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ORIGINAL ARTICLE

Prevalence of Iron Overload and Chelation Therapy in patients of Thalassemia Major and Knowledge and Attitude of Population towards Iron Chelation Therapy in Sargodha, Pakistan

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ABSTRACT

Background: Whether familial or sporadic, whatever the cause may be, among hereditary hematological disorders, thalassemia accounts for 4.5 out of 10,000 live births worldwide[1]. Among other complications that these patients have, magnitude of transfusion related iron overload ranges from 9.5%-18%[2]. Prevalence of post transfusion iron overload in Southeast Asia is much higher than Western world[3].
Aim: To measure the prevalence of iron overload and chelation therapy in patients of thalassemia major and knowledge and attitude of population towards iron chelation therapy in Sargodha, Pakistan.
Methods: This was a cross-sectional study conducted at Rai Hospital, a private-sector hospital and Hilal-e-Ahmar Hospital in Sargodha. Total 390 patients of beta thalassemia major aged 1 month-20 years were included in the study. Data Records of 390 patients of beta thalassemia major, confirmed on hemoglobin electrophoresis admitted in the hospital from June 2021 to June 2022 were included. Data were collected on socio-demographics clinical and laboratory profile. Analysis was done in SPSS
Results: The mean age of the participants was 8.79 years, and 51.2 % were males and 48.9 % were females. Iron overload was found in 85.64% % of patients. Highest figure was 18,666ng/ml. Mean serum ferritin level was 3815 ng/ml. 30.2% received regular chelation therapy; 35.3%was on irregular chelation therapy and in 34.3% it was not started yet. Significant factors associated with iron overload and lack of chelation therapy were highly constrained socioeconomic status, repeated and frequent transfusions, lack of knowledge of importance of chelation therapy and unawareness of complications of transfusion.
Conclusion: Considering the prevalence and perils of disease itself and treatment related complications government should focus on establishing thalassemia centers nationwide to cater problems faced by these patients where trained doctors and paramedical staff can counsel them regarding disease prognosis and importance of iron chelation therapy in course of disease.
Keywords: Prevalence, iron overload, thalassemia major, chelation therapy

INTRODUCTION

Hemoglobinopathies account for 5% of congenital hematological disorders[1]. These include thalassemia, sickle cell anemia, sickle cell trait and hemoglobin E. In India and Pakistan prevalence of thalassemia is 3-4%[6] and 5-7% [4] respectively. There are estimated 60,000 births per year of thalassemia carriers[1]. Thalassemia variants include beta thalassemia (major, intermedia and trait), alpha thalassemia, sickle cell variety and HBE variant. All patients of thalassemia, after 06 months of birth become anemic because of genetic mutations in synthesis of globin chains resulting in defective hemoglobin[5]. Severity of anemia in beta thalassemia depends upon the type of mutation. In heterozygous state (thalassemia minor/trait) patients have mild microcytic anemia and almost never require transfusion. In heterozygous state (thalassemia major) patients are almost invariably dependent on blood transfusion to maintain adult hemoglobin between 9-10mg/dl. Spectrum of thalassemia intermedia lies in between thalassemia minor and major.

Anemia in patients of thalassemia major is treated with lifelong transfusion therapy. Long term transfusion is associated with iron overload in these patients. On one hand to treat anemia, with each transfusion 250mg of iron is transfused[6] and on the other hand body itself tries to compensate this in the form extra medullary hematopoiesis. Extra medullary hematopoiesis results in skeletal changes, hepato-splenomegaly and in severe forms can cause spinal cord compression. Along with long term, repeated and frequent transfusions, other factors responsible for iron load in these patients are body's inability to excrete extra iron and alteration in iron metabolism and hepcidin levels. Hepcidin maintains plasma iron level by binding to ferroportin 1 which is an iron export protein on enterocytes, macrophages and prevents iron

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secretion in plasma. Normally in anemia hepcidin levels are reduced to increase plasma iron concentrations as in thalassemia intermedia[7]. In thalassemia major due to increased plasma iron concentrations secondary to multiple transfusions hepcidin levels are raised but body is unable to excrete that iron resulting in increased transferrin saturation and increased plasma labile iron which is highly toxic and starts depositing in cardiac, hepatic and endocrinal glands and tissues[8,9,10]. Thus it results in cardiomyopathy, hepatic cirrhosis, diabetes mellitus, hypogonadism, dwarfism and bronze skin[11]. It also induces continuous state of inflammation in body by producing oxygen free radicals[12]. Iron overload is diagnosed in patient if serum ferritin level (Normal-7-142ng/ml from6 months-15 years, males-12-300ng/ml, females-10-150ng/ml)[13] is more than 1000ng/ml (mild) [14], if more than 2000ng/ml then it is moderate and if more than 4000ng/ml, it is severe.

