Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4050

ASSESSMENT OF SERUM FERRITIN IN BETA THALASSEMIA MAJOR PATIENTS: INSIGHTS FROM A THALASSEMIA CENTRE IN PAKISTAN

Wahid Ullah[1*], Parveen Anjum[2], Attiya Sultana[3], Marium Khan[4], Rafia Amin[5]

[1*]Lecturer at Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University, Islamabad, Pakistan
[2]Charge Nurse at Victoria Hospital, Bahawalpur, Punjab Pakistan
[3]Charge Nurse at Sadiq Muhammad Khan Abbasi Hospital, Bahawalpur, Punjab, Pakistan
[4]Charge Nurse at Parvaiz Elahi Institute of Cardiology, Bahawalpur, Punjab, Pakistan
[5]Charge Nurse at Sadiq Muhammad Khan Abbasi Hospital, Bahawalpur, Punjab, Pakistan

[*]**Corresponding Author:** Wahid Ullah
[*]Lecturer at Faculty of Rehabilitation and Allied Health Sciences (FRAHS), Riphah International University, Islamabad, Pakistan, wahid.ullah@riphah.edu.pk, Phone: 00923055006199

ABSTRACT
Beta thalassemia major is the most common monogenic hereditary hemoglobinopathy which poses a major health burden in Pakistan. Regular transfusion of RBC's (erythrocytes) required for survival of these patients which consequently leads to inevitable iron overload, which is demonstrated by elevated serum ferritin levels. Developing of iron excess may lead to organ damage and other life-threatening diseases. The aim of this study was to evaluate iron overload in beta thalassemia major patients in one of the thalassemia centers of Pakistan by means of their serum ferritin levels. The study was also conducted to approximate the current situation of iron overload in them. 155 blood samples of clinically diagnosed beta thalassemia major patients were collected from Jamila sultana foundation (JSF) for their serum ferritin estimation. Serum ferritin measurement was performed using indirect enzyme linked immune sorbent (ELISA) based serum ferritin assay kit. Demographics data of the patients were statistically analyzed by using SPSS 2.O and association between age, gender and serum ferritin levels were established. 85.4% of the beta thalassemia major patients showed very high ferritin levels. The mean serum ferritin level was found to be 4442 ± 2882 ng/ml. 44.4% patients had serum ferritin between 1000 to 2500 ng/ml, while 43.05% patients had values above 2500 ng/ml. Majority of the patients with high serum ferritin concentration (>2500 ng/mL) were aged more than 11 years. These levels reflect inadequate chelation and vulnerability to develop iron overload related complications. There is an urgent need to rationalize the chelation therapy and to create focus about the consequences of iron overload in these patients. The study showed elevated levels of serum ferritin beta thalassemia major patients which provide a normal bleak view.

Keywords: Beta thalassemia major, Iron overload. Ferritin level, red blood cells (RBCs)

INTRODUCTION
The term "thalassemia," which is derived from the Greek words "Thalassa" (sea) and "Haem" (blood) describes abnormalities linked to impaired synthesis of the α- or β-globin subunits of hemoglobin (Hb) A (α2; β2). Thomas Cooley and Lee in 1925 first described the term *β* Thalassemia major that

is why it is also known as Cooley's Anemia (1). Beta-thalassemia is an hereditary hemolytic anemia due to abnormal production of the chains of adult hemoglobin (Hb A) (2).Beta thalassemia is the most common monogenic hereditary hemoglobin disorder, which poses a major health burden in Pakistan (3). These alleles are inherited as pathologic alleles of one or more of the globin genes found on chromosomes 11 (β) and 16 (α)(4)..The absence or nonexistent of (beta^0) chains of the hemoglobin tetramer is the paramount cause of β thalassemia major (5).

β-Thalassemia major is a never-ending transfusion-dependent anemia because it requires regular transfusions (5). As each unit of packed cell unit contains approximately 200 mg of iron, a patient who is receiving 25 units per year would accumulate 5 gram of iron by the year in the absence of chelation (1).The human body have no physiological mechanism to actively remove excess iron. The accumulative effects of iron overload will accelerate significant morbidity and mortality, if left untreated (6).This "free" & excess iron has the ability to catalyze the production of abundantly injurious molecules from regular metabolic byproducts like hydrogen peroxide, such as the hydroxyl radical (OH) (7).The injurious reactive hydroxyl radical (OH) is free & can enter any cell and damage DNA, proteins, and lipids (8).

