Pakistan J. Zool., pp 1-6, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190630160603

Assessment of Patients with β-Thalassemia Major, Undergoing Tertiary Care at a Regional Thalassemia Center in Pakistan

Yasir Sharif[1], Saba Irshad[1]*, Ammara Muazzam[1], Muhammad Hamza Tariq[1], Ambreen Kanwal[1], Sana Rasheed[1], Mabel Baxter Dalrymple[2] and Anam Tariq[1]

[1]*Institute of Biochemistry and Biotechnology, University of the Punjab-54590, Lahore, Pakistan.*
[2]*The University of Manchester, Oxford Road, Manchester, M13 9PL, England, United Kingdom.*

Article Information
Received 30 June 2019
Revised 22 August 2019
Accepted 12 December 2019
Available online 04 June 2020

Authors' Contribution
YS, SR, AT performed the experimental work. SI supervised and designed the work. MBD reviewed the manuscript. MHT, AM, AK provided technical supports and participated in drafting the manuscript.

Key words
Beta thalassemia major, Serum ferritin, Liver enzymes, Blood cells count, Secondary complications

ABSTRACT

To assess iron overload, disturbed liver and hematological profile and secondary complications in β thalassemia major (BTM) patients, the current study was carried on 408 subjects including 204 patients and 204 controls. For all 408 individuals; complete blood count (CBC), blood group, serum ferritin level and liver function tests were performed. Secondary complications were assessed by physical examination of pallor, splenomegaly, ascites, and hepatomegaly. The average±SD values of patients' CBCs and liver enzymes were: red blood cells 3.07x 10^{12}±0.769x 10^{12}/L, white blood cells 8.89x 10^{9}±2.849x 10^{9}/L, hemoglobin 8.01± 1.027 g/dL, platelets 321.68x 10^{9}±1.027x 10^{9}/L, serum ferritin 2773.3±1071.9ng/mL, alanine transaminase 117.12±32.001U/L, aspartate transaminase 84.77±18.223U/L and bilirubin 1.02±0.139mg/dL. CBC of control group revealed that all of the studied parameters were normal in them and BTM patients showed significant deviation from control in both hematological and hepatic profile ($P < 0.05$). Examination for secondary complications revealed that Pallor sign was observed in 79.6% of patients, followed by splenomegaly (64.9%) and hepatomegaly (9%). As far as the control group is concerned no complication was found in that group. Current study provides sufficient evidence to justify advanced therapies to overcome secondary complications, iron overload, disturbed hepatic and hematological profile of patients and overall offers insight into improving the quality of treatment for β-thalassemia major patients.

INTRODUCTION

Thalassemia is defined as an inherited disorder of hemoglobin that is portrayed by decreased synthesis of at least one of the globin chains, prompting imbalanced globin synthesis. β thalassemia is due to the imperfection in β globulin chain generation and reaches from clinically quiet heterogeneous thalassemia minor to extreme transfusion-dependent thalassemia major (Desouky *et al*., 2009; Omar *et al*., 2005).

It is estimated that hemoglobinopathies has 270 million carriers worldwide. Out of this large number, 80 to 90 million carriers are of β thalassemia (Williams *et al*., 2012). As per the recent information, 0.3 to 0.4 million kids experience the serious issue of hemoglobin defect during childbirth, of which, 23,000 are affected with β-thalassemia. Eminently, proof recommends that feasible 90% of these kids are conceived in low pay zones of the world (Kountouris *et al*., 2014; Williams *et al*., 2012). In Pakistan nearly 9.8 million carriers of β-thalassemia exist with 5-7% carrier rate (Ansari *et al*., 2012). β thalassemia major ultimately causes anemia which is both hypochromic and microcytic in nature, in which mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) is lower than the normal persons (Karmi *et al*., 2016). Degradation of erythrocytes and repetitive blood transfusion results in iron accumulation in different essential organs like pancreas, spleen, endocrine organs, liver and heart (Rasool *et al*., 2016; Shanaki *et al*., 2016). Iron overload in hepatocytes causes damage to the liver which can be checked by measuring the level of liver enzymes that is enhanced in toxicity of liver, induced by iron accumulation within it. Untreated thalassemia ultimately leads to a collection secondary complications including hepatosplenomegaly, pallor, poor musculature, jaundice, leg ulcer, growth retardation, masses development due to hematopoiesis, genum valgum, skeleton changes (Galanello *et al*., 2010), ascites, edema, haemosiderosis (severe iron accumulation in blood), splenomegaly (Taher *et al*., 2010), heart diseases, cirrhosis, pseudoxanthoma

