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Assessment and Comparison of Thyroid Function Test and 2 D Echo Findings with Serum Ferritin Levels among Children with B Thalassemia Major

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Abstract

Introduction: Beta-thalassemia major is a severe hereditary blood disorder that requires regular blood transfusions and chelation therapy to manage iron overload, which can affect organs like the heart and thyroid. Thyroid dysfunction, particularly hypothyroidism, is common in these patients, as is cardiac impairment. The study aims to assess the relationship between iron overload, thyroid function, and cardiac health in children with beta-thalassemia major.

Objective: The objective is to evaluate how serum ferritin levels, a marker of iron overload, correlate with thyroid function and cardiac abnormalities in paediatric patients with beta-thalassemia major.

Methods: This cross-sectional study (June–December 2023) includes 80 participants: 40 children with Beta Thalassemia Major and 40 healthy controls. Cases, requiring regular transfusions, exclude those with lung/cardiac diseases or recent illness. Blood samples for serum ferritin and pre-transfusion haemoglobin (cases) will be analysed, and echocardiography will assess cardiac parameters and pulmonary artery pressure in all participants.

Results: Beta-thalassemia major patients showed significantly lower weight, height, and BMI compared to controls, along with larger cardiac dimensions, indicating cardiac remodeling from iron overload. Elevated TSH levels were found in 29% of cases, suggesting subclinical hypothyroidism, though Free T3 and Free T4 levels showed no significant differences. Ferritin levels were high in all patients but did not strongly correlate with thyroid dysfunction or cardiac abnormalities.

Conclusion: Children with beta-thalassemia major show impaired growth, increased risk of subclinical hypothyroidism, and cardiac remodelling, all associated

with iron overload. Regular monitoring of thyroid function and cardiac health, alongside effective chelation therapy, is essential to manage the complications of iron overload.

Keywords: Beta-thalassemia; iron overload; serum ferritin; thyroid dysfunction; cardiac health; paediatric;

Introduction

Beta-thalassemia major is a severe hereditary blood disorder that presents significant challenges in paediatric health due to its profound effect on red blood cell production. This condition is characterized by a genetic defect that disrupts the synthesis of the beta-globin chain in haemoglobin, leading to severe anaemia [1]. Children with this condition require lifelong blood transfusions, along with chelation therapy to manage the iron overload that results from frequent transfusions. Excessive iron accumulation in vital organs such as the heart, liver, and endocrine glands is a leading cause of complications, including heart disease, liver dysfunction, and various endocrine abnormalities [2].

Among the endocrine complications commonly seen in children with beta-thalassemia major, thyroid dysfunction is particularly prevalent. The thyroid gland, responsible for producing hormones that regulate metabolism, growth, and development, is vulnerable to damage from iron deposition. Consequently, many children with beta-thalassemia major develop hypothyroidism, a condition characterized by insufficient thyroid hormone production. This can further impair growth and development, emphasizing the need for regular monitoring through thyroid function tests (TFTs) to detect and manage these abnormalities early on [3].

In addition to thyroid dysfunction, cardiac complications are a leading cause of death in patients with beta-thalassemia major. Excess iron deposited in the myocardium can lead to cardiomyopathy, heart failure, and arrhythmias [4]. Two-dimensional echocardiography (2D Echo), a non-invasive imaging technique, is commonly used to assess the heart's structure and function. This allows clinicians to evaluate the impact of iron overload on the heart and guide appropriate treatment strategies. Regular monitoring of cardiac health is crucial, given the severe outcomes associated with iron-induced cardiac complications [5].

Serum ferritin levels are widely used as a marker for iron overload in patients with beta-thalassemia major. Elevated ferritin levels indicate excess iron in the body, making it essential to monitor these levels regularly to assess the effectiveness of chelation therapy and prevent iron-related complications. Although serum ferritin provides an accessible and cost-effective means of estimating iron burden, it is influenced by factors such as inflammation and liver disease, and may not always accurately reflect total body iron stores. As a result, additional assessments may be needed to evaluate organ-specific iron deposition [6].