Serum transferrin, which is the primary iron transport protein, may reach its limit in its capacity to bind and detoxify iron as iron loading increases. After then, the portion of iron in plasma that is not bound by transferrin may invigorate the production of free hydroxyl radicals(OH), which spread oxygen-related harm (9).Overconsumption of iron is extremely noxious to all cells of the body and can cause severe and irreversible organic damage, such as cirrhosis, diabetes, heart disease, splenomegaly and hypogonadism (9).

Thalassemia is a global public fitness hassle. In the next 20 years, there could be an expected 900,000 births of clinically extensive thalassemia issues. Worldwide, the Asian, Indian, and Middle Eastern areas account for 95% of thalassemia births (10). This speedy growth in the thalassemia population from these regions is converting the scientific photograph of thalassemia global. Today's epidemiology of thalassemia is strikingly specific from that of the past (10, 11).The prevalence rate in Pakistan is 5-8%, and 5000 children are diagnosed with beta-thalassemia in Pakistan every year. Male race is the main reason for the high prevalence in Pakistan. There are 25,000 children registered with the Thalassemia Society of Pakistan but the actual figure is much higher which could be around one million, as many live in villages where they are not registered with any thalassemia center (12).

Previous studies have shown a significant relationship between high serum ferritin levels and growth failure of the beta thalassemia major patients (13) (14).The aim of this study is to evaluate the iron overload and growth status of the beta thalassemia patients in one of the thalassemia centers in Pakistan and to find out the relationship between them.

MATERIALS AND METHODS

This was a cross sectional study conducted on 155 patients of both Male and Female genders with confirmed diagnosis of Beta Thalassemia Major with recent multiple transfusions at Jamila Sultana Foundation (JSF) during the period from 01 September 2023 to 01 January 2024. The written and informed consents were obtained from all 155 patients to collect their demographic data.

All the patients are in the age limit between 06 moths to 30 years old. We categorized the subjects into three age groups because that provided us more sophisticated statistical analysis; 1 to 6 years old between 7 to 13 years old and, over 14 years old. The diagnosis of Beta Thalassemia was confirmed by their Hb electrophoresis. This study was approved by Research Ethical Committee of Jamila Sultana Foundation (JSF).

The inclusion criteria of this included patients suffering from beta thalassemia major which was confirmed by means of their Hb Electrophoresis. The age limit was considered between 1 to 30 years old and was categorized into three age groups because that provided us more comprehensive statistical analysis and the patients having proper history of Iron overload was considered as inclusion criteria. The exclusion criteria included history of Iron overload other than Beta Thalassemia major such as Iron Deficiency Anemia (IDA), Arrhythmia, Liver Cirrhosis, history of cardiac and metabolic diseases.

The estimation of serum ferritin levels was performed by collecting blood samples of all 155 patients in a gel tube separator by means of clean venipuncture technique. The serum ferritin tests were performed using ELISA Assay Kit (enzyme-linked immunoassay kit) in the department of clinical chemistry in Riphah international university pathology laboratory.

A standardized and validated pro forma was used by the interviewer to gather demographics information from all the patients during their hospital stay for regular blood transfusion. The pro forma was pre tested with some patients in JSF to ensure its feasibility and accuracy. The clinical details of patients were recorded in a pro forma, taking into account the Age, Gender, frequency of transfusions, MCV levels, MCH levels, HB A, HB A2, HB F percentages and Hemoglobin levels.

RESULTS

This study included a total of 155 cases of beta thalassemia major patients out of which 81 (52%) Females and 74 (48%) were Males. The mean age was 16.3 ± 1.2 years and at the time of data collection the age of patients of beta thalassemia ranged from 1 to 30 years old with only one subject having an age of just one month. The mean spleen size (in cm) of the subjects was 12.3 ± 0.8 with a mean Hemoglobin (g/dl) level of 11.1 ± 0.6 after the transfusion.

