thalassaemia Major (TM) has significantly improved. The majority of patients now live well into adulthood, thanks to advanced healthcare and effective chelation therapies, enabling them to surpass the age of 50.[7] However, this enhanced longevity brings with it a heightened prevalence of complications. Excessive iron accumulation in various organs, including the liver, joints, skin, heart, and endocrine glands, results in complications such as liver cirrhosis, arthritis, cardiomyopathy, congestive heart failure, and endocrinopathies.[8] Endocrinopathies, notably prevalent in transfusion-dependent thalassaemia(TDT), comprises of hypopituitarism, hypothyroidism, hypoparathyroidism, hypogonadism, impaired glucose tolerance, and growth abnormalities.[9] Disturbances in glucose homeostasis encompass a spectrum of conditions, ranging from heightened insulin resistance and mild glucose intolerance to the onset of overt diabetes mellitus. In individuals with milder forms of these disorders, symptoms may not be readily apparent, with impaired glucose tolerance (IGT) being a prevalent condition, affecting up to 24.1% of patients.[10–12]

In this study, our primary objective is twofold: firstly, we aim to investigate the association between plasma glucose levels and serum ferritin (SF) levels. Secondly, to identify potential dysregulation of glucose homeostasis in patients with iron overload, utilizing a cutoff value of serum ferritin >2500 ng/mL as risk factor for development of endocrinopathies. By addressing these objectives, we seek to elucidate the relationship between iron overload and impaired glucose metabolism, providing valuable insights into potential endocrine complications associated with elevated serum ferritin levels.

Methodology

This cross-sectional study was conducted at the Thalassaemia Centre of Pakistan Institute of Medical Sciences (PIMS), Islamabad, over a period of three months. Ethical approval was obtained from the Ethical Review Board Committee of Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad under notification number F.1-1/2015/ERB/SZABMU/1166, and informed consent was obtained from all participants or their legal guardians. Demographic variables including age and gender were recorded during data collection. Other variables such as the number of annual blood transfusions and age at TDT diagnosis were also documented. Ferritin levels, a marker of iron overload, were obtained from medical records. These levels were used to categorize participants having iron overload as risk factor for endocrinopathies based on a cutoff of 2500 ng/mL, as indicated in previous literature(6,13). Glucometabolic status was assessed using an oral glucose tolerance test (OGTT) conducted at 8:00 a.m. using standard protocols(14). Participants were classified as having normal glucose tolerance, impaired glucose tolerance (IGT), or diabetes mellitus (DM) based on American Diabetes Association criteria.[15]

Inclusion criteria for this study comprised individuals diagnosed with transfusion-dependent thalassaemia at least two years prior, aged two years or older, and receiving a minimum of twelve blood transfusions annually for at least two years. Exclusion criteria included recent initiation of transfusions within the last two year, a history of cancer or liver disease, medication use affecting glucose metabolism, prior marrow transplantation, diagnosis of primary hemochromatosis, hemolytic anemias other than BTM absence of consent from participants or their legal guardians, and presence of vital instability. Additionally, individuals with a family history of diabetes were excluded from participation. The statistical analysis for this study was conducted using IBM SPSS Statistics, Version 27.

Results

The study involved 118 participants, split into two groups based on iron overload status: 62 individuals in the iron overload group with SF levels>2500 ng/mL and 56

individuals in the iron overload group with ferritin levels<2500 ng/mL. In the iron overload group with SF levels>2500 ng/mL, the mean age was 13.25 years (SD ± 5.00), with an average of 19.21 transfusions per year (SD ± 8.63). These participants were diagnosed at a mean age of 13.14 months (SD ± 30.60), and their mean 2-Hour OGTT was 156.18 (SD ± 23.38). Conversely, in the iron overload group with SF levels<2500 ng/mL, the mean age was 9.68 years (SD ± 3.30), with an average of 16.90 transfusions per year (SD ± 6.72). They were diagnosed at a mean age of 9.58 months (SD ± 8.98), and their mean 2-Hour OGTT was 139.68 (SD ± 15.23). All patients were taking iron chelation therapy (ICT) while dosing and frequency of ICT was beyond the scope of this study.

Table 1:Demographic characteristics (n=118)

		Iron overload (SF<2500 ng/mL)	Iron Overload (SF>2500 ng/mL)
		N (%)	N (%)
Sex	**Male**	24(38.7%)	32(57.1%)
	Female	38(61.3%)	24(42.9%)
			Mean±SD
Age		9.68±3.30	13.25±5.00
Number of transfusions per year		16.90±6.72	19.21±8.63
Age of diagnosis in months		9.58±8.98	13.14±30.60
2-Hour PG (OGTT)		139.68±15.23	156.18±23.38

In transfusion-dependent thalassaemia patients, those with SF levels more than 2500 ng/mL showed a higher percentage of Impaired Glucose Tolerance (75.0%, n=42) compared to those with SF levels less than 2500 ng/mL (54.8%, n=34). Additionally, a small percentage of patients with SF levels more than 2500 ng/mL had Diabetes Mellitus (7.1%, n=4) as shown in Figure 1.