* Corresponding author: saba.ibb@pu.edu.pk
0030-9923/2020/0001-0001 $ 9.00/0

elasticum and hepatocellular carcinoma (Borgna Pignatti *et al*., 2005). The aim of the current study is to assess various hepatic biochemical, hematological parameters and secondary complications in β thalassemia major (BTM) patients.

MATERIALS AND METHODS

A total of 408 samples were collected, out of which 204 were β-thalassemia major patients and the 204 were healthy blood donors (controls). All these patients (already diagnosed with BTM by CBC test, hemoglobin electrophoresis and family history whilst the complicated cases were diagnosed through molecular identification methods) and controls were enrolled in Sundas blood bank and thalassemia center, situated in Lahore, Pakistan. We investigated several parameters from patients' medical records including history, age, gender, blood group and frequency of blood transfusions. Informed consent form was taken from each patient while the study was approved by ethical committee of University of the Punjab, Lahore. To study secondary complications, physical examinations like pallor, splenomegaly, ascites and hepatomegaly were performed and then following blood tests were conducted.

For CBC parameters (hemoglobin level, WBC, RBC and platelets count) 3cc sample was collected in EDTA vacutainer tube and CBC was performed on Sysmex anlayser (Sysmex kx-21). The sample was suctioned into the Sysmex; passed between two terminals through a gap, so thin that just a single cell can sit back. The impedance changes as a cell go through. The adjustment in impedance results in cell check and volume (Ike *et al*., 2010). The ferritin was measured on 'Architect 1000 SR' the architect ferritin estimation is an immunoassay which determine the presence of ferritin in serum using chemiluminescent microparticle immunoassay (CMIA) technology. ABO and Rh Blood grouping of thalassemia patients was performed using antisera A, B and Anti D (Reid *et al*., 2004).

To perform the hepatic biochemical profile which includes liver function tests (LFTs), the samples were collected in red top vacutainer tubes to obtain serum. The above tests were analyzed using Thermoscientifc kits, on Spinlab 300 spectrophotometer. Total bilirubin was performed by photometric test using 2, 4-dichloroaniline (DCA). At the end, absorbance was taken at 546 nm. alanine transaminase (ALT) and aspartate transaminase (AST) were measured by enzymatic colorimetric method and absorbance for the test was taken at 340 nm. To analyze the data statistically, SPSS 18.0 software was used. An estimation of $p \leq 0.05$ was taken as statistically significant.

RESULTS

In the current study, the mean age of β thalassemia major (BTM) patients was 6.34 ± 2.272 (Mean± Standard Deviation) years which was 32.84± 3.915 years in normal blood donors. Among 204 patients, 139 were male whilst 65 were female and in controls group, 161 were male and 43 female. This depicts male to female ratio 2.13:1 in BTM patients whilst it was 3.74:1 in the control group.

The age of first blood transfusion in patients was observed 1.2 years in male and 1.3 years in female. 119 (58.34%) patients were undergoing blood transfusions on a monthly basis, followed by 69 (33.82%) patients, undergoing transfusions after every three weeks. Whereas 9 (4.41%) and 7 (3.43%) patients received blood transfusions fortnightly and weekly, respectively (Table I). Blood grouping showed that "B" blood group was the most common among both BTM patients and controls. Out of 204, 15 and 16 subjects were found to be negative for Rh antigen in patients and control groups, respectively (Table II).

Table I. Frequency of blood transfusion in BTM patients (n=204).

Frequency of transfusion	Number of Patients		Total
	Female (% within gender)	Male (% within gender)	
Weekly	03(4.6%)	04(2.88%)	07(3.43%)
Fortnightly	05(7.69%)	04(2.88%)	09(4.41%)
3 weeks	20(30.77%)	49(35.25%)	69(33.82%)
Monthly	37(56.92%)	82(58.99%)	119(58.33%)

Table II. Blood grouping in patients and controls.