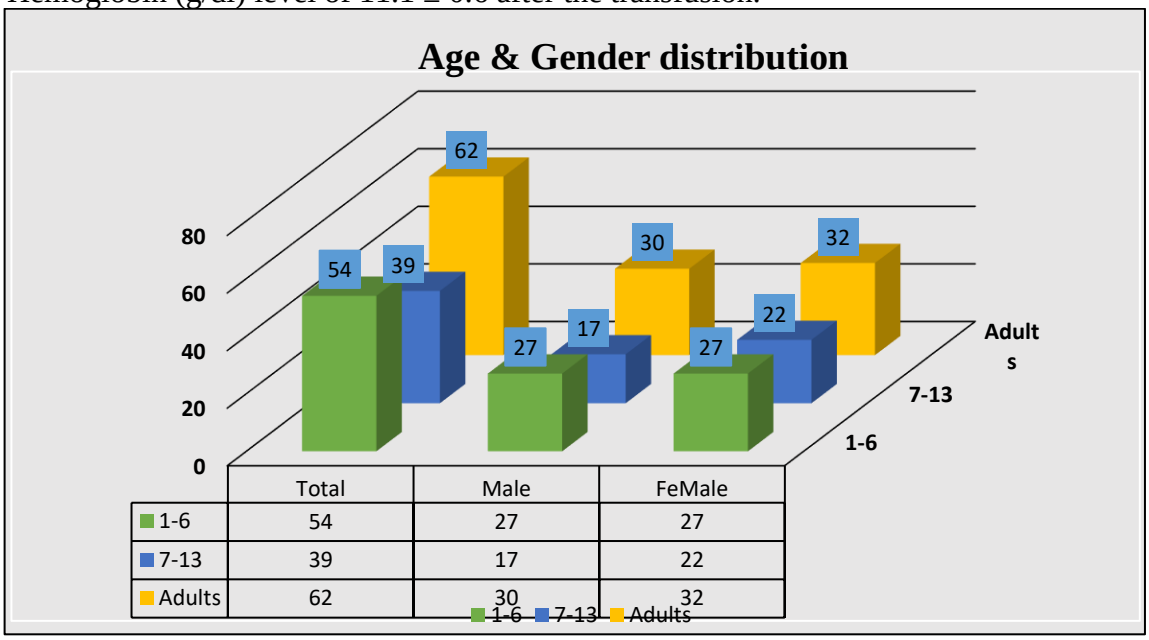

Figure 1.0: shows the study subjects categorized into 3 age groups.

The age and gender distribution chart shows the study subjects categorized into three age limits i.e. 1 to 6 years old between 7 to 13 years old and, over 14 years old. The age group of 1-6 years old have a total of 54 patients out of which 27 are male and 27 are females. The age group of 7-13 years old have a total of 39 patients out of which 17 are male and 22 are females. The adults group have a total of 62 patients out of which 30 are male and 32 are females.

Table 1.0: Descriptive Statistics

Parameter	Mean ± SD	Median (IQR)
Age (years)	16.3 ± 1.2	16 (15-17)
Spleen Size (cm)	12.3 ± 0.8	12 (11-13)
Hemoglobin Level (g/dL)	11.1 ± 0.6	11.2 (10.5-11.6)
Serum Ferritin Level (ng/mL)	4442 ± 2882	4858 (1810-6542)
Iron Overload Status		
- Mild	30%	-
- Moderate	50%	-
- Severe	20%	-

The mean serum ferritin concentration was 4442 ± 2882 ng/ml (range 3588.0–7625.14 ng/ml). The ferritin concentration was less than ≤ 1500 ng/mL in 17 (10%) Male and 15 (9%) Female patients and 62 (40%) Male and 71 (45%) Female patients were with serum ferritin levels more than or equal to ≥ 1500 ng/mL. Majority of the patients with high serum ferritin concentration (>2500 ng/mL) were aged more than 11 years.

