Proceedings S.Z.M.C. Vol: 37(4): pp. 58-63, 2023. PSZMC-908-37-4-2023

Body Iron Status and Its Complications in Patients With Beta Thalassemia Major: A Cross-Sectional Study

[1]Shafaq Ismail, [2]Shaiza Ijaz, [1]Spenta Kakalia, [3]Muhammad Ashraf Chaudhry, [1]Menahil Mukhtar, [1]Laiba Fayyaz

ABSTRACT

Introduction: Thalassemia is a genetic condition and is one of the commonest single-gene hereditary disorders. As it is among a group of hemoglobinopathies, it consists of a decrease in either alpha or beta globin chain synthesis. This eventually leads to the inappropriate ratio of alpha or beta globin chains causing ineffective erythropoiesis. Ineffective erythropoiesis thus causes chronic anemia and haemopoietic expansion to compensate for it.

Aims & Objectives: This study aimed to investigate the serum ferritin levels of patients with beta thalassemia major presenting in a tertiary care hospital in Lahore, Pakistan and study the complications that occur with an abnormal iron load.

Place and Duration of Study: It was conducted at CMH Hospital, Lahore, and completed over a period of one and a half year, from June 2021 to January 2023.

Material & Methods: It was a descriptive cross-sectional study. The sample size of 32 was calculated using Raosoft calculator, with 90% Confidence Interval, 9% margin of error and 10.5% response distribution.Data was collected using an interviewer-administered questionnaire that compromised of 4 parts, demographics and socio-economic background of patient and caretakers; medical history and anthropometric measurement of the patient; details regarding serum ferritin levels and chelation; and lastly, laboratory (for example, TSH, T4, HbA1c and ALT for all patients, as well as FSH and LH for only those patients greater than 10 years of age), ultrasound, and cardiac echocardiography findings.Collected data was entered and analyzed in SPSS version 24. P value of <0.05 was taken as significant.

Results: In this study, thirty-two patients with beta thalassemia major were included with mean age of 7.79 ± 4.57 years. Mean serum ferritin was 3410±2629µg/l and lack of compliance to chelation had significant association with serum ferritin values (p=0.05). Serum ALT levels showed that hepatic dysfunction was the most common endocrine complication in 17 (53.1%) patients. There was a significant association between compliance and echocardiography findings (p=0.04). No significant association of compliance was found with ultrasound findings, serum TSH and T4 levels, serum ALT levels and serum FSH and LH levels.

Conclusion: This study concluded that high ferritin levels ultimately lead to significant complications.

Keywords: Ferritin, Iron overload, Beta-Thalassemia Major.

INTRODUCTION

Thalassemia, one of the most common single gene hereditary disorders, is associated with significant mortality and morbidity worldwide[1]. In Pakistan, the prevalence of beta thalassemia major is estimated to be five to seven percent and approximately 50,000 patients are currently enrolled with different thalassemia centers throughout the country[2]. The carrier rate, that is persons with thalassemia minor, varies between five to seven percent; with almost 9.8 million carriers in the overall population[3].Thalassemia is part of a group of disorders called hemoglobinopathies. It is characterized by a decrease in globin chain synthesis (alpha or beta) resulting in inappropriate ratio of alpha or beta globin chains, ineffective erythropoiesis, chronic anemia, and haemopoietic expansion to compensate for it[1]. In this study, we have focused on beta thalassemia only. The definitive cure is successful bone marrow transplant. If this is not possible, the patient has to remain on lifelong blood transfusions, leading to multiple complications. The complications are many and affect various organs throughout the body. Hemochromatosis (iron overload) due to repeated blood transfusions results in iron deposition in multiple organs especially the heart, pancreas, liver and kidney. Deposited iron contributes to cardiomyopathy, liver dysfunction, hypogonadism, hypothyroidism, hypoparathyroidism, diabetes

1.[1]Department of Pediatrics, CMH Lahore Medical College, Lahore.
2.[2]Department of Physiology, CMH Lahore Medical College, Lahore.
3.[3]Department of Community Medicine, CMH Lahore Medical College, Lahore.

