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CORRELATION OF HEMOGLOBIN F LEVEL WITH DISEASE SEVERITY IN BETA-THALASSEMIA SYNDROME

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ABSTRACT

Background: Beta Thalassemia Major is a major health problem and approximately ten thousand thalassemic children are registered in different thalassemia centers in Pakistan. Clinical severity and transfusion frequency is varied person to person. Elevated Hemoglobin F (HbF) in thalassemia patients is considered less clinical severe then low level.

Objectives: Present study is designed to evaluate correlation between HbF level with disease severity.

To determine correlation of HbF with Hematological parameters in Beta Thalassemia major patients

Material and method: It were descriptive cross-sectional study conducted at institute of paramedical sciences and Peshawar institute of medical sciences, Peshawar. Study duration

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was from 24 Jun 2022 to 8 Dec 2022. Co-inheritance of β Thalassemia major with other hemoglobinopathy were excluded. Total of 101 thalassemia Patients history, demographic information, and clinical detail were obtained under the supervision of clinical Hematologist. Two mL of venous blood were taken in EDTA vacutainer tube for complete blood count and HbF level, 2 mL blood were also taken in heparin vacutainer tube for serum ferritin level. CBC was performed on automated hematology analyzer (XN-1000, Sysmex, Japan) and hemoglobin electrophoresis was performed on Sebia capillary 2 analyzers. Serum ferritin was performed on automated biochemistry analyzer (Cobas e622 Roch Germany).

Results: Out of 101 thalassemia patients 55(55.5%) were male and 46(46.5%) were female with mean age 8.73 (±6.71) with mean age at 1st transfusion of study population were 19.04(±23.96) month with maximum age at 1st transfusion were 144 months. The mean Hb of the studied population were 6.731313 (±2.0306) g/dl with maximum Hb were 13.000 g/dl with the mean RBC of the studied population were 2.74273 (±1.17436) (m/ ul) with maximum RBC, s was 9.300 m/ ul.). The mean Ferritin of the studied population were 3538.85(±2758.599) ng/mL with maximum ferritin were 15536 ng/mL. The mean HbA2 of the studied population were 3.310101 (±5.18157) % with maximum HbA2 were 51.0000 %. The mean HbF of the studied population were 76.111111 (±28.3777) % with maximum HbF were 99.0000 %**Conclusion:** In the present study, concluded that in patients with β thalassemia major, there is significant correlation between HbF and HbA2 and also HbA1 in hematological parameters so there is increasement of severity with level of HbF and HbA2 and also there is significant correlation between HbA2 and HbA1.But no correlation was found between HbF and HbA2 in clinical parameter.

Key Words: Thalassemia syndrome, Thalassemia major, Thalassemia intermedia, Thalassemia minor, HBF, HbA2

INTRODUCTION

Thalassemia syndrome

Thalassemia is heterogenic autosomal recessive inherited hemoglobin disorder characterized by reduced or absent production of one or more globin chains synthesis. The disease was first described by an American pediatrician Cooley in 1925. Thalassemia is a Greek word “Thala’s” mean sea and “Emia” mean blood. Because it was first time diagnosed in the

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patients Italian and Greek coasts nearby Mediterranean Sea.Thalassemia is classified according to the type of globin chain which are not synthesized, such as alpha gene defect term as an alpha thalassemia, beta gene defect as beta Thalassemia, delta gene defect as delta Thalassemia and gamma gene defect as a gamma Thalassemia. Beta Thalassemia is further classified based on clinical severity into three sub types. B Thalassemia minor, β Thalassemia intermediate and β Thalassemia major. (1,2)

Beta Thalassemia Minor /Trait/carrier

It is heterozygous state of β thalassemia when person inherited one normal β gene from one parent and either β^0 or β^+ thalassemia allele from another partner. Frequency of BTT in Pakistan is 5 to 8%. These patients are mostly shows not symptoms but may develop anemia during stress (severe infections), increased demand (pregnancy, menstruating teenager female) and coexisting conditions (Iron deficiency, vitamin B12/folate deficiency). β Thalassemia trait is diagnosed with hemoglobin electrophoresis with high HbA2 (>3.5%) level than normal. Complete blood count reveals lower or borderline hemoglobin level (10 to 12 g/dL) with low MCV and MCH but normal MCHC and RDW level. Erythrocytes is relatively high (erythrocytosis > 5.0 million/uL) with low HCT. Peripheral blood smear mostly shows microcytic hypochromic Rbc,s, polychromasia, basophilic stippling and occasional target cells(3–6)