Table 2.0: Correlation Matrix

	Serum Ferritin Level	Transferrin Saturation	Liver Iron Concentration	Hemoglobin Levels
Serum Ferritin Level	1.00	0.85	0.72	-0.65
Transferrin Saturation	0.85	1.00	0.62	-0.55
Liver Iron Concentration	0.72	0.62	1.00	-0.48
Hemoglobin Levels	-0.65	-0.55	-0.48	1.00

The iron overload status category defines that 30% of the individuals in the study exhibit very low levels of excess iron accumulation and 50% of individuals fall into the category, of having moderate levels of excess iron in their bodies. This may indicate a greater accumulation of iron than in the coarse fraction. Severe iron overload category represents the 20% of individuals in the study with the highest rate of iron over accumulation, indicating a significant and potentially more serious condition of iron overload.

The Correlation Matrix section represents that Serum Ferritin Level shows a strong effective correlation (0.85) with Transferrin Saturation. Moderate superb correlation (0.72) with Liver Iron Concentration. Moderate negative correlation (-0.65) with Hemoglobin Levels. Transferrin Saturation shows very strong high-quality correlation (0.85) with Ferritin Level. Moderate high-quality correlation (0.62) with Liver Iron Concentration. Moderate poor correlation (-0.55) with Hemoglobin Levels. Liver Iron Concentration shows moderate effective correlation (0.72) with Ferritin Level. Moderate nice correlation (0.62) with Transferrin Saturation. Moderate terrible correlation (-0.48) with Hemoglobin Levels. Hemoglobin Levels shows moderate negative correlation (-0.65) with Ferritin Level. Moderate bad correlation (-0.55) with Transferrin Saturation. Moderate bad correlation (-0.48) with Liver Iron Concentration.

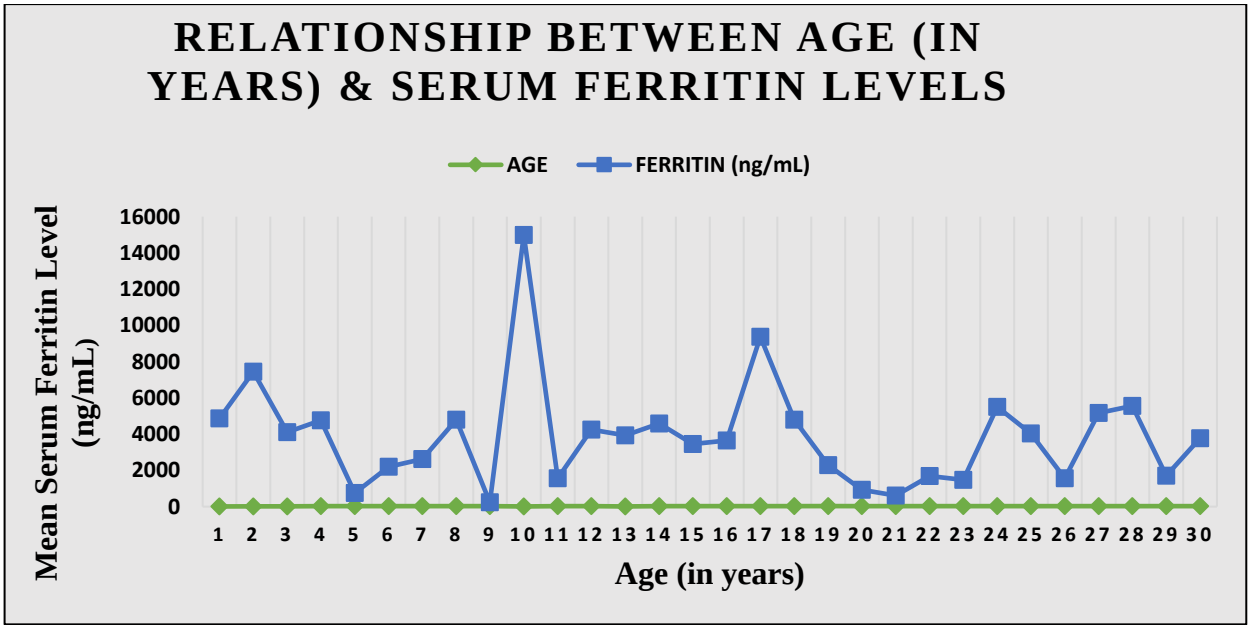

Figure 2.0: Shows the relationship between Age (in years) and Serum Ferritin levels(ng/ml) of the study participants.