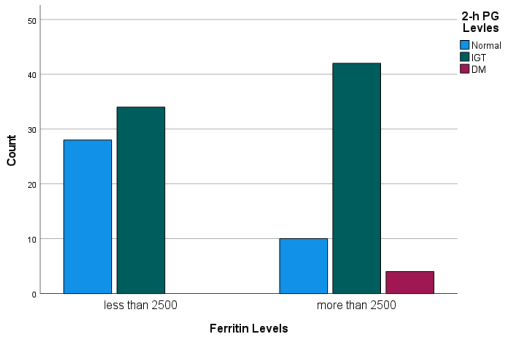

Fig. 1: Glucose homeostasis status

The data analysis employed linear regression to investigate the causal relationship between the independent variable (serum ferritin levels) and the dependent variable (glucose levels). Results revealed that the independent variable significantly predicts the dependent variable, as evidenced by $F(1,116) = 21.4$, $p < 0.05$. This finding indicates a substantial impact of serum ferritin levels on blood glucose levels. Additionally, the coefficient of determination (R^2) was found to be .156, suggesting that the model accounts for 15.6% of the variance in glucose levels, as explained by serum ferritin levels in the blood.

Following the establishment of cut-offs for both serum ferritin levels and 2-hour oral glucose tolerance test (OGTT), a chi-square test was conducted that also revealed significant results (χ^2 (2, N=118) = 13.097, p = 0.001).

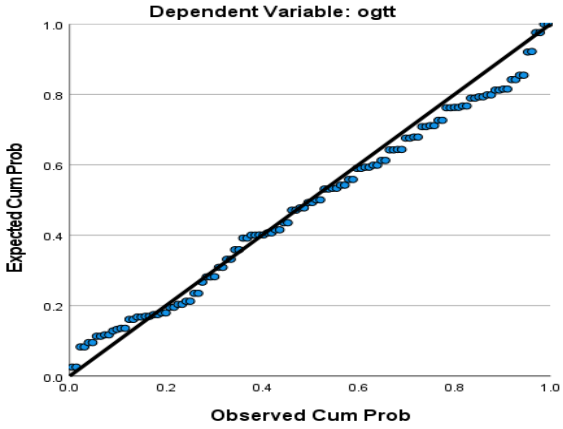

Fig. 2: Normal p-p Plot of Regression Standardized Residual

Discussion

In our study 52 percent of the participants were females. This finding aligns with the inheritance pattern of thalassemia, an autosomal recessive disorder, where both males and females have a similar risk of inheriting the condition from their carrier parents.

Interestingly, other studies have reported varying percentages of female participants, with some studies showing as high as 72.2 percent females, while others have reported a higher proportion of males.[16,17] These discrepancies could be attributed to differences in sample demographics, geographical locations, or study methodologies.

We found that individuals having SF levels below 2500 ng/mL were diagnosed with thalassaemia at a mean age of 9.58 months (±8.98) and those with SF levels exceeding 2500 ng/mL were diagnosed at a mean age of 13.14 months (±30.60). Interestingly, our findings align with those reported in several other studies. A study indicated that the majority of subjects were diagnosed with

Table II: Regression Analysis

Regression Weight	Beta Co-efficient	R^2	F	P-value	Null Hypothesis rejected
SF→GL	0.004	0.156	21.4	0.00	Yes

thalassaemia before reaching 2 years of age, with a significant proportion diagnosed before 1 year old.[18]

Similarly, study by Galanello and Origa noted that severe β-thalassaemia patients are typically diagnosed between 6 and 24 months of age.[16] Furthermore, study by Trehan et al. reported that a considerable portion of patients with severe β-thalassaemia were diagnosed before 1 year of age, with another third diagnosed between 12 and 24 months of age.[19] These findings collectively suggest that thalassaemia diagnosis often occurs within the first two years of life, highlighting the importance of early detection and screening efforts. Thus, early diagnosis remains crucial for timely intervention and management of thalassaemia to mitigate the risk of complications and improve patient outcomes.