Beta Thalassemia Intermedia

Patient with TI don't need regular transfusion or need transfusion time to time. They do not show any symptoms until adulthood. Cholelithiasis, Pallor, mild to moderate jaundice, liver, increase in spleen size, moderate jaundice, leg ulcer, extramedullary masses of hyperplastic erythroid marrow are the Clinical feature of BTI . Individual with TI present later then TM.(7).This condition is neither as critical like BTM nor as mild as BTT. Such patients might present with anemia the severity could lie from non-symptomatic carrier state to the severe symptomatic state dependent on blood transfusion. Beta thalassemia intermediate is diagnosed with hemoglobin electrophoresis with HbA2 normal or >4% level than normal. Complete blood count reveals lower hemoglobin level (7 to 10g/dL) with low MCV and MCH.Erythrocytes is relatively low (erythrocytopenia) < 4.5 million/ ul with low HCT. Peripheral blood film reveals similar to beta Thalassemia major but with less severe changes.

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β thalassemia Major

It is monogenic hematologic disorder in which synthesis of globin chains are completely absent, results in severe TDA, hepatosplenomegaly, and bone deformities. Therefore, it is also known as transfusion dependent β thalassemia and it is clinically severe form of β thalassemia. Complete blood count reveals lower Hb level (<7g/dl), hematocrit and total RBC,s count, with low MCV, MCH and MCHC. But RDW is elevated in BTM patients. Peripheral blood film reveals anisopoikilocytosis, marked hypochromia, microcytosis with target cells, tear drop cell, spherocytes, polychromasia, basophilic stippling, fragmented and nucleated red blood cells. Leucoerythroblastic blood picture in BTM patients is due to tendency of these patient to infections and ineffective erythropoiesis. Bone marrow is not usually recommended for diagnosis except with few conditions. Bone marrow reveals hypercellular marrow with erythroid hyperplasia. Myelopoiesis is hyperactive with normal maturation. Megakaryopoiesis and lymphopoiesis is normal. Beta thalassemia major diagnosis is based on hemoglobin electrophoresis. It is revealed that low level of HbA1 with elevated Hemoglobin F and variable HbA2 levels. (8)

Figure 1: β Thalassemia major patient at FATMID foundation Peshawar Pakistan

Epidemiology

β thalassemia major is a world major health problem especially in certain regions such as Mediterranean, south Asia and Middle East. The prevalence of β thalassemia major is increased day by day due to increased migration ratio worldwide including Northern Europe and North America. Currently β thalassemia major childbirth rate is approximately 60000 per year globally. This ratio in Pakistan is approximately 6000 per Annam. Till now about one hundred thousand β thalassemia major patients are registered in Pakistan. This number might be high due to unavailability of national registry for thalassemia major patients. These patients

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required five hundred blood donation per month which occupy firth part of total hospital blood donor requirement. These patients required approximately 7.8 billion rupees for comprehensive supportive treatment such as RBC transfusion, iron chelation and other supportive medications(2,9,10)

Clinical Presentation

Typically, children with β thalassemia major present with severe anemia, iron overload, bone deformities, enlarged spleen, growth retardation, infection, alloimmunization, frontal bossing and overgrowth of maxilla produce thalassemia or "chipmunk" facies Due to the deposition of iron in various endocrine glands, liver, heart it will cause failure of these visceral organs. Hypopituitarism, Hypothyroidism, Hyperparathyroidism, Testicular/Ovarian failure, Diabetes is the complications seen due to deposition of iron in multiple endocrine glands leads to damage of those glands.

Growth retardation also occurs due to deposition of iron in pituitary gland.(11)

Another clinical feature is hypersplenism, HIV infection (due to regular transfusion), chronic hepatitis, cirrhosis, venous thrombosis, and osteoporosis and cardiac disease including dilated myocardiopathy and arrhythmias.