To combat this situation iron chelation therapy is part and parcel of treatment of transfusion dependent thalassemia .It is indicated in patients with history of multiple blood transfusions(20-25 units)[15] or serum ferritin more than 1000ng/ml or dry weight of liver exceeding 3-5 mg/g due to iron deposition[16,17].

The rationale of this study is to measure the frequency of iron overload and chelation therapy in patients of thalassemia major and knowledge and attitude of population towards iron chelation therapy in Sargodha, Pakistan.

MATERIAL AND METHODS

Study design: This cross-sectional study was carried out in Rai Hospital, a private-sector hospital and Hilal-e-Ahmar Hospital in Sargodha from January 2021 to December 2021. Sample size of 378 was calculated using Raosoft sample size calculator 2004 by Raosoft .Inc. At Confidence interval 95% and margin of error 5%

with response distribution of 56.8%[8] .To increase power of study and to compensate for missing and loss to follow up patients sample size is increased to 390 patients.

Inclusion criteria: All patients between 6month -20 years of age, diagnosed cases of beta thalassemia major (on hemoglobin electrophoresis or high performance liquid chromatography).

Exclusion criteria: All patients of thalassemia intermedia and minor, Thalassemia major with comorbid

Data collection Procedure: All patients meeting the inclusion criteria and admitted for blood transfusion or presenting in outpatient department were included in the study after taking informed consent from the patients and guardians as pediatric patients were included in the study. Demographic information including age, gender, frequency of blood transfusion, duration of transfusion dependence, frequency of chelation therapy, compliance to chelation therapy, knowledge of complications of transfusion, awareness of importance of chelation therapy was obtained on predesigned Performa. Serum ferritin was done to determine iron overload.

Data Analysis: Data was analyzed in SPSS version 25. Quantitative variables in the study like age were presented as mean+/-SD. Categorical variables that is gender, , frequency of blood transfusion, duration of transfusion dependence, frequency of chelation therapy ,compliance to chelation therapy ,knowledge of complications of transfusion, awareness of importance of chelation therapy outcome variables were presented as frequency and percentage. Data was stratified for age, gender.

RESULTS

Out of 390 patients included in the study, 200(51.2%) were males and 190(48.7%) were females (Table 1). Mean Age of the patients was 8.79 years. Mean duration of transfusion was 7.93 years .Mean age at start of blood transfusion was 08 months. Because of variable ages, age ranges were defined. Transfusion was started between 1 month-12 months, 1.1-2 years, 3.3-4 years and 4.1-20 years in 308(78.9%), 32(8.2%), 20(5.12%), 20(5.12%) and 10(2.56%) patients respectively Table 2. Highest representation of patients was observed within the age range of 6 month-12 months (n=308) followed by age range of 1.1-2 years (n=32). However, the prevalence of iron excess was not significantly associated with gender (P>0.05) but there was a positive association of duration of transfusion with the iron overload.

Table 1: Gender statistics

Gender	Cases
Male	200(51.2%)
Female	190(48.7%)
Total	390

Table 2: Age at the start of Transfusion

6 month-12 months	308(78.9%)
1.1-2 years	32(8.2%)
2.2-3 years	20(5.12%)
3.3-4 years	20(5.12%)
4.1-20 years	10(2.56%)

Table 3: Frequency of Blood Transfusion

Frequency of Blood Transfusion	Cases
Every 14 days	144(36.9%)
Every 28 days	176(45.1%)
Every 56 days	36(9.2%)
Variable period	34(8.7%)
Total	390

144(36.9%) patients received transfusion after every 14 days (twice in a month). 176(45.1%) received transfusion after every 28 days (once a month). 36(9.2%) received transfusion after every 56 days or after 02 months. 34(8.7%) included in study used to transfuse irregularly at variable time period Table 3.