The serum ferritin level were observed to be high in patients with 10 years of age as depicted in Figure 2.0. The mean serum ferritin level in males was found to be ±3691.99 ng/mL and it is comparable to the mean serum ferritin level in females i.e. ±4799.86 ng/ mL. Beta Thalassemia patients showed very high increase in their serum ferritin levels.

DISCUSSION

Beta thalassemia major is a common hereditary hemoglobinopathy disorder in Pakistan mostly affecting children. Multiple transfusions over decades have improved the life expectancy of beta thalassemic patients but excess iron accumulation in the body is still an unavoidable complication suffered by the patients. Therefore, effective iron chelation therapy and close monitoring of iron overload is crucial in these patients to improve their quality of life.

The assessment of serum ferritin concentrations in this study has been used to create awareness of iron excess and its life-threatening conditions in the body. This is why it is also been referred as "second disease" during treatment because it invades other diseases and complications in the body(15). Many other studies have concluded that increase in the size of liver and liver cirrhosis is associated with increase in serum ferritin levels(16).

In our study serum ferritin levels of 155 patients were statistically analyzed. In this study unfortunately only 20% of the patients had values below 1500 ng/ml ferritin levels. It was reported in a study conducted in Bhopal, India that 12.5% of participants had serum ferritin levels of less than 1000ng/ml (17). However, a similar study conducted in Western India reported that only 2% of the patients were with serum ferritin level less than 1000 ng/ml (18).

Nevertheless another study conducted in Peradeniya Teaching Hospital in Sri Lanka shows that only 15% of the patients had serum ferritin values below 1500 ng/ml which is close enough to our study (3). Beta thalassemia major patients should maintain their serum ferritin levels below or less than < 1500 ng/ml.

The mean serum ferritin concentration of the patients was 4442 ± 2882 ng/ml which is statistically significant when compared to the normal reference range of ferritin level (19). Normal reference values of serum ferritin for men and women are 12-300 ng/mL and 12-150 ng/mL, respectively(20). Therefore, right adherence to iron chelation therapies and close monitoring of iron overload is obligatory. However, Choudhry VP et al in India reported mean serum ferritin levels to be 6723 ng/ml even higher than in our study (21). Introduction of iron chelation therapies such as deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX) drugs might provide effective management of iron overload in Pakistani beta thalassemia major patients (22).

Although similar sort of studies conducted in south Asian countries have also reported high mean serum ferritin levels when compared to the values of this study. It is said that mean serum ferritin levels reported to be 4236.5 ng/ml, 6723 ng/ml, 3272.5 ng/ml and 2992.2 ng/ml in studies conducted in Pakistan(15), India (21), Saudi Arabia(23) and Srilanka (3) respectively. Those differences in iron excess can be explained on the basis of developed and better healthcare facilities in those countries and also socio background of the patients. The data on comparative mean serum ferritin levels expressed in ng/ml in different countries across south Asian countries is summarized in table 1.0.

The liver is a major site of iron overload, accounting for 70% or more of the body's iron content. Transfusion iron overload of hepatic iron and total body iron correlate closely with total body iron. Estimation of direct hepatic iron concentration is the most accurate method of estimating iron overload. However, this method and data were not available in our study. The indirect method with serum ferritin level measurement was reliable, easy, low cost and had no side effects(24).The distribution of beta thalassemia gene is not uniform in Pakistan. The highest frequency of β thalassemia gene is reported as gene carrier rate of 5-7% and roughly a pool of 9.8 million carriers in the general population (25)

Table 3.0: Comparative mean serum ferritin levels across different countries in south Asia.