Our study revealed that the serum ferritin (SF) levels differed based on mean age of individuals, with individuals having SF levels below 2500 ng/ml having a mean age of 9.68 years (±3.30), while those with SF levels exceeding 2500 ng/ml had a mean age of 13.25 years (±5.00). Additionally, our study found that SF levels also varied based on the average number of transfusions per year, with individuals having SF levels below 2500 ng/ml receiving an average of 16.90 transfusions per year (±6.72), and those with SF levels exceeding 2500 ng/ml receiving an average of 19.21 transfusions per year (±8.63). These findings align with study by Cario et al. which reported that 60% of patients undergoing transfusions have SF levels below 2500 ng/ml in the first decade of life, while 52% of patients older than ten years presented with ferritin levels above 2500 ng/ml, indicating the impact of age on SF levels.[20] Similarly, a study conducted by Ullah et al. in Pakistan found that the majority of beta thalassaemia patients with high SF concentrations (>2500 ng/mL) were aged more than 11 years.[21] Furthermore, a study conducted in Pakistan by SE Malik et al. highlighted the long-term implications of transfusion therapy, with a mean duration of transfusion of 13.6 years and a mean serum ferritin level of 5219.94 ng/ml, underscoring the cumulative effect of transfusions on iron accumulation.[22] Another study has emphasized the risk of iron toxicity with repeated transfusions, particularly after 10–20 consecutive transfusions, which may lead to secondary hemochromatosis.[23] Moreover, a study demonstrated a significant positive correlation between SF levels and the amount of transfusion received, indicating that intensive transfusion therapy contributes to iron loading proportional to the received blood volume.[18] Study by Mishra et al. supported these findings by highlighting that SF levels increase with the frequency of blood transfusions and the age of the patient, pointing out the importance of monitoring SF levels in thalassaemia patients to prevent iron overload-related complications.[24]

Overall, this phenomenon can be elucidated by the correlation between the frequency and duration for which transfusions are taking place, where an escalation in both factors corresponds to increased iron accumulation in the body, consequently heightening the risk of iron overload. It also demonstrates the complex interplay between age, transfusion therapy, and SF levels in thalassaemia management, emphasizing the need for tailored treatment strategies and close monitoring to optimize patient outcomes.

In our study, we adopted a serum ferritin (SF) level of 2500 ng/mL as a cutoff for identifying the risk of endocrinopathies, particularly impaired glucose tolerance (IGT) and diabetes mellitus (DM), based on previous literature. This cutoff was chosen in alignment with findings from other studies. Study by Ibrahim et al. demonstrated that endocrine dysfunctions were associated with SF levels in the range of 2501–3500 ng/mL, with the minimum range at which DM appeared being 1501–2500 ng/mL, suggesting that an upper bound of 2500 ng/mL can be considered a risk factor.[13] Similarly, Study by Taher et al. concluded that SF levels >2500 ng/mL can reliably predict endocrine diseases such as DM and IGT.[6] Our findings support the use of a SF level of 2500 ng/mL as a clinically relevant cutoff for identifying thalassaemia patients at increased risk of developing endocrinopathies. By identifying individuals above this threshold, healthcare providers can implement proactive monitoring and interventions to mitigate the risk of glucose metabolism disorders and improve overall patient outcomes.

Our study investigated the association between serum ferritin (SF) levels and glucose metabolism disorders in transfusion-dependent thalassaemia patients. We found that patients with SF levels exceeding 2,500 ng/mL exhibited a higher percentage of impaired glucose tolerance (IGT) compared to those with SF levels below 2500 ng/mL (75.0% vs. 54.8%, respectively). Additionally, a small percentage of patients with SF levels above 2500 ng/mL had diabetes mellitus (DM) (7.1%).

These findings are consistent with several other studies examining similar associations. A study reported an overall prevalence of impaired fasting glucose (IFG) and diabetes in thalassemic children under 18 years of 30% and 2%, respectively.[25] Similarly, another study observed a prevalence of IGT and DM of 2.1% to 37.9% and 1.0% to 26.8%, respectively, in beta thalassaemia major patients.[26] Study by Zhang et al. reported a prevalence of IFG, IGT, and DM of 40.68%, 14.41%, and 14.41%, respectively, further emphasizing the high prevalence of glucose metabolism disorders in thalassaemia patients.[27] In contrast, study by Dewiyanti et al. found no association between serum ferritin levels and impaired glucose tolerance or diabetes mellitus, despite using a higher ferritin cut-off point than for other endocrine disorders.[28] These contrasting findings highlight the complexity of the relationship between SF levels and glucose metabolism disorders, suggesting potential variability across different patient populations, methodological differences or compliance to ICT.

Conclusion

Overall, our study adds to the body of evidence indicating an association between elevated serum ferritin levels and an increased risk of impaired glucose tolerance and diabetes mellitus. Secondly, our findings confirm that the risk of endocrinopathies, particularly impaired glucose tolerance, escalates when serum ferritin levels exceed the cutoff of 2500 ng/mL. These results emphasize the importance of regular monitoring of serum ferritin levels in thalassaemiapatients undergoing regular blood transfusions to detect and manage potential glucose metabolism disorders early. Further research is imperative to elucidate the underlying mechanisms and explore potential interventions aimed at mitigating

The limitations of our study include its cross-sectional design, which makes definitive conclusions about associations and correlations challenging. Conducted solely at a single tertiary care hospital, the generalizability of our findings is limited without confirmation from multicenter studies with larger sample sizes. Additionally, we lacked hepatic iron data (Gold standard for investigating Iron overload) due to the absence of liver biopsies, primarily due to invasiveness and financial constraints. Patient height, weight, and BMI data were not obtained, and compliance with iron chelation therapy was not investigated.

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