Blood Group	Controls (n= 204)	Patients (n= 204)
O+	57(27.94%)	59(28.92%)
A+	43(21.07%)	37(18.14%)
B+	72(35.29%)	73(35.78%)
AB+	16(7.84%)	20(9.80)
O-	5(2.45%)	6(2.94%)
A-	4(1.96%)	2(0.98%)
B-	6(2.94%)	5(2.45%)
AB-	1(0.49%)	2(0.98%)
Total Rh-	16(7.84%)	15(7.35%)
Total Rh+	188(92.16%)	189(92.64%)

Table III. Hepatic biochemical and hematological profile of patients and controls.

Parameter	Controls (n= 204)	Patients (n= 204)	P value
	Mean ± S.D (Range)	Mean ± S.D (Range)	
Bilirubin (mg/dL)	0.96 ±0.20 (0.7-1.3)	1.02 ±0.13 (0.8-1.7)	<0.001
Alanine transaminase (IU/L)	33.15 ±3.71 (20-50)	117.12 ±32.00 (35-220)	<0.001
Aspartate transaminase (IU/L)	30.01 ±9.58 (10-50)	84.77 ±18.22 (30-140)	<0.001
Hemoglobin (g/dL)	14.29 ±0.87 (12.7-17.1)	8.01 ±1.02 (4.5-10.4)	<0.001
Serum ferritin (ng/mL)	154.7 ±30.5 (90-250)	2773.3 ±1071.9 (1081-9817)	<0.001
White blood cells x 10^9/L	7.53 ±4.99 (2.5-77.4)	8.89 ±2.84 (4.2-16.3)	<0.005
Red blood cells x 10^{12}/L	4.73 ±0.44 (4.6-5.2)	3.07 ±.769 (2.1-5.2)	<0.001
Platelets x 10^9/L	313.14 ±39.27 (230-420)	321.68 ±1.02 (109-654)	<0.005

Hepatic biochemical profile showed that the mean level of bilirubin, alanine transaminase (ALT) and aspartate transaminase (AST) was significantly increased in BTM patients. Average value of bilirubin was 0.96 ±0.206 mg/dL in the control group which was raised to 1.02 ±0.139 mg/dL in patients group (P<0.001). ALT and AST concentration was also raised in BTM patients with a mean value of 33.15 ±3.71 IU/L and 30.01 ±9.583 IU/L (in controls) to 117.12 ±32.001 IU/L (P<0.001) and 84.77 ±18.223 IU/L (P<0.001), respectively (Table III).

Data from hematological profile of BTM patients showed that their mean serum ferritin level was 2773.3 ±1071.9 ng/mL that was significantly higher (as expected) than the mean value of normal donors i.e. 154.7 ±30.5 ng/mL. Contrary to this, mean level of hemoglobin and red blood cells (RBCs) was significantly reduced to 8.01±1.027 g/dL (P<0.001) and 3.07± 0.769x 10^{12}/L (P<0.001) respectively which had a mean value of 14.29± 0.876 g/dL and 4.73± 0.445x 10^{12}/L in control group. The mean values of white blood cells (WBCs) and platelets count in donors were 7.53 ±4.999 x 10^9/L and 313.14 ±39.27 x 10^9/L correspondingly that was significantly enhanced to 8.89 ±2.849 x 10^9/L (P<0.005) and 321.68 ±1.027 x 10^9/L (P<0.005) in BTM patients (Table III).

In regard to secondary complications, 79.66% patients presented pallor condition. Splenomegaly and hepatomegaly were dominant sign and seen in 64.9% and 9% patients respectively. Ascites, edema and Jaundice was also seen rarely in patients however bruises, central nervous system (CNS) disorders and lymphadenopathy was not inspected in any of the BTM patient (Fig. 1).

DISCUSSION

β-thalassemia is a genetic disorder with global prevalence, that ultimately results in death. Treatment of this disease includes blood transfusion, usage of antioxidants, chelation therapies and inducers of fetal haemoglobin. Typically, transfusions and chelation are most commonly administered; however, frequent blood transfusions increase the risk of transmission of infections.