Pathophysiology

In β thalassemia major due to deficient or complete absent of β globin chains the production of gamma chains will increase and excess of alpha chains occurs and hence HbF increased which effects oxygen affinity (increase) then tissue hypoxia occurs which result in compensatory erythroid hyperplasia in bone marrow and skeletal changes.(12)

Due to Lack of β globin chain production the Hemoglobin synthesis decrease which lead to the formation of microcytic hypochromic red cells Excessive red blood cells destruction in spleen occurs which leads to splenomegaly. (13)

In BTM patients the absorption of iron from intestine is increased due to ineffective erythropoiesis, also these patients need regular blood transfusion therapy which causes iron overload .and hence it damages the parenchymal cells of liver ,pancreas ,gonads and heart as a result various disease occurs such as diabetes mellitus ,cirrhosis, infertility and heart

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failure.(14)

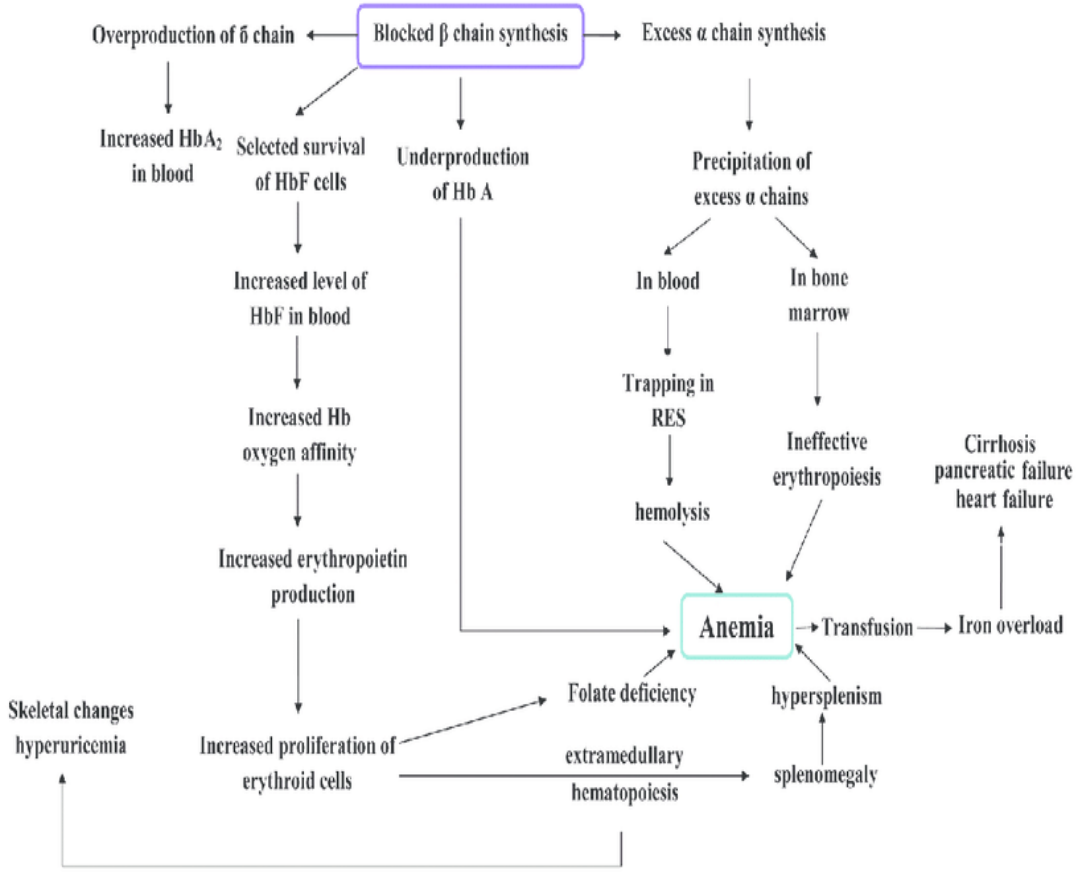

Figure 2: Pathophysiology of Beta Thalassemia Major

Laboratory diagnosis

β Thalassemia can be diagnosed on different laboratory examination such as CBC, blood smear, HB electrophoresis.

Complete Blood Count [CBC]

CBC was carried out by automated hematology analyzer by which we can check hemoglobin level and size of RBCs. It also counts cells and talks about the information on the size and structure. It is the 1st step to investigate the suspected case of thalassemia Low HB and low MCV is the 1st indication of thalassemia. After ruling out iron deficiency as the cause of anemia.(15)

Peripheral blood smear

Morphological changes of effected individuals show microcytic, hypochromic, anisocytosis, poikilocytosis and nucleated RBC, s (erythroblasts), basophilic stippling, tear drop cells

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Qualitative and quantitative Hb analysis

Cellulose acetate electrophoresis and HPLC was performed to identify the amount and type of hemoglobin. Pattern of hemoglobin changes according to β thalassemia type. HbA1 is fully absent and HbF constitutes the 92-95% of the total Hb in homozygous Beta thalassemia. HbA2 may be increased or normal.(16)

Treatments: -

A) Supportive treatment:

RBC transfusion therapy

Patients with BTM require regular blood transfusion therapy to maintain fresh, normal RBCs as well as to correct anemia and suppress ineffective erythropoiesis. it prevents the patient from bone deformities, hepatosplenomegaly and allow the patient for normal survival.