Correspondance:
Dr. Shaiza Ijaz, *Demonstrator, Department of Physiology, CMH Lahore Medical College, Lahore.*
Email: shaizaijaz@gmail.com

Submission Date: 15th July 2023
1st Revision Date:30th August 2023
Acceptance Date:7th November 2023

mellitus and short stature[4-7]. These complications affect quality of life, and can lead to mortality, with cardiac complications being the most common cause of death[8].

To prevent iron overload, all blood transfusion dependent thalassemia patients should be on iron chelation therapy. This can be effectively done by administration of iron chelating drugs. Deferoxamine (DFO), which is the first line chelator, is administered subcutaneously over a period of 8 hours, as an infusion for 6 out of 7 days, usually given during night hours[9]. The other drugs are orally administered. Despite its effectiveness in preventing life threatening complications, given the cumbersome administration, compliance can be low. Serum ferritin level measurements are easily available and relatively inexpensive, is used to monitor total body iron levels. Specific investigations to screen for organ dysfunction should also be carried out regularly[10]. Multiple studies have been done in various countries, including Sri Lanka and India to demonstrate a relationship between iron overload and various organopathies encountered in beta thalassemia patients, butdata from Pakistan is scarce. We believe this study was crucial, as it will add to the meager statistics present in Pakistan. Given the scanty data from Pakistan on this topic, this study is very important in helping us understand and manage complications from this disease better.Theprimary objective of this study was to determine the prevalence of organ dysfunction in patients with beta thalassemia and its association with serum ferritin levels. The secondary objective was to assess the compliance to chelation therapy.

MATERIAL AND METHODS

This descriptivecross-sectional study was conducted at the Thalassemia Center, CMH Lahore.

The study received approval of the Ethical Review Committee of CMH Lahore Medical College, with the number 764/ERC/CMH/LMC. Sample size of 32 was calculated using the Raosoft calculator, with 90% confidence interval, 9% margin of error and 10.5% response distribution. Inclusion criteria were children with beta thalassemia major (diagnosed by hemoglobin electrophoresis), age up to 18 years, and receiving blood transfusions. Exclusion criteria included patients with thalassemia minor, and patients receiving blood transfusion for any other hematological disorder. Prior to enrollment in the study, informed written consent was obtained.

The primary team members carried out interviews. Data was collected using an interviewer-administered questionnaire that compromised of 4 parts. The first part covered demographics and socio-economic information. The second part comprised of medical history, including history ofco-morbidities, Hepatitis B and/or Hepatitis C, any known disabilities and family history of blood disorders. Information was also gathered about age at which blood transfusions were started and frequency of transfusions. In the third part, serum ferritin levels were recorded, and compliance to chelation was assessed. The last section was used to record laboratory, ultrasound abdomen and cardiac echocardiogram (echo) findings.The lab tests and abdominal ultrasounds were done from CMH Diagnostics Centre, while echocardiograms were performed at the Army Cardiac Center, CMH Lahore. For all patients, laboratory investigations included serum TSH as well as free T4 to identify thyroid functioning, serum HbA1C for diabetes mellitus, and serum ALT to determine liver dysfunction.Serum FSH and LH were only obtained in patients older than ten years to assess gonadal development. The data collection tool was designed for the study (appendix) by the primary team, based on available literature. Two pediatricians, independent of the study, prior to starting the study, reviewed it.

Statistical Analysis:

Collected data was entered in SPSS version 24. Quantitative data was presented as mean and standard deviation while qualitative data as frequency and percentages. P value of <0.05 was taken as significant.