Iron overload was determined by measuring serum ferritin levels in blood samples. Mean serum ferritin level was 3815 ng/ml. Iron overload was found in 85.64% of patients. To categorize in mild (>1000ng/ml), moderate (>2000ng/ml), and severe (>4000ng/ml), ranges were defined for measured serum ferritin levels. In 14.35% of patients ferritin level was less than 1000ng/ml. Iron overload in 20.51% patients was mild, in 35.8% moderate and in 29.23% severe (Table 4). Out of total 390 patients 118(30.2%) received regular chelation therapy after every 3 weeks. 138(35.3%) patients used to take chelation therapy at variable intervals (after 4,6 or 8 weeks or more). In 114(29.23%) patients it was not started yet (Table 5, 6). In 56(14.35%) patients with serum ferritin less than 1000ng/ml, average serum ferritin was 565.9ng/ml. 52 patients among these used to get transfusion after 28 days and 17(4.35%) were on regular chelation therapy and in 35(8.97%) out of these 52 patients chelation therapy was not started yet. In 4(1.02%) patients out of 56 patients transfusion was done after every 14 days and they were on regular chelation therapy. Among 80(20.51%) patients with mild iron overload ,60(15.38%) used to get transfusion after 28 days and among these in 35(8.97%) patients chelation was not started yet, 10(2.56%) were on chelation therapy at variable intervals and 15(3.84%) were on regular chelation therapy after every 3 weeks. In patients with mild iron overload 20(5.12%) used to be transfused every 14 days and in these patients 5(1.28%) were on regular and 5(1.28%) on irregular chelation therapy and in 10(2.56%) patients it was not started yet. Among 140(35.89%) patients with moderate iron overload, 52(13.33%) were transfused every 28 days and 80(20.51%) after every 14 days and 8 on irregular intervals. Out of 62(15.89%) patients 4(1.02%) were on regular and 25(6.41%) were on irregular chelation and in 33(8.46%) it was not started yet. Among 70(17.94%) who were transfused fortnightly, 38(9.74%) were on regular and 25(6.41%) were on irregular chelation and in 7(1.79%) it was not started yet. Among 114(29.23%) patients with severe iron overload, 45(11.53%) were transfused every 28 days and 69(17.69%) after every 14 days. Out of 55(14.1%) patients 5(1.28%) were on regular and 29(7.43%) were on irregular chelation and in 21(5.38%) it was not started yet. Among 59(15.1%) who were transfused fortnightly, 29(7.43%) were on regular and 21(5.38%) were on irregular chelation and in 9(2.30%) it was not started yet. Data clearly showed that the frequency of iron overload increased with frequent transfusions. Also, in patients with serum ferritin less than 1000ng/ml, most (n=52) used to get transfusion after 28 days. Highest number of patients (n=140) had moderate iron overload followed by the patients who had severe iron overload (n= 114) among which 80(20.51%) and 69(17.69%) were transfused every 14 days respectively .Among moderate and severe iron overload groups 20.76% were females and 15.1 were males and 16.4% were females and 12.82 % were males respectively. This data shows prevalence of iron overload was slightly higher in females as compared to males. But statistically it was not significant.

Table 4: Serum Ferritin Levels

Serum Ferritin(ng/ml)	Cases
Less than 1000	56(14.35%)
1001-2000	80(20.51%)
2001-4000	140(35.8%)
>4001	114(29.23%)

Table 5: Frequency of Chelation Therapy

Frequency of Chelation Therapy	Cases
Every 3 weeks	118(30.2%)
After 4 or 6 or 8 weeks	138(35.3%)
Not started yet	134(34.3%)

Table 6: Compliance to Chelation therapy

Compliance to Chelation therapy	Cases
Regular	118(30.2%)
Irregular	138(35.3%)

285(73.07%) patients and their families had knowledge of complications of transfusion and 105(26.92%) had no knowledge

of complications of transfusion. 245(62.8%) were aware of importance of chelation therapy and 145(37.17%) had no awareness regarding importance of chelation therapy (Table 7, 8).