The major problem associated with regular transfusion in BTM patients are iron overload, TTI, alloimmunization, febrile transfusion reactions and delayed hemolytic transfusion reactions. CT is given for BTM patients to manage iron overload and avoid death associated with iron overload also to extend life expectancy.(17)

Splenectomy

It is used as alternatives for blood transfusion therapy which increases Hb levels and improves growth and Quality of life, but it also has several disadvantages like sepsis and thrombosis.

B) Curative treatment

it is most cost-effective treatment for BTM children's which can improve overall survival and quality of life.

Gene therapy

Gene therapy is done for the first time in 1980s for hemoglobinopathy. In gene therapy we deliver normal globulin gene into stem cells by a suitable vector. But the main complexities in gene therapy is to design of suitable vector that are systematic, non-dangerous, and safe vectors, the safe and systemic hemopoietic stem cell mobilization from bone marrow to blood circulation by GM-CSF or G-CSF, genetic manipulation, transplant manipulated-HST into the patient. And combination and assembly of different regulatory DNA elements associated with high expression of Gemma globin. A suitable vector which carries the gene such as lentiviral

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vector are the best choice for transfection (exogenous gene transfection to mammalian cells) (18)

Bone marrow transplantation

HSCT are well known treatment for BTM patients while it is less use for beta thalassemia intermedia. The eligibility decision for bone marrow transplantation is usually complicated and is related to quality of life and also expected survival time of transplanted patient when compared to the supportive treatment.(19)

C) HbF induction

HbF induction is given to reduce the transfusion requirements and prevent related difficulties of iron overload. Hb level is improved in patients by using HbF inducing agents like hydroxyurea and thalidomide.

Mechanism of action: - it decreases the precipitation of alpha globin chain due to which reduction occurs in ineffective erythropoiesis.

Thalidomide activate the expression of globin gene and proliferation of erythroid cell .

Hydroxyurea capsules are available in strength of 500mg, and thalidomide capsules are available in strength of 100mg and 50mg in Pakistan.

BTM Patients received a combination of HU 500mg everyday (>30kg) or every alternate day (<30kg). Thalidomide 100mg once daily weight greater than >30kg.

The combinatorial treatment (thalidomide and HU) is mostly effective in children with BTM.(20)

Asma Abdul Ghani et al (2020) conducted a study on Hematological and Biochemical status of β Thalassemia in Pakistani and Afghani patients. Total 100 patients were studied including 50 Pakistani and 50 Afghani patients of both genders. Result revealed that a significant decrease occurs in hematological parameters in both populations. LFTs and RFTs were increased in both populations as compared to control. They found that the prevalence of beta thalassemia is more in Afghani patients as compared to the Pakistani patients because lack of awareness, availability of beta Thalassemia screening facility and inter tribe marriages.

In 2016 Raffaella Origa, et al. worked on beta thalassemia syndrome and from his research study he concluded that the severity of β-thalassemia is mainly related with the degree of α-globin chain excess and influenced by genetically factors unlinked to globin genes as well as

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environmental conditions and management. Transfusion and iron chelation therapy decrease the severity of BTM.

Retno Dwi Wulandari1 et al. worked on Hematologic and Clinical Features of Thalassemia Patients with Early or Late Onset Transfusion in East Java, Indonesia the CBC of thalassemia patients showed lower HbA, MCV, MCH, increased RDW- CV and increased HbA2 (>3.5%) and HbF (>1.5%). The aim of their study to compare hematologic HbA2 and HbF profile as well as height, weight, BMI and spleen size in thalassemia patients. Grouped based on transfusion started on early or later age. Results showed that hematologic, HbA2 and HbF profile were not different in both groups. Height, weight was below third percentile in both groups.