RESULTS

The study enrolled thirty-two patients (6 months – 18 years of age) with beta thalassemia major. Mean age was 7.79 ± 4.57 years; 15 (46.9%) patients were males. Blood transfusion was required once monthly in 27 (84.4%), and twice monthly for five (15.6%) patients. For all patients, the primary caregiver was the mother, all of whom defined themselves as a homemaker. Most mothers, 13 (40.6%) had no formal education, 10 (31.3 %) were educated up to Matriculation, whereas nine (21.8%) were educated up to Intermediate level and above. In 21 (65.6%) families, the monthly income was less than PKR 50,000. In 30 (93.8%) patients, parents had a consanguineous marriage. Mean serum ferritin was 3410±2629µg/l. Only three (9.37%) children had serum ferritin levels less than 1000 µg/l. However, 13 (40.62%) had serum ferritin between 1000 and 2499 µg/l and 16 (50%) patients had serum ferritin levels at or above 2500 µg/l. (Table-1) On assessment of compliance to chelation, 19 (59.3%)

patients were compliant to therapy, while three (23%) were not taking any chelation.

Value	Frequency	Normal/ Abnormal
29	1	Normal
45	1	Normal
715	1	Abnormal
1200	1	Abnormal
1324	1	Abnormal
1587	1	Abnormal
1868	1	Abnormal
2000	4	Abnormal
>2000	5	Abnormal
2500	1	Abnormal
2597	1	Abnormal
2775	1	Abnormal
3066	1	Abnormal
3764	1	Abnormal
4082.2	1	Abnormal
4570	1	Abnormal
4852	1	Abnormal
5084	1	Abnormal
5149	1	Abnormal
5575	1	Abnormal
5771	1	Abnormal
6298	1	Abnormal
6556	1	Abnormal
10726	1	Abnormal
11000	1	Abnormal

Table-1: Serum Ferritin Values Among Patients With B – Thalassemia Major (n = 32)

Compliance to Iron Chelation	Ferritin		P – Value
	Normal	High Ferritin	
Always	0	19	0.05
Monthly	0	2	
NA[1]	2	5	
Not Complaint	0	4	
Total	**2**	**30**	

Table-2: Lack Of Compliance In Association To Chelation Therapy And Serum Ferritin Levels Among Patients With B – Thalassemia Major (n = 32)

Compliance to Iron Chelation	Echocardiography Findings			P – Value
	Normal	Abnormal	Valvulardysfunction	
Always	7	0	12	0.047
Monthly	2	0	0	
NA[1]	3	0	4	
Not Compliant	3	1	0	
Total	**15**	**1**	**16**	

Table-3: Compliance To Chelation And Echo cardiography Findings Among Patients With B-Thalassemia Major (n = 32)

These, two were less than one year of age with ferritin levels less than 1000 µg/l, while one of them was more than a year old but had serum ferritin levels less than 1000 µg/l; four(30.7%) identified lack of awareness as the reason for poor or no compliance (Fig-1). Lack of compliance was significantly associated with serum ferritin (p=0.055) (Table-2).In 31 (96.9%) patients, there was no co-morbidity, and no complications of beta thalassemia were reported. Similarly, 28 (87.5%) patients complained of no known disabilities. However, laboratory reports and radiological evidence proved otherwise. Serum ALT levels showed that hepatic dysfunction was the most common biochemical complication in 17 (53.1%) patients. Hypothyroidism was found in three (9.4%) patients and hyperthyroidism in only one (3.1%);five (15.6%) patients had diabetes mellitus. Only seven (21.9%) participants who were above the age of 10 years were tested for gonadal function with serum FSH and LH levels. Serum FSH and LH was low in three (9.4%), while serum FSH was high in four (12.5%) and LH was high in one (3.1%). Hepatitis B was positive in one (3.1%), Hepatitis C in four (12.5%), while both were positive in one (3.1%) patient. Hepatomegaly and splenomegaly were found in six (18.8%) patients had both on ultrasound, while two (6.3%) had hepatomegaly and four (12.5%) had splenomegaly individually. Isolated valvular dysfunction was found on cardiac echo in 16 (50%) patients, while valvular dysfunction, along with abnormalities of chambers was found in only one (3.1%) patient. There was a significant association between high serum ferritin levels, which was mostly due to lack of compliance, and echo findings (p=0.047) (Table-3). However, there was no significant association of compliance with ultrasound findings; serum TSH and T4 levels; serum ALT levels and serum FSH and LH levels.