Table 7: Knowledge of Complications of Transfusion

Knowledge of Complications of Transfusion	Cases
Yes	285(73.07%)
No	105(26.92%)
Total	390

Table 8: Awareness of Importance of Chelation Therapy

Awareness of importance of chelation therapy	Cases
Yes	245(62.8%)
No	145(37.17%)
Total	390(%)

DISCUSSION

Daily millions of patients of varying age groups get blood transfusion worldwide[2]. Exact data on how many patients of beta thalassemia major are transfused daily in Pakistan is not available. One of the complications of transfusion is iron overload which further has its own dreadful consequences[8,9,10] . This study was aimed to measure the prevalence of iron overload and chelation therapy in patients of thalassemia major and knowledge and attitude of population towards iron chelation therapy in Sargodha, Pakistan. Among 390 patients included in the study iron excess was found in 85.64% patients which is a significantly high figure. This data is in line with study done by Karunaratna et al in which 87.4% of the patients had iron overload[14].

There was slight female predominance which was more than 5% in patients with moderate iron excess. This data is supported by the study done by Din G et al[18] and Fung et al in 2007[19] in which females were in highest numbers in iron excess as compared to males. But a study done by Belhoul KM et al in 2013 demonstrate opposite results in which females show significantly low serum ferritin levels in comparison to males[20]. But statistically the difference was not significant.

A study done by Thuret et al showed serum ferritin more than 2500ng/ml in 16% of the patients whereas in our study more than 64% of the patients had either moderate or severe iron overload[21]. AS per usual course of disease, most of the patients were transfusion dependent after 6 months of age and in our most 308(78.9%) patients transfusion started between 06-12months of age and mean age at the start of transfusion was 8 months with mean duration of transfusion in patients of iron excess 7.93 years. This strongly suggested a positive relationship of iron overload with early age and long duration of transfusion. About 60-80% patients who had moderate to severe iron overload, were transfused fortnightly and there was strong association between iron excess and frequent transfusion which means increased number of blood cells transfused per year. This finding is supported by the study done by Shander et al in 2009[22]. In our study highest serum ferritin was 18,666ng/ml in a female patient.

In patients who are chronically and frequently transfused warrant chelation therapy but dilemma is that this important treatment is missing from the treatment plan of most of the patients. Only 30.2% were receiving regular chelation therapy and that too in inadequate doses as there serum ferritin was not maintained below 1000ng/ml. 35.3% of the patients were on irregular chelation therapy and in 34.3% it was not even started yet .In a study done by Shah et al, 67% were taking some form of iron chelation and only 2% had effective chelation[23]. In another study done by De Sanctis et al compliance to chelation therapy was poor in 51% of the patients and it was one of the most significant factors in patients developing complications of overload[24]. In another study done in India 92.5% of patients were on regular chelation while study by Arif F et al showed regular chelation in just 10.3% of patients[25]. In our patients the mean duration of transfusion was 7.93 years but 34.3% of patients had not received chelation therapy even once since the start of blood transfusion. After a year of start of transfusion, chelation therapy should be offered to all patients and make sure that they are getting the iron chelators in effective dose and at regular intervals. It is a sad state of affairs that a lifesaving procedure of blood transfusion which improves morbidity and mortality in thalassemia major patients is becoming a major cause of death in these patients in the form iron deposition in cardiac and hepatic tissues. Iron levels in the body correlate with the serum ferritin levels as shown by the study done by Gamberini et a[26]. Data clearly shows that effective iron chelation is not available to all patients either due to poor patient compliance owing to poor socioeconomic status or negligence on part of families or health care professionals or slackness on part of government. But dynamics in Southeast are changing in countries where iron chelators are available in hospitals and there are supervised regular follow up of majority of patients[27]. Lack of knowledge and understanding about thalassemia, its complications, treatment of the disease and its complications and compliance to therapy in patients, their parents and families is the one of most important contributory factor for deteriorating health of these patients. Though 73.07% patients and their families had knowledge of complications of transfusion which was higher than the statistics shown by Basu M[28] but unfortunately it was inadequate in almost all of these patients and their families.

Study conducted by Miri-Moghaddam et al[29] in school students showed adequate knowledge in 14.7% of the population which was far low in term of figures but better in terms of information and knowledge as our study population mostly belonged to rural areas. 62.8% were informed of significance of chelation therapy but still only 30.2% patients were getting regular iron chelation therapy.