The thyroid gland is a common target for iron deposition in beta-thalassemia major, given its rich blood supply and high metabolic activity. Hypothyroidism, both overt and subclinical, is the most frequently reported thyroid dysfunction in these patients. Overt hypothyroidism is characterized by elevated levels of thyroid-stimulating hormone (TSH) and reduced free thyroxine (T4) levels, while subclinical hypothyroidism involves elevated TSH levels with normal T4 levels. Left untreated, hypothyroidism can lead to growth delays, developmental issues, and other metabolic complications in children [7].

Previous studies have established a positive correlation between iron overload and thyroid dysfunction in beta-thalassemia major, with serum ferritin levels linked to both overt and subclinical hypothyroidism [8]. Thus, regular thyroid function tests, including TSH and T4 measurements, are essential to monitor thyroid health and initiate hormone replacement therapy when necessary. However, the precise serum ferritin threshold that increases the risk of thyroid dysfunction remains uncertain and likely varies among individuals, influenced by factors such as age, transfusion duration, and chelation adherence [9].

Cardiac complications are among the most serious consequences of iron overload in beta-thalassemia major, often leading to heart failure, arrhythmias, and sudden death. Iron deposition in the myocardium causes progressive damage, making early detection and intervention critical [10]. 2D Echo is a valuable tool for assessing cardiac function in these patients, providing detailed information on parameters such as left ventricular ejection fraction (LVEF), wall thickness, and diastolic function [11].

Studies have shown a significant relationship between high serum ferritin levels and abnormal 2D Echo findings, with elevated ferritin levels associated with reduced LVEF, increased left ventricular size, and impaired diastolic function [12]. These findings highlight the importance of regular cardiac screenings in children with beta-thalassemia major, especially in those with persistently high ferritin levels, to prevent irreversible heart damage.

Serum ferritin, despite its limitations, is the most commonly used marker for estimating iron overload in clinical practice [13]. Ferritin is an acute-phase reactant, meaning its levels can be elevated due to inflammation, infection, or liver disease, potentially leading to overestimation of iron stores. Nonetheless, serum ferritin remains widely used, with levels above 1,000 ng/mL often considered indicative of an increased risk of iron-related complications [14].

While serum ferritin provides a useful estimate of iron burden, it is important to recognize its limitations and consider additional assessments when necessary to accurately evaluate iron overload in vital organs, A comprehensive assessment of thyroid function, cardiac health, and iron overload in children with beta-thalassemia major can contribute to early detection, prevention, and management of complications, ultimately improving outcomes for children living with this condition [15].

The aim of this study is to evaluate the relationship between iron overload, thyroid function, and cardiac health in children with beta-thalassemia major. It involves analyzing thyroid function tests (TSH and T4), two-dimensional echocardiography (2D Echo) findings, and serum ferritin levels. The study seeks to assess how serum ferritin correlates with thyroid and cardiac dysfunction, exploring potential risks of hypothyroidism and cardiac abnormalities. Insights gained could improve early detection and management strategies, ultimately enhancing clinical outcomes for affected children.

Materials and Methods

This cross-sectional study, conducted from June to December 2023, includes 80 participants: 40 children with Beta Thalassemia Major and 40 healthy, age- and sex-matched controls, selected via purposive sampling. Cases require regular blood transfusions, excluding those with pre-existing lung/cardiac diseases or recent illness. Clinical histories, including transfusion details

and iron chelation therapy, will be documented. Blood samples for serum ferritin and pre-transfusion haemoglobin (cases) will be analysed using the Cobas 6000 analyser. Echocardiography will assess posterior wall thickness, left ventricular diameters, septal thickness, and tricuspid regurgitation velocity for pulmonary artery pressure estimation.