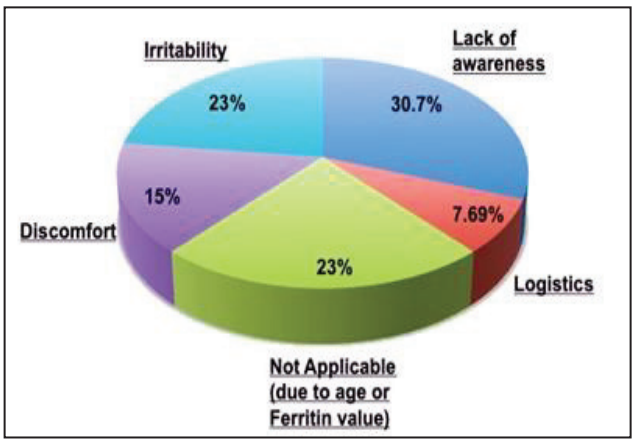

Fig-1: Reasons for lack of compliance

DISCUSSION

This study points out the extent of iron overload and its associated complications in patients with beta thalassemia major in an urban setting inPakistan.

As the definitive treatment of bone marrow transplantation is only available to a small proportion of patients, most depend on regular blood transfusions, leading to iron overload. Several known complications of iron overload can be prevented by adequate chelation[11].

The most significant finding in this study was that 29 (90.7%)patients had higher than normal serum ferritin levels. Regular blood transfusions were being carried out to sustain hemoglobin levels of patients with beta thalassemia major.Because of poor compliance to iron chelation therapy, iron overload has been demonstrated in various organ systems. In our study, from the organs studied, hepatic dysfunction was found to be the most common, in 17 (53%). This was followed by cardiac valvular dysfunction in 17 (53.1%), diabetes mellitus in five (15.4%), hypothyroidism in three (9.4%), and hypogonadism in three (9.4%).

Assessing iron overload in patients with beta thalassemia major receiving regular blood transfusions is an essential part of their management. Although protocols may differ slightly, it is accepted to begin chelation therapy after a certain number of blood transfusions have been administered (10-12 at our center), or after serum ferritin has surpassed an acceptable normal limit (1000µg/l at our center). Blood transfusions are given with the intention to keep the mean hemoglobin at 12g/dl. For this, pre-transfusion hemoglobin is taken at 9-10.5 g/dl and post-transfusion hemoglobin to be less than 14-15 g/dl[12].

There are numerous methods for assessment of body iron status. Specific tests target the organs; for example, T2 MRI to assess for cardiac status or a biopsy for liver involvement. Despite these often complicated and expensive specific tests, serum ferritin remains the most common entity to assess body iron status due to its low cost and availability[13]. In this study, a dismal two (6.2%) had normal serum ferritin level. Mean serum ferritin levels was 3410µg/l; half of them had values greater than 2500µg/l. Ferritin values are considerably higher those reported in Sri Lankan and Indian studies, where mean serum ferritin level was 2992µg/l and 2767µg/l, respectively[7, 14]. Although it is difficult to understand why this difference exists, it could be attributed to higher literacy in both these countries compared with Pakistan, where higher literacy might lead to better understanding of the disease process and chelation[15].Formal education had not been attained by 13 (40.6%) mothers in this study. When we compare this with a similar study conducted in Karachi, ferritin levels in this study were lower than their reported level of 4236.5± 2378.3 ng/ml[16].Being a single centered study, we cannot completely explain these discrepancies. It could be attributed to availability of chelation agents, which are given free of cost to patients at our center. This combined with awareness regarding the disease and its treatment might have contributed to lower ferritin levels than in other local studies. There is a paucity of more local studies for us to compare this with.