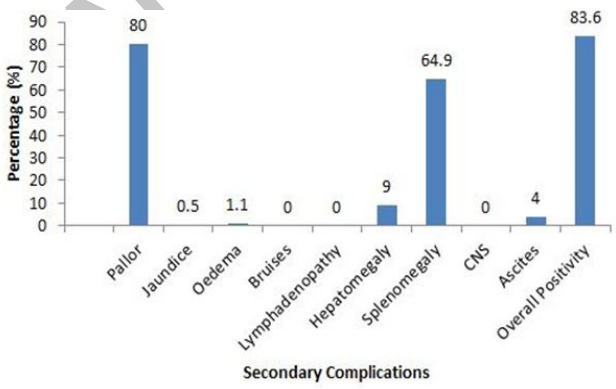

Fig. 1. Secondary complications among the patients of β-thalassemia major.

The mean age of the patients of β thalassemia was 6.34±2.272 years which was consistent with the literature (Ansari *et al*., 2012; Ishaq *et al*., 2012; Shafique *et al*., 2020). Serum ferritin level in 204 patients was greater than 1000µg/L with a mean Ferritin level of 2773.32 ±1071.908 ng/mL that is also comparable with previous studies where reported ferritin level was 3225 ± 1594 ng/mL (Munir *et al*., 2012) and 3682 ± 1693 ng/mL (Kandhari *et al*., 2005). Increased intestinal iron absorption, repeated blood transfusions, peripheral hemolysis and ineffective erythropoiesis are unavoidably linked to iron accumulation within different organs like liver, kidney, heart and endocrine glands (Rasool *et al*., 2016; Shanaki *et al*., 2016). This iron accumulation leads to liver fibrosis, endocrine abnormalities, heart disease and cirrhosis (Origa, 2017). In our study bilirubin level was found to

be increased as compared to the control group, which is also similar to the previous studies (Kumar *et al*., 2017; Mohammad *et al*., 2012). This disarrangement of bilirubin is owing to peripheral hemolysis, that is quick to the point that it surpasses the liver ability to utilize the bilirubin, prompting increased bilirubin (Cappellini *et al*., 2018), that is because of decline in function of enzyme cytochrome c oxidase, disrupting the respiration by mitochondria (Al Haddad, 2012). According to Suman *et al*. (2017) when the number of blood transfusions crosses 30 and serum ferritin level gets more than 1000 ng/mL and it ultimately results in derangement of liver enzymes. As in all of the patient's serum ferritin level was more than 1000 ng/mL, therefore, two of the most important enzymes of liver i.e. ALT and AST were examined to be increases in our BTM group, high ALT and AST has also been reported previously (Mansi *et al*., 2008; Mohammad *et al*., 2012; Shams *et al*., 2010). Barton (2007) also examined that there was a positive correlation between serum ferritin and liver enzymes.

β thalassemia is a genetic disease of hemoglobin, therefore, it leads to the acute form of anemia, in which the body lacks healthy RBCs and hemoglobin (Malloy *et al*., 1937). In our study mean hemoglobin level in BTM patient was also lower than the healthy blood donors, that is also in line with previous studies (Ayyash *et al*., 2018; Bashir *et al*., 2010). As β thalassemia is characterized by irregularity in hemoglobin level it causes a decrease in concentration of RBCs in BTM patients. In the current study, the concentration of RBCs was observed to be lower than the normal people, which are also reported in previously published data (Akula, 2017; Munir *et al*., 2013). Contrary to RBCs, WBCs and platelets were described to be increased in the patient group of current study, which is also in cohort with previous studies (Ayyash *et al*., 2018; Munir *et al*., 2013).

Repeated blood transfusion ultimately results in an array of secondary complications. Although our analysis shows that a few patients were suffering from edema, ascites, jaundice and lymphadenopathy, other secondary complications were evident. Pallor, hemochromatosis, splenomegaly and hepatomegaly were observed among the patient group. Furthermore, the current study showed that pallor is present in almost 80% patient, illustrative of pallor appearance as a validated test for diagnosis of β-thalassemia (Yalcin *et al*., 2007). In the current study, 64.9% of the total patients exhibited the complication of splenomegaly which is in line with previously reported occurrence of 63.2% (Shah *et al*., 2005) and 64.9% (Chaudhary *et al*., 2012). Hepatomegaly is another complication of untreated β-thalassemia, in which manifestation varies from 27.37% (Din *et al*., 2014) to 74.8% (Saeed *et al*., 2015) but in the current study it was found in 9% of the total patients. Coincidence of different secondary complications revealed that pallor sign was the most prevalent and this condition with splenomegaly are linked to anemic, hemolytic and iron overload in BTM patients.