Reference	Country	Mean Serum Ferritin level ng/mL	% of Patients with Serum Ferritin less than <1000 ng/mL
Shah et al.(3)	Western India	-	2.00%
Mishra and Tiwaria (3)	Bhopal, India	-	12.5%
Karunaratna, Ranasingha(3)	Srilanka	2992.2 ng/ml	-
Choudhry et al. (21)	India	6723 ng/ml	-
Riaz et al. (15)	Pakistan	4236.5 ng/ml	-
Al Jaouni et al. (23)	Saudi Arabia	3272.5 ng/ml	-

CONCLUSION

This study concludes that 80% of the patients have high mean serum ferritin levels which consequently leads to iron overload in the body that cause many serious other diseases and life-threatening complications. There is a strong need to create awareness among the thalassemia patients on the effects of iron overload in their bodies. Proper iron chelation therapies and close monitoring of ferritin levels could improve the quality and expectancy of life of these patients. The problems of low education levels, poverty, and inadequate facilities of healthcare serve as the main stumbling blocks in productive treatment of iron overload in thalassemic patients which is major cause of morbidity and mortality in beta thalassemia major.

CONFLICT OF INTEREST

This study has no conflict of interest.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. All the samples were processed by our own cost.

LIMITATIONS

The height and weight (consequently leading the body mass index (BMI)) of the patients was not obtained. The dose and frequency of Deferoxamine (DFX) infusions or any other iron chelation therapy is not investigated. Due to the small sample size in our study, we may encounter challenges in defending our interpretations. Hence, it is recommended to consider a larger sample size to conduct a more comprehensive assessment.

ACKNOWLEDGEMENTS

Moreover, we would like appreciate the staff of Jamila Sultana Foundation (JSF) for their kind behavior and cooperation regarding data collection.

REFERENCES

1. Riaz H, Riaz T, Khan MU, Aziz S, Ullah F, Rehman A, et al. Serum ferritin levels, socio-demographic factors and desferrioxamine therapy in multi-transfused thalassemia major patients at a government tertiary care hospital of Karachi, Pakistan. BMC research notes. 2011;4(1):1-5.
2. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. Blood reviews. 2009;23(3):95-104.
3. Mishra AK, Tiwari A. Iron overload in beta thalassaemia major and intermedia patients. Maedica. 2013;8(4):328.
4. Shah N, Mishra A, Chauhan D, Vora C, Shah N. Study on effectiveness of transfusion program in thalassemia major patients receiving multiple blood transfusions at a transfusion centre in Western India. Asian journal of transfusion science. 2010;4(2):94.