This means affected individuals and their families have the knowledge of the disease and its course but in bits and pieces which is a cause of concern as this is a significant factor hampering effective and optimal treatment of the disease and its complications.

Based on above discussion it is clear that statistics differ in various regions because of different geographical distribution and burden of disease and cultural and social values of population. The need of hour is the availability and implementation of prenatal screening, regular and safe blood transfusion followed by regular optimal chelation therapy.

CONCLUSION

Blood transfusion is inevitable in patients of thalassemia major and it definitely improves survival and lowers mortality if given according to set guidelines. But if goes unchecked it can wreak havoc on patients in form iron overload. Transfusion dependent iron load is very common and iron chelation therapy required to control this complication is not available to all patients due to multiple factors like socioeconomic constraints, unavailability of facility or negligence on part of health practitioners or government. So, considering the prevalence and perils of disease itself and treatment related complications government should focus on establishing thalassemia centers nationwide to cater problems faced by these patients where trained doctors and paramedical staff can counsel them regarding inheritance patterns, disease prognosis, the physical, social and psychological impact of the disease in affected children and their families and importance of iron chelation therapy in course of disease. Standardized governmental policies to resolve this issue and targeted campaigns like seminars, workshops for doctors and civilians, affected families on how to follow the course of disease, collaboration with social media, information cells involving central influential figures to educate masses, are required so that mortality and morbidity in these patients can be reduced. If blood transfusion is given according to guidelines and patients are monitored for complications and treated timely for the complications then not only the prognosis in these patients is

improved but economic burden in the form of costly treatment is reduced as well.

Contribution of authors: SF: principal author, **UAA:** corresponding author, **JA**: data collection, **SJ**: Statistical analysis, **NF**: Editing