β-thalassemia patients require a regular transfusion of foreign blood for their survival in our study most of the patients (58.33%) received blood transfusion every month, followed by 33.82% (69 patients) who are being transfused every 3rd week. Moreover, it is found that blood group B was predominant, in both patients and controls. This is consistent with a study from Faisalabad, Punjab, in which B blood group was most common (Munir *et al*., 2013). Previous data from Lahore, Punjab, reported "O" as the principal blood group affected by β-thalassemia (Nazir *et al*., 2014), and from Jamshoro, Sindh, blood group "A" was the most prevalent blood group (Laghari *et al*., 2018). In terms of the Rh antigen, less than 8.0% (patients and controls) were found negative for Rh antigen which is also in accordance with previous studies of Pakistan (Laghari *et al*., 2018; Nazir *et al*., 2014).

CONCLUSION

The present study concluded that a large number of β-thalassemia major patients undergo tertiary care, requiring regular transfusion of blood and other treatment. This study recommend proper iron chelation therapy (to reduce secondary complications), as serum ferritin levels among the patients exceeded the normal range. Iron chelation therapy assists in preventing the accumulation of excess iron in organs throughout the body, in order to maintain their proper function. Furthermore, as thalassemia major patients have elevated levels of HbF for protective purpose thus HbF enhancing drugs such as hydroxyurea, could present therapeutic potential. In conclusion, the current study provides sufficient evidence to justify advanced therapies to overcome secondary complications, iron overload, disturbed hepatic and hematological profile of patients, inform changes to present public health policies and overall offers insight into improving the quality of treatment for β-thalassemia major patients.

ACKNOWLEDGMENT

Sundas Foundation (blood bank and thalassemia center) provided free interaction with patients who provided their data and donated blood for further blood testing.

Declaration of interests

Authors have no conflicts of interest to disclose and

assure that all authors have read/approved the manuscript.

Funding statement

The study was funded by Institute of Biochemistry and Biotechnology, University of the Punjab and Sundas Foundation, Lahore.

REFERENCES

Akula, M., Ch, K.R. and Jampani, S., 2017. Hematological and biochemical profiles in beta-thalassemia patients with literature review. *Int. J. Curr. Advan. Res*., **6**: 6745-6748.

Al Haddad, R.M., 2012. *Molecular, biochemical and hematological investigations of β-thalassemic children in gaza governorate*. The Islamic University-Gaza.

Ansari, S.H., Shamsi, T.S., Ashraf, M., Farzana, T., Bohray, M., Perveen, K., Erum, S., Ansari, I., Ahmed, M.N. and Ahmed, M., 2012. Molecular epidemiology of β-thalassemia in Pakistan: Far reaching implications. *Indian J. Hum. Genet*., **18**: 193-197. https://doi.org/10.4103/0971-6866.100762

Ansari, S.H., Shamsi, T.S., Khan, M.T., Perveen, K., Farzana, T., Erum, S. and Ansari, I., 2012. Seropositivity of hepatitis c, hepatitis b and hiv in chronically transfused ββ-thalassaemia major patients. *J. Coll. Physic. Surg. Pak*., **22**: 610-611.

Ayyash, H. and Sirdah, M., 2018. Hematological and biochemical evaluation of β-thalassemia major (βtm) patients in gaza strip: A cross-sectional study. *Int. J. Hlth. Sci*., **12**: 18-24.

Barton, J.C., 2007. Chelation therapy for iron overload. *Curr. Gastroenterol. Rep*., **9**: 74-82. https://doi.org/10.1007/s11894-008-0024-9

Bashir, F.Y. and Sadoon, O.A., 2010. Serum ferritin level in transfusion dependent beta thalassemia patients in mousl. *Annls. Coll. Med. Mousl*., **36**: 72-78. https://doi.org/10.33899/mmed.2010.8922

Borgna Pignatti, C., Cappellini, M., De Stefano, P., Del Vecchio, G., Forni, G., Gamberini, M., Ghilardi, R., Origa, R., Piga, A. and Romeo, M., 2005. Survival and complications in thalassemia. *Annls N. Y. Acad. Sci*., **1054**: 40-47. https://doi.org/10.1196/annals.1345.006