Results

In the 5 to 10 years age group, male cases (16) significantly outnumber male controls (8), while female controls (11) slightly exceed female cases (7). For the 10 to 15 years group, male controls (14) outnumber male cases (6), but female cases (11) are higher than female controls (7). This pattern suggests a greater representation of male cases in younger children, whereas male controls dominate in the older age group.

Table 1: Weight, Height, and BMI comparison between cases and controls

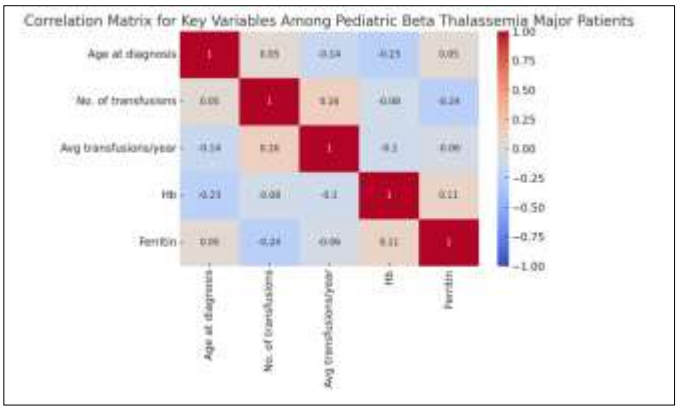
Variables	Cases	Control	Total	p-value
Weight (kgs)	24.12 ± 7.54	31.90 ± 9.93	28.01 ± 8.74	<0.001
Height (cms)	120.03 ± 12.41	135.38 ± 14.71	127.70 ± 13.56	<0.001
BMI	15.62 ± 1.58	16.93 ± 2.33	16.27 ± 1.96	0.004

The weight, height, and BMI of paediatric Beta Thalassemia Major patients are significantly lower than those of healthy controls. Cases have a mean weight of 24.12 ± 7.54 kg compared to 31.90 ± 9.93 kg in controls, with $p < 0.001$. Height and BMI are also lower in cases (120.03 cm, 15.62) than controls (135.38 cm, 16.93), indicating impaired growth ($p < 0.001$, 0.004).

Table 2: 2D Echocardiography comparison between cases and controls

Variables	Cases	Control	Total	p-value
PWT-D (cm)	0.62 ± 0.22	0.44 ± 0.07	0.53 ± 0.15	<0.001
PWT-S (cm)	0.60 ± 0.14	0.51 ± 0.08	0.55 ± 0.11	<0.001
LVID-D (cm)	5.70 ± 0.64	2.73 ± 0.55	4.22 ± 4.10	0.017
LVID-S (cm)	2.53 ± 0.24	1.79 ± 0.44	2.16 ± 0.34	<0.001

Pediatric Beta Thalassemia Major patients exhibit significantly larger cardiac dimensions compared to healthy controls. Cases have higher Posterior Wall Thickness in diastole (0.62 cm vs. 0.44 cm) and systole (0.60 cm vs. 0.51 cm), and larger Left Ventricular Internal Diameters in diastole (5.70 cm vs. 2.73 cm) and systole (2.53 cm vs. 1.79 cm), all with p-values < 0.001. This indicates substantial cardiac remodeling, likely due to iron overload.



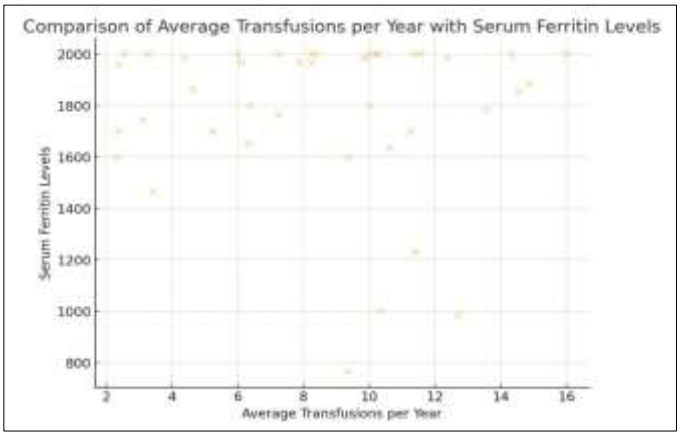
Graph 1: Correlation Matrix for key Variables among Pediatric Beta Thalassemia Major Patients