The most common complication from iron overload reported in our study was hepatic dysfunction (17, 53.1%) determined by abnormal ALT levels;six (25%) of whom had ultrasonographic evidence of hepatomegaly. There is no local data to compare this with. In Sri Lanka and Iran, delayed puberty was found to be most prevalent organ dysfunction[13, 17].There was no statistical association between serum ferritin levels and hepatic dysfunction (or hepatomegaly) in our study. Hepatic dysfunction, although mainly caused by iron overload in our patients, has also shown to be attributed side effect of chelating agents[18]. Hepatitis B and C, which was reported in 18% patients, can also contribute to hepatic dysfunction[19]. This supports the literature than liver involvement in persons with beta thalassemia major could be multifactorial[20].

Cardiac valvular dysfunction was noted in 17 (53.1%) of our study population, on cardiac echo. A statistically significant relationship was found between high serum ferritin and echocardiographic findings of valvular and chamber dysfunction of heart, indicating sensitivity of cardiac tissue to toxic effects of iron overload. However, out of 382 subjects enrolled in a study in Dubai, only seven (1.8%) suffered from iron overload cardiomyopathy, based on T2 MRI[6]. Given that cardiac complications are the most common cause of mortality in children with beta thalassemia major in Pakistan and worldwide, protocols should focus not only on adequate chelation, but also evaluation of cardiac status in thalassemia patients. Although the gold standard is T2 MRI, electrocardiography and echocardiography can be used in resource-limited settings[8, 21].

Besides liver and cardiac complications, this study also looked at the prevalence of diabetes mellitus, hypothyroidism and gonadal dysfunction. Other complications like parathyroid disorders were not tested for at because of cost constraints, which is probably true for most centers seeing patients with beta thalassemia major in Pakistan. Diabetes mellitus, assessed by HbA1C was found in five

(15.6%) patients; hypothyroidism in three (9.4%), and hyperthyroidism in only one (3.1%) patient. Unfortunately, no local study was found in the last five years for comparison of our findings. In a Sri Lankan study, none of the 54 patients had diabetes or hypothyroidism[13]. Another parameter assessed in our study was gonadal function by evaluating FSH and LH values. Gonadal dysfunction was found in only nine (21.9%) of children. On the contrary, among 21 patients in Sri Lanka, nine (42.8%) suffered from pubertal delay[13].Likewise, Saffariet.al. evaluated gonadal development by assessing serum FSH, LH andtestosterone levels, along with carrying out Tanner staging to estimate breast and testicular development. Impaired pubertal development was the commonest endocrine finding in their study[17].Tanner staging was not carried out, and we had a small proportion of patients around pubertal age; hence, we cannot draw conclusions on the significance of gonadal dysfunctionfound in our results. More studies are required to judge the influence of elevated body iron status on pubertal growth in patients with beta thalassemia major. It is well established that effective chelation has a significant effect in reducing iron overload. Although a serum ferritin of less that 2,500µg/l is usually aimed for, some studies suggest that lower levels of about 1000µg/l might confer further benefit[22]. Despite this, serum ferritin levels often remain high, as in this study and in similar studies in developing countries[5, 7, 16, 17, 21]. Not surprisingly, a statistically significant association was found between compliance to chelation and serum ferritin levels in this study. This corresponds to results from a systemic review conducted in 2022 on 37 articles from different parts of world[23]. This emphasizes the need to create awareness among caretakers regarding the importance of adherence to iron chelating therapy. In this study, 13(40.6%) reported poor compliance. Although it was hypothesized that most would report discomfort and difficulty in administration, surprisingly four(30.7%) reported a lack of knowledge about its importance. Sidhuet.al. in India, found only 11% non-adherent to chelation, mainly because of difficulty in administration[24]. There were some limitations of this study. It was a single center study with a small sample size. Given the location in an urban setting in the second largest city of Pakistan, the authors feel that the results of this study are generalizable to children with beta thalassemia major in Pakistan. Due to financial constraints, the authors were unable to consistently use gold standard testing to evaluate iron overload.