Cappellini, M.D., Porter, J.B., Viprakasit, V. and Taher, A.T., 2018. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? *Blood Rev*., **4**: 300-311. https://doi.org/10.1016/j.blre.2018.02.001

Chaudhary, H.T. and Ahmad, N., 2012. Frequency of platelet aggregation defects in children suffering fromg β-thalassemia. *Saudi J. Hlth. Sci*., **1**: 92-98. https://doi.org/10.4103/2278-0521.100962

Din, G., Malik, S., Ali, I., Ahmed, S. and Dasti, J.I., 2014. Prevalence of hepatitis c virus infection among thalassemia patients: A perspective from a multi-ethnic population of Pakistan. *Asian Pac. J. trop. Med*., **7**: 127-133. https://doi.org/10.1016/S1995-7645(14)60218-2

Desouky, O.S., Selim, N.S., El-Bakrawy, E.M. and El-Marakby, S.M., 2009. Biophysical characterization of β-thalassemic red blood cells. *Cell Biochem. Biophys*., **55**: 45-53. https://doi.org/10.1007/s12013-009-9056-5

Galanello, R. and Origa, R., 2010. Beta-thalassemia. *Orphanet J. Rare Dis*., **5**: 1-15. https://doi.org/10.1186/1750-1172-5-11

Ike, S.O., Nubila, T., Ukaejiofo, E.O., Nubila, I.N., Shu, E.N. and Ezema, I., 2010. Comparison of haematological parameters determined by the sysmex kx-2in automated haematology analyzer and the manual counts. *BMC clin. Pathol*., **10**: 1-5. https://doi.org/10.1186/1472-6890-10-3

Ishaq, F., Hasnain Abid, F.K., Akhtar, A. and Mahmood, S., 2012. Awareness among parents of β-thalassemia major patients, regarding prenatal diagnosis and premarital screening. *J. Coll. Physic. Surg. Pak*., **22**: 218-221.

Kandhari, A., Sachdeva, A., Jain, N., Arya, S.C., Khanna, V.K. and Yadav, S.P., 2005. Pulmonary dysfunction in beta thalassemia: *Am. Soc. Hematol*., **11**: 3851.

Karim, M.F., Ismail, M., Hasan, A.M. and Shekhar, H.U., 2016. Hematological and biochemical status of beta-thalassemia major patients in bangladesh: A comparative analysis. *Int. J. Hematol. Oncol. Stem Cell Res*., **10**: 7-12.

Kountouris, P., Lederer, C.W., Fanis, P., Feleki, X., Old, J. and Kleanthous, M., 2014. Ithagenes: An interactive database for haemoglobin variations and epidemiology. *PLoS One*, **9**: e103020. https://doi.org/10.1371/journal.pone.0103020

Kumar, S., Singh, D. and Garg, A., 2017. An epidemiological study on the clinico-hematological profile of pediatric patients with congenital hemolytic anemia. *Int. J. Contemp. Pediat*., **4**: 374-377. https://doi.org/10.18203/2349-3291.ijcp20170021

Laghari, Z., Baig, N., Charan, T., Lashari, K. and Suhag, R., 2018. Distribution of ABO blood groups and rhesus factor in ß-thalassemia patients at Thalassemia Care Center Nawabshah, Pakistan. *Sindh Univ. Res. J*., **50**: 123-128. https://doi.

org/10.26692/Surj/2018.01.0021

Malloy, H.T. and Evelyn, K.A., 1937. The determination of bilirubin with the photoelectric colorimeter. *J. biol. Chem*., **119**: 481-490.

Mansi, K.M. and Aburja, T.A., 2008. Lipid profile in Jordanian children with β-thalassemia major. *Int. J. Hematol. Oncol.,* **18**. 93-98.

Mohammad, I.I. and Al-Doski, F.S., 2012. Assessment of liver functions in thalassaemia. *Tikret J. pharmaceut. Sci*., **8**: 87-95.

Munir, B., Tahira Iqbal, A.J. and Muhammad, F., 2013. Effect of β-thalassemia on hematological and biochemical profiles of female patients. *Pak. J. Life Soc. Sci*., **11**: 25-28.