The correlation matrix shows weak relationships between age at diagnosis, transfusion frequency, hemoglobin (Hb) levels, and serum ferritin in pediatric Beta Thalassemia Major patients. Age at diagnosis is weakly negatively correlated with Hb levels (-0.23), and ferritin shows a weak negative correlation with transfusion frequency (-0.24). Other correlations, such as between ferritin and Hb (0.11), are non-significant. The heatmap visually represents these weak correlations using color gradients.

Table 3: Comparison of Thyroid Profile (Free T3, Free T4, and TSH) Between Pediatric Beta Thalassemia Major Patients and Healthy Controls

Thyroid profile	Cases (Mean ± SD)	Controls (Mean ± SD)	p-value
Free T3 (ng/dL)	134.62 ± 29.43	132 ± 21.71	0.651
Free T4 (ng/dL)	1.22 ± 0.26	1.31 ± 0.16	0.096
TSH (µIU/mL)	4.41 ± 2.74	2.84 ± 0.70	0.001

A statistical comparison of thyroid hormone levels in paediatric Beta Thalassemia Major patients and healthy controls shows no significant difference in Free T3 levels (p = 0.651). However, there is a trend towards lower Free T4 levels in cases (p = 0.096). A highly significant difference in TSH levels (p = 0.001) indicates elevated TSH in patients, suggesting potential thyroid dysfunction in this group.



Graph 2: Comparison of Average Transfusions per Year with Serum Ferritin levels

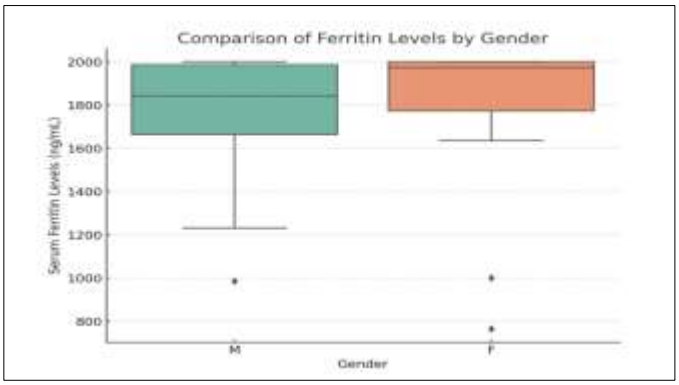
The scatter plot shows a very weak negative correlation (-0.06) between Average Transfusions per Year and Serum Ferritin Levels, suggesting little to no significant linear relationship between these variables. This indicates that transfusion frequency has minimal impact on serum ferritin levels in this dataset of paediatric Beta Thalassemia Major patients.

Table 4: Thyroid Profile Distribution by Age Group for Cases and Controls

Groups	Age group	Euthyroid	Subclinical hypothyroidism	Overt hypothyroidism
Case	5-10 years	13	10	2
	10-15 years	7	5	2
Control	5-10 years	21	2	1
	10-15 years	17	0	0
Total		58	17	5
Chi-square statistic: 18.75 and p-value: 0.0046				

The distribution of thyroid profiles shows a higher prevalence of subclinical hypothyroidism among Beta Thalassemia Major cases, especially in the 5-10 years age group. In contrast, controls are predominantly euthyroid. A chi-square test (χ^2 = 18.75, p = 0.0046)

confirms a significant association between thyroid dysfunction, age group, and case-control status.