CONCLUSION

This single center study adds to the data related to beta thalassemia major patients in an urban center of Pakistan. We concluded that patients of beta thalassemia major suffered from elevated serum ferritin levels, which was in turn found to be related to compliance to chelation. Therefore, we realized that iron overload is a significant problem in children living with beta thalassemia major in our setting. Compliance to chelation needs to be addressed by doctors and health policy makers. Access and affordability of chelating agents, along with education is the order of the day.

REFERENCES

1. Manouras A, Pararas N, Antonakis P, Lagoudiannakis EE, Papageorgiou G, Dalianoudis IG, et al. Management of major bile duct injury after laparoscopic cholecystectomy: a case report. Journal of medical case reports. 2009;3(1):1-6.
2. Renz BW, Bösch F, Angele MK. Bile duct injury after cholecystectomy: surgical therapy. Visceral medicine. 2017;33(3):184-90.
3. Abid KJ, Ishaque S, Naeem I, Ch TS. Management of major bile duct injury after laparoscopic cholecystectomy: a case report. International Journal of Recent Advances in Multidisciplinary Research. 2017;04(03):2398-402.
4. Pandit N, Yadav TN, Awale L, Deo KB, Dhakal Y, Adhikary S. Current Scenario of Postcholecystectomy Bile Leak and Bile Duct Injury at a Tertiary Care Referral Centre of Nepal. Minimally invasive surgery. 2020;2020(4382307):1-6.
5. Lalisang TJM, Situmorang I, Ibrahim F, Widianto P, Marbun VMG. Management of post-cholecystectomy bile duct injuries without operative mortality at Jakarta tertiary hospital in Indonesia–A cross-sectional study. Annals of Medicine and Surgery. 2021;62:211-5.
6. Pesce A, Palmucci S, La Greca G, Puleo S. Iatrogenic bile duct injury: impact and management challenges. Clinical and experimental gastroenterology. 2019;12:121-8.
7. Sugrue M, Coccolini F, Bucholc M, Johnston A. Intra-operative gallbladder scoring predicts the conversion of laparoscopic to open cholecystectomy: a WSES prospective collaborative study. World Journal of Emergency Surgery. 2019;14(1):1-8.
8. Stilling NM, Fristrup C, Wettergren A, Ugianskis A, Nygaard J, Holte K, et al. Long-term outcome after early repair of iatrogenic bile duct injury. A national Danish multicentre study. Hpb. 2015;17(5):394-400.
9. Patrono D, Benvenga R, Colli F, Baroffio P, Romagnoli R, Salizzoni M. Surgical management of post-cholecystectomy bile duct injuries: referral patterns and factors influencing early and long-term outcome. Updates in surgery. 2015;67(3):283-91.