Conflict of interest: Nothing to declare

REFERENCE

1. Aziz, S. S., Hamad, B. K., Hamad, H. O., Qader, M. I., Ali, E. N., Muhammed, R. H., & Shekha, M. S. (2022). Estimation of the prevalence of Hemoglobinopathies in Erbil governorate, Kurdistan region of Iraq. *Iraqi Journal of Hematology*, *11*(1), 19.
2. Shah, F. T., Sayani, F., Trompeter, S., Drasar, E., & Piga, A. (2019). Challenges of blood transfusions in β-thalassemia. *Blood reviews*, *37*, 100588.
3. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020 Dec;105(6):692-703. doi: 10.1111/ejh.13512. Epub 2020 Sep 21. PMID: 32886826; PMCID: PMC7692954.
4. Ehsan, H., Wahab, A., Anwer, F., Iftikhar, R., & Yousaf, M. N. (2020). Prevalence of transfusion transmissible infections in beta-thalassemia major patients in Pakistan: a systematic review. *Cureus*, *12*(8).
5. Nasiri, A., Rahimi, Z., & Vaisi-Raygani, A. (2020). Hemoglobinopathies in Iran: an updated review. *International Journal of Hematology-Oncology and Stem Cell Research*, *14*(2), 140.
6. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. *Biochim Biophys Acta.* 2009;1790:694–701.
7. Gardenghi S, Marongiu MF, Ramos P, et al. Ineffective erythropoiesis in beta-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood. 2007;109:5027–35
8. Krittayaphong, R., Viprakasit, V., Saiviroonporn, P., Siritanaratkul, N., Siripornpitak, S., Meekaewkunchorn, A., ... & Wood, J. (2017). Prevalence and predictors of cardiac and liver iron overload in patients with thalassemia: a multicenter study based on real-world data. *Blood Cells, Molecules, and Diseases*, *66*, 24-30.
9. Gamberini, M. R., De Sanctis, V., & Gilli, G. (2008). Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. *Pediatric endocrinology reviews: PER*, *6*, 158-169.
10. Sharif, Y., Irshad, S., Tariq, A., Rasheed, S., & Tariq, M. H. (2019). Association of frequency of hereditary hemochromatosis (HFE) gene mutations (H63D and C282Y) with iron overload in beta-thalassemia major patients in Pakistan. *Saudi medical journal*, *40*(9), 887.
11. Melchiori L, Gardenghi S, Rivella S. Beta-Thalassemia: HiJAKing Ineffective Erythropoiesis and Iron Overload. Adv Hematol. 2010;2010:938640–938640
12. Hershko C, Konijn AM, Link G. Iron chelators for thalassaemia. Br J Haematol. 1998;101:399–406.
13. Vichinsky E: Consensus document for transfusion-related iron overload. Semin Hematol 2001; 38(Suppl. 1):2–4
14. Karunaratna, A. M. D. S., Ranasingha, J. S., & Mudiyanse, R. M. (2017). Iron overload in beta thalassemia major patients. *Int J Blood Transfus Immunohematol*, *7*, 33-40.
15. Piomelli S. The management of patients with Cooley's anemia: transfusions and splenectomy. Semin Hematol. 1995;32:262–8.
16. Wintrobe MM, John P, Greer M. Wolters Kluwer Health. Lippincott Williams & Wilkins; 2009. Wintrobe's Clinical Hematology.
17. Wang, C. J., Kavanagh, P. L., Little, A. A., Holliman, J. B., & Sprinz, P. G. (2011). Quality-of-care indicators for children with cell disease. Pediatrics, 128(3), 484-493.sickle
18. Din, G., Malik, S., Ali, I., Ahmed, S., & Dasti, J. I. (2014). Prevalence of hepatitis C virus infection among thalassemia patients: a perspective from a multi-ethnic population of Pakistan. Asian Pacific journal of tropical medicine, 7, S127-S133.
19. Fung, E. B., Harmatz, P., Milet, M., Ballas, S. K., De Castro, L., Hagar, W., ... & Multi-Center Study of Iron Overload Research Group. (2007). Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: A report from the multi-center study of iron overload. *American journal of hematology*, *82*(4), 255-265.
20. Belhoul KM, Bakir ML, Kadhim AM, Dewedar HE, Eldin MS, Alkhaja FA. Prevalence of iron overload complications among patients with b-thalassemia major treated at Dubai Thalassemia Centre. Ann Saudi Med. 2013 Jan-Feb;33(1):18-21. doi: 10.5144/0256-4947.2013.18. PMID: 23458935; PMCID: PMC6078582
21. Thuret, I. (2013). Post-transfusional iron overload in the haemoglobinopathies. *Comptes rendus biologies*, *336*(3), 164-172.
22. Shander, A., Cappellini, M. D., & Goodnough, L. T. (2009). Iron overload and toxicity: the hidden risk of multiple blood transfusions. *Vox sanguinis*, *97*(3), 185-197.
23. Shah, N., Mishra, A., Chauhan, D., Vora, C., & Shah, N. R. (2010). 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*, *4*(2), 94.
24. De Sanctis, V., Eleftheriou, A., & Malaventura, C. (2004). Prevalence of endocrine complications and short stature in patients with thalassaemia major: a multicenter study by the Thalassaemia International Federation (TIF). *Pediatric endocrinology reviews: PER*, *2*, 249-255.
25. Saxena, A., Sharif, M., Siddiqui, S., & Singh, S. (2017). Knowledge, practice and experiences of parents with a thalassemic child. *Int J Contemp Pediatr*, *4*(5), 1630-1633.
26. Gamberini, M. R., De Sanctis, V., & Gilli, G. (2008). Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. *Pediatric endocrinology reviews: PER*, *6*, 158-169.
27. Azman, N. F., Abdullah, W. Z., Mohamad, N., Bahar, R., Johan, M. F., Diana, R. & Zilfalil, B. A. (2016). Practice of iron chelation therapy for transfusion-dependent thalassemia in Southeast Asia. *Asian Biomedicine*, *10*(6), 537-547.
28. Basu, M. (2015). A study on knowledge, attitude and practice about thalassemia among general population in outpatient department at a Tertiary Care Hospital of Kolkata. *Journal of Preventive Medicine and Holistic Health*, *1*(1), 6-13.
29. Miri-Moghaddam, E., Motaharitabar, E., Erfannia, L., Dashipour, A., & Houshvar, M. (2014). High school knowledge and attitudes towards thalassemia in southeastern Iran. *International Journal of Hematology-Oncology and Stem Cell Research*, *8*(1), 24.