Graph 3: Comparison of Serum Ferritin Levels by Gender among cases

Serum ferritin levels are slightly higher in females (1796.22 ng/mL) than males (1773.86 ng/mL), with more variability in females. Despite this, the chi-square test ($\chi^2 = 0.14$, $p = 0.71$) indicates no statistically significant difference in mean ferritin levels between male and female patients, as the p-value exceeds 0.05.

Table 5: Association of Thyroid Status with Ferritin level in cases

Thyroid Status	1000-2500	<1000
Euthyroid	22 (95.7%)	1 (4.3%)
Subclinical hypothyroid	16 (94.1%)	1 (5.9%)

The Mann-Whitney U test shows a p-value of 0.80, suggesting no statistically significant difference in serum ferritin levels between the Euthyroid and Hypothyroid groups. This indicates that ferritin levels are similar across both thyroid function groups in this dataset.

Discussion

Beta-thalassemia major is a severe inherited blood disorder that disrupts beta-globin production, causing anaemia. Children with this condition require lifelong blood transfusions and chelation therapy to manage iron overload, which can damage vital organs like the heart and thyroid. Thyroid dysfunction, especially hypothyroidism, is common, impairing growth and development. Regular thyroid function tests and cardiac monitoring through 2D echocardiography are essential. Serum ferritin levels, while used to estimate iron overload, may be inaccurate due to other factors like inflammation, requiring additional assessments [16].

During the study, 62 children attended for regular transfusions, with 40 meeting inclusion-exclusion criteria. Of these, 28 children (56%) were aged 5–10 years, and 12 (44%) were aged 10–15 years. The mean age was 9.84 years. A study at a tertiary care center found the common age group to be 10–12 years (51.43%) with a mean of 13.46 ± 3.67 years. Male predominance was noted, with 54% boys, similar to other studies. Indian literature, such as Trehan et al., reported male preponderance up to 69.5%, likely due to gender disparities in health-seeking behaviour for chronic conditions [17].

In this study, 54% of cases were diagnosed after the age of 1 year, while 46% were diagnosed before 1 year, indicating early disease presentation. The mean age of diagnosis was 1.35 ± 1.06 years, with a minimum age of 4 months and a maximum of 5 years. In a similar study by Trehan et al., PGI Chandigarh on children with thalassemia major, the average age of presentation was 17.2 ± 19.8 months, which aligns with our findings. In that study, 52% of cases were diagnosed before 1 year of age, comparable to the early presentation rate in this study [17].

Kattamis et al. (1975) reported the age of presentation for thalassemia major to be 13.1 months, ranging from 2 to 36 months. In contrast, a retrospective cross-sectional study by Joseph et al. conducted at two hospitals in Mangalore found the mean age at diagnosis to be 2.3 ± 2.4 years, which is higher than the mean age of 1.35 ±

1.06 years observed in our study. These variations may reflect differences in healthcare access, diagnostic practices, and awareness across regions and time periods. Trehan et al. reported a mean age of 1.43 ± 1.65 years, Kattamis et al. found 1.09 years, and Joseph et al. observed a higher mean age of 2.3 ± 2.4 years. Our study reports a mean age of 1.35 ± 1.06 years. These findings illustrate variations in the age of diagnosis, with Joseph et al. showing a notably higher age than other studies [17,18,19].

In our study, 60% of the cases had received more than 100 transfusions, while 40% had less than 100. In comparison, a study by Pattanshetti et al. reported that 94.2% of patients had over 100 transfusions, and only 5.8% had fewer, indicating a higher frequency of transfusions compared to our findings. Regarding transfusion frequency per year, 44% of the cases in our study received more than 10 transfusions annually, while 56% had fewer than 10. In a Sri Lankan study, 72% of cases received more than 10 transfusions per year, slightly higher than our study, suggesting more frequent transfusions in that population. These differences may reflect variations in treatment practices and healthcare access across different regions [20].