Nazir, S., Faraz, A., Shahzad, N., Ali, N., Khan, M.A., Iqbal, M., Khan, M.F., Ahmed, T., Rakha, A. and Sabzwari, J., 2014. Prevalence of HCV in β-thalassemia major patients visiting Tertiary Care Hospitals in Lahore, Pakistan. *Advan. Life Sci*., **1**: 197-201.

Omar, A., Abdel, E.K., Gendy, W., Marzouk, I. and Wagdy, M., 2005. Molecular basis of beta-thalassemia in alexandria. *Egypt J. Immunol*., **12**: 15-24.

Origa, R., 2017. B-thalassemia. *Genet. Med*., **19**: 609-619. https://doi.org/10.1038/gim.2016.173

Rasool, M., Malik, A., Jabbar, U., Begum, I., Qazi, M.H., Asif, M., Naseer, M.I., Ansari, S.A., Jarullah, J. and Haque, A., 2016. Effect of iron overload on renal functions and oxidative stress in beta thalassemia patients. *Saudi med. J*., **37**: 1239-1242. https://doi.org/10.15537/smj.2016.11.16242

Reid, M. and Lomas-Francis, C., 2004. *The blood group antigen facts book.* Elsevier academic press, New York. https://doi.org/10.1016/B978-012586585-2/50027-5

Saeed, U., Waheed, Y., Ashraf, M., Waheed, U., Anjum, S. and Afzal, M.S., 2015. Estimation of hepatitis b virus, hepatitis c virus, and different clinical parameters in the thalassemic population of capital twin cities of Pakistan. *Virol. Res. Treat*., **6**: S31744. https://doi.org/10.4137/VRT.S31744

Shafique F., Ali S., Andleeb S., Rauf A., Kazmi S.A., Idrees S., Farooq F., Khan F.N., Saad-ul-Hassan M., Raja A.M. and Khalid S., 2020. Prevalence of hepatitis B and C and assessment of responsible risk factors among the vulnerable β-thalassemic patients of Azad Kashmir, Pakistan. *Pakistan J. Zool.*, **52**: 793-796.

Shah S.M.A., Khan M.T., Ullah Z. and Ashfaq N.Y., 2005. Prevalence of hepatitis b and hepatitis c virus infection in multitransfused thalassaemia major patients in North West Frontier Province. *J. Pak. med. Assoc*., **3**: 281-284.

Shams, S., Ashtiani, M.T.H., Monajemzadeh, M., Koochakzadeh, L., Irani, H., Jafari, F. and Mohseni, A., 2010. Evaluation of serum insulin, glucose, lipid profile, and liver function in β-thalassemia major patients and their correlation with iron overload. *Lab. Med*., **41**: 486-489. https://doi.org/10.1309/LMS0EQOUZSII2BNE

Shanaki, M., Ehteram, H., Nasiri, H., Azad, M., Kouhkan, F., Pakzad, R. and Mobarra, N., 2016. Assessment of liver and kidney functional parameters along with oxidative stress and inflammatory biomarker in patients with β-thalassemia major. *Iran. J. Ped. Hematol. Oncol*., **6**: 249-260.

Suman, R.L., Sanadhya, A., Meena, P. and Goyal, S., 2017. Correlation of liver enzymes with serum ferritin levels in β-thalassemia major. *Int. J. Res. med. Sci*., **4**: 3271-3274. https://doi.org/10.18203/2320-6012.ijrms20162278

Taher, A.T., Musallam, K.M., Karimi, M., El-Beshlawy, A., Belhoul, K., Daar, S., Saned, M., Cesaretti, C. and Cappellini, M.D., 2010. Splenectomy and thrombosis: The case of thalassemia intermedia. *J. Thromb. Haemost*., **8**: 2152-2158. https://doi.org/10.1111/j.1538-7836.2010.03940.x

Williams, T.N. and Weatherall, D.J., 2012. World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harb. Perspect. Med*., **2**: a011692. https://doi.org/10.1101/cshperspect.a011692

Yalçin, S.S., Ünal, S., Gümrük, F. and Yurdakök, K., 2007. The validity of pallor as a clinical sign of anemia in cases with beta-thalassemia. *Turk. J. Pediat*., **49**: 408-412.