10. Halbert C, Altieri MS, Yang J, Meng Z, Chen H, Talamini M, et al. Long-term outcomes of patients with common bile duct injury following laparoscopic cholecystectomy. Surgical endoscopy. 2016;30(10):4294-9.
11. Vickers NJ. Animal communication: when I'm calling you, will you answer too? Current biology. 2017;27(14):R713-R5.
12. Dominguez-Rosado I, Sanford DE, Liu J, Hawkins WG, Mercado MA. Timing of surgical repair after bile duct injury impacts postoperative complications but not anastomotic patency. Annals of surgery. 2016;264(3):544-53.
13. Törnqvist B, Waage A, Zheng Z, Ye W, Nilsson M. Severity of acute cholecystitis and risk of iatrogenic bile duct injury during cholecystectomy, a population-based case–control study. World Journal of Surgery. 2016;40(5):1060-7.
14. Buddingh KT, Nieuwenhuijs VB, van Buuren L, Hulscher JB, de Jong JS, van Dam GM. Intraoperative assessment of biliary anatomy for prevention of bile duct injury: a review of current and future patient safety interventions. Surgical endoscopy. 2011;25(8):2449-61.
15. Ghazanfar S, Qureshi S, Leghari A, Taj MA, Niaz SK, Quraishy MS. Endoscopic management of postoperative bile duct injuries. JPMA-Journal of the Pakistan Medical Association. 2012;62(3):257-62.
16. Elhamel A, Nagmuish S, Elfaidi S, Dalal HB. Handling of biliary complications following laparoscopic cholecystectomy in the setting of Tripoli Central Hospital. HPB. 2002;4(3):105-10.
17. Ibrarullah M, Sankar S, Sreenivasan K, Gavini S. Management of bile duct injury at various stages of presentation: experience from a tertiary care centre. Indian Journal of Surgery. 2015;77(2):92-8.
18. Kaman L, Sanyal S, Behera A, Singh R, Katariya RN. Comparison of major bile duct injuries following laparoscopic cholecystectomy and open cholecystectomy. ANZ journal of surgery. 2006;76(9):788-91.
19. Eisenstein S, Greenstein AJ, Kim U, Divino CM. Cystic duct stump leaks: after the learning curve. Archives of Surgery. 2008;143(12):1178-83.
20. Aksoz K, Unsal B, Yoruk G, Buyrac Z, Haciyanli M, Akpinar Z, et al. Endoscopic sphincterotomy alone in the management of low-grade biliary leaks due to cholecystectomy. Digestive Endoscopy. 2009;21(3):158-61.
21. Karanikas M, Bozali F, Vamvakerou V, Markou M, Chasan ZTM, Efraimidou E, et al. Biliary tract injuries after lap cholecystectomy—types, surgical intervention and timing. Annals of translational medicine. 2016;4(9):1-9.
22. Chaudhary A, Manisegran M, Chandra A, Agarwal A, Sachdev A. How do bile duct injuries sustained during laparoscopic cholecystectomy differ from those during open cholecystectomy? Journal of Laparoendoscopic & Advanced Surgical Techniques. 2001;11(4):187-91.
23. Kelsoe J, Greenwood T, Akiskal H, Akiskal K. The genetic basis of affective temperament and the bipolar spectrum. International Clinical Psychopharmacology. 2012;28:e5-e6.
24. Sidhu S, Kakkar JS,Dewan P,2 BansalN, Sobti PC.Adherence to Iron Chelation Therapy and Its Determinants.Int J Hematol Oncol Stem Cell Res. 2021 Jan 1; 15(1): 27–34

The Authors:

Dr. Shafaq Ismail,
House Officer,
Department of Pediatrics,
CMH, Lahore.

Dr. Shaiza Ijaz,
Demonstrator,
Department of Physiology,
CMH Lahore Medical College, Lahore.

Dr. Spenta Kakalia,
Assistant Professor,
Department of Pediatrics,
CMH Lahore Medical College, Lahore.

Dr.Muhammad Ashraf Chaudhry,
Professor,
Department of Community Medicine,
CMH Lahore Medical College, Lahore.

Dr. Menahil Mukhtar,
House Officer,
Department of Pediatrics,
CMH, Lahore.

Dr. Laiba Fayyaz,
House Officer,
Department of Pediatrics,
CMH, Lahore.

Authorship:

SI: Synopsis writing, Data Collection, Data Entry, Literature Review, Manuscript Writing, Editing & Finalizing
SI: Project Idea, Data Collection, Literature Review, Manuscript Writing, Editing & Finalizing
SK: Project Idea, Data Collection, Literature Review, Manuscript Writing, Editing & Finalizing
MAC: Input with initial design, Data Analysis & Manuscript Editing
MM: Input with initial design, Data Collection & Literature Review
LF: Input with initial design, Data Collection & Literature Review