In our study, 84% of cases were underweight with a BMI <18.5 kg/m², and the mean BMI was 15.71, showing moderate significance (P = 0.036). A similar study at a Dehradun tertiary care center found 77% underweight, while Bhopal's study reported 17.64% using WHO BMI charts. Asadi-Pooya et al. (2004) found that 12.4% of thalassemia patients under 10 had a BMI below the 10th percentile, increasing to 46.5% for those over 10. The average weight in our study was 26.9 kg versus 32.98 kg in controls (P = 0.002), and the mean height was 127.2 cm compared to 137.58 cm (P = 0.002). The average transfusions per year ranged from 3.57 to 16.733, with a mean of 10.129, consistent with studies from Sri Lanka and Mangalore [21].

In our study, hemoglobin levels ranged from 5 to 9 g/dl, with a mean of 7.58 g/dl, similar to the findings of Rao JS et al. in the Mangalore study, where baseline hemoglobin was 7.3 ± 1.7 g/dl, and post-transfusion levels reached 10 g/dl, following guidelines to maintain levels above 9–10.5 g/dl. Ferritin levels ranged from 343 to 2000, with a mean of 1746.34 and a standard deviation of 421.25. Chelation therapy was given to 94% of cases. Cardiac parameters, evaluated via 2D echocardiography, showed strong significance for key measures like PWT-D, PWT-S, LVIDd, LVIds, ejection fraction, TR velocity, and septal thickness, aligning with other studies [22].

In our study, the TR velocity was 2.42 ± 0.25, which is below the threshold suggestive of pulmonary hypertension. According to Mohammed AM et al., a TR velocity greater than 2.5 indicates the presence of pulmonary hypertension. Although the mean TR velocity in our study is slightly lower than this threshold, it remains an important parameter to monitor in thalassemia patients, as elevated values can be a precursor to serious cardiac complications like pulmonary hypertension, warranting further clinical attention [23].

Conclusion

Children with Beta Thalassemia Major often experience impaired cardiac function, which is closely linked to high serum ferritin levels resulting from iron overload. Consistent blood transfusions and effective chelation therapy are crucial in managing this condition and reducing cardiac-related complications. Regular transfusions help maintain appropriate hemoglobin

levels, while chelation therapy removes excess iron, preventing damage to the heart and other organs. By ensuring these children receive consistent transfusions and proper chelation, we can significantly decrease the risk of morbidity and mortality, ultimately improving both their health outcomes and quality of life.