5. Karunaratna A, Ranasingha JS, Mudiyanse RM. Iron overload in beta thalassemia major patients. Int J Blood Transfus Immunohematol. 2017;7:33-40.
6. Hows J, Hussein S, Hoffbrand A, Wickramasinghe S. Red cell indices and serum ferritin levels in children. Journal of Clinical Pathology. 1977;30(2):181.
7. Berdoukas V, Farmaki K, Carson S, Wood J, Coates T. Treating thalassemia major-related iron overload: the role of deferiprone. Journal of blood medicine. 2012:119-29.
8. Choudhry V, Pati H, Saxena A, Malaviya A. Deferiprone, efficacy and safety. The Indian Journal of Pediatrics. 2004;71:213-6.
9. Shamsian BS, Esfahani SA, Milani H, Akhlaghpoor S, Mojtahedzadeh S, Karimi A, et al. Magnetic resonance imaging in the evaluation of iron overload: a comparison of MRI, echocardiography and serum ferritin level in patients with β-thalassemia major. Clinical imaging. 2012;36(5):483-8.
10. Al Jaouni SK. Survival and disease complication of thalassemia major: experience of 14 years at King Abdulaziz University Hospital, Jeddah, KSA. Journal of King Abdulaziz University: Medical Sciences. 2010;98(277):1-20.
11. Nf O. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739-61.
12. Ehsan H, Wahab A, Anwer F, Iftikhar R, Yousaf MN. Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. Cureus. 2020;12(8).
13. Prabhu R, Prabhu V, Prabhu R. Iron overload in beta thalassemia: a review. J Biosci Tech. 2009;1(1):20-31.
14. Brili SV, Castelanos SS, Aggeli CJ, Tentolouris CA, Pitsavos CE, Toutouzas PK, et al. The effect of iron overload in the hearts of patients with beta-thalassemia. Clinical cardiology. 1997;20(6):541-6.
15. Karunaratna A, Ranasingha JS, Mudiyanse RM. Iron overload in beta thalassemia major patients. Int J Blood Transfus Immunohematol. 2017;7:33-40.
16. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood, The Journal of the American Society of Hematology. 2011;118(13):3479-88.
17. Cao A, Galanello R. Beta-thalassemia. Genetics in medicine. 2010;12(2):61-76.
18. Cappellini MD. Exjade®(deferasirox, ICL670) in the treatment of chronic iron overload associated with blood transfusion. Therapeutics and clinical risk management. 2007;3(2):291-9.
19. Gutteridge JM, Rowley DA, Halliwell B. Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Detection of 'free'iron in biological systems by using bleomycin-dependent degradation of DNA. Biochemical Journal. 1981;199(1):263-5.
20. Bacon BR, Britton RS. The pathology of hepatic iron overload: a free radical–mediated process? Hepatology. 1990;11(1):127-37.
21. Hershko C, Konijn AM, Link G. Iron chelators for thalassaemia. British Journal of Haematology. 1998;101(3):399-406.
22. Vichinsky EP. Changing patterns of thalassemia worldwide. Annals of the New York Academy of Sciences. 2005;1054(1):18-24.
23. Weatherall D, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Health Organization. 2001;79(8):704-12.
24. Asif N, Hassan K. Management of thalassemia in Pakistan. Journal of Islamabad Medical & Dental College. 2016;5(4):152-3.
25. Hashemi A, Ghilian R, Golestan M, Akhavan GM, Zare Z, Dehghani MA. The study of growth in thalassemic patients and its correlation with serum ferritin level. 2011.
26. Saka N, ŞüKür M, Bundak R, Anak S, Neyzi O, Gedikoglu G. Growth and puberty in thalassemia major. Journal of Pediatric Endocrinology and Metabolism. 1995;8(3):181-6.
27. Riaz H, Riaz T, Khan MU, Aziz S, Ullah F, Rehman A, et al. Serum ferritin levels, socio-demographic factors and desferrioxamine therapy in multi-transfused thalassemia major patients at a government tertiary care hospital of Karachi, Pakistan. BMC research notes. 2011;4(1):1-5.

28. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. Blood reviews. 2009;23(3):95-104.
29. Mishra AK, Tiwari A. Iron overload in beta thalassaemia major and intermedia patients. Maedica. 2013;8(4):328.
30. Shah N, Mishra A, Chauhan D, Vora C, Shah N. Study on effectiveness of transfusion program in thalassemia major patients receiving multiple blood transfusions at a transfusion centre in Western India. Asian journal of transfusion science. 2010;4(2):94.
31. Hows J, Hussein S, Hoffbrand A, Wickramasinghe S. Red cell indices and serum ferritin levels in children. Journal of Clinical Pathology. 1977;30(2):181.
32. Berdoukas V, Farmaki K, Carson S, Wood J, Coates T. Treating thalassemia major-related iron overload: the role of deferiprone. Journal of blood medicine. 2012:119-29.
33. Choudhry V, Pati H, Saxena A, Malaviya A. Deferiprone, efficacy and safety. The Indian Journal of Pediatrics. 2004;71:213-6.
34. Shamsian BS, Esfahani SA, Milani H, Akhlaghpoor S, Mojtahedzadeh S, Karimi A, et al. Magnetic resonance imaging in the evaluation of iron overload: a comparison of MRI, echocardiography and serum ferritin level in patients with β-thalassemia major. Clinical imaging. 2012;36(5):483-8.
35. Al Jaouni SK. Survival and disease complication of thalassemia major: experience of 14 years at King Abdulaziz University Hospital, Jeddah, KSA. Journal of King Abdulaziz University: Medical Sciences. 2010;98(277):1-20.
36. Nf O. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739-61.
37. Ehsan H, Wahab A, Anwer F, Iftikhar R, Yousaf MN. Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. Cureus. 2020;12(8).