References

1. Galanello R, Origa R. Beta-thalassemia. Orphanet journal of rare diseases. 2010 Dec; 5:1-5.
2. Shawkat AJ, Jwaid AH. Clinical complications of beta-thalassemia major. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019 Dec 21;28(2):1-8.
3. Singhal A, Goyal H. Thyroid dysfunction in beta thalassemia major patients. Thyroid Research and Practice. 2020 May 1;17(2):70-5.
4. Chiew JY, Thiruchelvam J, Rahmat MA, William SP, Shafien ZI, Banerjee KG. The key complications of beta thalassemia major: a review and update. Int J Res Med Sci. 2021 Jun; 9:1846-52.
5. Chen R, Zhu M, Sahn DJ, Ashraf M. Non-invasive evaluation of heart function with four-dimensional echocardiography. PloS one. 2016 May 4;11(5):e0154996.
6. Kaddah AM, Abdel-Salam A, Farhan MS, Ragab R. Serum hepcidin as a diagnostic marker of severe iron overload in beta-thalassemia major. The Indian Journal of Pediatrics. 2017 Oct; 84:745-50.
7. Jerome J. Prevalence of Hypothyroidism, Hypoparathyroidism and Delayed Puberty in Children with β-Thalassemia Major (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
8. Akıncı B, Yenigurbuz FD, Üstyol A, Ökdemir D. Thyroid Dysfunction in Beta-Thalassemia Major: Is It Related to Autoimmunity or Iron Overload? Acıbadem Üniversitesi Sağlık Bilimleri Dergisi. 2023 Jul 10;14(3):377-82.
9. Jonklaas J. Optimal thyroid hormone replacement. Endocrine reviews. 2022 Apr 1;43(2):366-404.
10. Yadav PK, Singh AK. A review of iron overload in beta-thalassemia major, and a discussion on alternative potent iron chelation targets. Plasmatology. 2022 May; 16:26348535221103560.
11. Ari ME, Ekici F, Çetin İİ, Tavil EB, Yaralı N, Işık P, Hazırolan T, Tunç B. Assessment of left ventricular functions and myocardial iron load with tissue Doppler and speckle tracking echocardiography and T2* MRI in patients with β-thalassemia major. Echocardiography. 2017 Mar;34(3):383-9.
12. Chen MR, Ko HS, Chao TF, Liu HC, Kuo JY, Bulwer BE, Yeh HI, Hung CL. Relation of myocardial systolic mechanics to serum ferritin level as a prognosticator in thalassemia patients undergoing repeated transfusion. Echocardiography. 2015 Jan;32(1):79-88.
13. Kaddah AM, Abdel-Salam A, Farhan MS, Ragab R. Serum hepcidin as a diagnostic marker of severe iron overload in beta-thalassemia major. The Indian Journal of Pediatrics. 2017 Oct; 84:745-50.
14. Ho PJ, Hiwase D, Ramakrishna R, Viiala N, Solterbeck A, Traficante R, Zor E, Gervasio OL, High LM, Ross DM, Bowden DK. Cardiac and hepatic siderosis in myelodysplastic syndrome, thalassemia and diverse causes of transfusion-dependent anemia: the TIMES study. Hemasphere. 2019 Jun 1;3(3): e224.
15. Garcia-Casal MN, Pasricha SR, Martinez RX, Lopez-Perez L, Pena-Rosas JP. Are current serum and plasma ferritin cut-offs for iron deficiency and

overload accurate and reflecting iron status? A systematic review. Archives of medical research. 2018 Aug 1;49(6):405-17.

16. Shawkat AJ, Jwaid AH. Clinical complications of beta-thalassemia major. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019 Dec 21;28(2):1-8.
17. Trehan A, Sharma N, Das R, Bansal D, Marwaha RK. Clinicoinvestigational and demographic profile of children with thalassemia major. Indian Journal of Hematology and Blood Transfusion. 2015 Mar; 31:121-6.
18. Kattamis C, Metaxotou-Mavromati A, Wood WG, Nash JR, Weatherall DJ. The heterogeneity of normal Hb A2-β thalassaemia in Greece. British Journal of Haematology. 1979 May;42(1):109-23.
19. Joseph N, Pai S, Sengupta S, Bharadwaj S, Dhawan S, Khare K. A clinico-epidemiological study of thalassemia cases in India. Journal of Natural Science, Biology and Medicine. 2018 Jul 1;9(2).
20. Pattanashetti MA, Pilli GS, Pattanashetti LA. Blood Transfusion Profile of Beta Thalassemia Major Patients Attending a Tertiary Care Hospital. Annals of Pathology and Laboratory Medicine. 2017 Apr 19;4(2): A208-211.
21. Asadi-Pooya AA, Karamifar H. Body mass index in children with beta-thalassemia major. Turk J Haematol. 2004 Dec 1;21(4):177-80.
22. Rao JS, Nagendra K. A study conducted in a tertiary care hospital to assess the cardiac function in paediatric beta thalassemia major patients and correlation with serum ferritin levels which are indicative of chelation status of the patients. Indian Journal of Child Health. 2023;10(6):73-6.
23. Mohammad AM. Echocardiographic evaluation of thalassemia intermedia patients in Duhok, Iraq. BMC cardiovascular disorders. 2014 Dec; 14:1-7.