In 2020 Vamsi K. et al. worked on An Evaluation of Bone Health Parameters in Regularly Transfused Beta-Thalassemia Major Patients that beta thalassemia patients have low Hb level and need regular transfusion. The bone defects occurs with the fluctuation in both hematological and biochemical parameters .they done cross sectional observational study to determine and correlate the bone mineral density with biochemical and hematological parameters in 50 beta thalassemia major patients(age>6years) who regularly transfused .it was concluded that continues investigation of BMD ,hematologic and biochemical parameters in regular transfused BTM patients may show ongoing deficiency may helping to maintain regular transfusion with supplementation of calcium and vitamin D for strong bone health.

In 2022 Asaad M. A. et al.had done work on the An Overview on Thalassemia and Challenges during COVID-19 in Department of Medical Laboratory Sciences, College of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates the aim of their study is to find out the genetic difference and etiology between α and β-thalassemia, hematological and pathophysiological difference in β-thalassemia major, to form challenges during COVID-19 crisis. In their research work they concluded that treatment of thalassemia depends upon its severity they also guide researchers to do study and meta-analysis study during COVID-19 crisis especially during infection and post vaccination of COVID-19.

In 2019 Farrukh T. Shaha, et al. in Whittington Health NHS Trust, London, UK b Hospital of the University of Pennsylvania, Philadelphia worked on Challenges of blood transfusions in β-thalassemia patients. They stated in their research paper that regular blood transfusion is essential for BTM patients, and they are supported by ICT throughout their life. In many

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countries patient with BTM do not have safety practices and regular blood transfusion. Due to under transfusion the outcomes of Beta thalassemia patients in both health and quality of life will decreased. He concluded that RBC transfusion is an essential lifesaving treatment for patients with severe BT.ICT is also needed because due to iron overload many complications are raised.AS a result transfusion generates medical complications and may place significant burden on health care system especially in low- and middle-income nations that can't afford it. Under transfusion also result in poor patient outcomes like life expectancy and quality of life. To make standardized and improve adequate blood transfusion services we should provide quality control and ICT among worldwide population with BTM patients.

From October 2021 to April 2022 Akanksha Garg1 et al. worked Safety and Efficacy of Thalidomide and Hydroxyurea Combination in Beta Thalassemia Patients from 2017 to 2021.

MATERIAL AND METHODS

Study design

It was descriptive observational study design to evaluate the association of hemoglobin F levels with β Thalassemia Major disease clinical severity.

Study setting

Current study was carried out at Institute of Paramedical Sciences, Khyber Medical University, Peshawar Institute of Medical Sciences (PIMS) and FATMID Thalassemia Center Peshawar-Pakistan.

Study duration

It was six a month study conducted from 24, Jun 2022 to 1, Dec 2022

Sample size calculation

Total of 101 beta Thalassemia major patients were studied. Sample was calculated with online sample size calculator (www.raosoft.com/samplesize.html)

Margin of error was 5%,

Confidence level was 95%

Disease distribution: 7%

Population size: 20

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Sample selection

Inclusion criteria

All diagnosed BTM patients labeled so based on a valid hemoglobin electrophoresis report were included in the study. Those β Thalassemia Intermedia patients who become transfusion dependent were also included in the study.

Exclusion criteria

All co-inheritance of β Thalassemia Major / Intermedia patients present with other hemoglobinopathy such as alpha Thalassemia, Sickle cell anemia, Hemoglobin E, and Hemoglobin C disease were excluded from the study.

Methods

Complete blood count estimation

Venous blood of 1.5 ml was collected in purple top vacutainer tube (EDTA) for to performed complete blood count. Complete blood count was performed through automated hematology analyzer (Sysmex XP-300™).

Hemoglobin electrophoresis

Hemoglobin electrophoresis was performed on EDTA vaccutainer tube by using automated hemoglobin electrophoresis analyzer (D10, Bio Rad, USA).

Serum Ferritin level

First, we collected 2ml of blood in gel tube from the patients in aseptic technique and then centrifuged at a speed of 1300 r/min for 15 min. The serum ferritin test was performed by an automated chemistry analyzer (Cobas e622, Roch, Germany)

Data Analysis method

SPPS version 22 was used for data analysis. Statistic frequencies were calculated for description and presented in tabulated form**.**

RESULTS

A total of 101 Thalassemia patients were studied, among them 55 (55.5%) were male and 46(46.5%) were female (Figure 3). The mean age of the studied population were 8.73 (±6.71) years with maximum age were 45 years. The mean weight of the studied population was 22.58 (±8.88) kg with maximum wight were 50 kg. The mean age at 1st transfusion of the studied population were 19.04 (±23.96) month with maximum age at 1st transfusion were 144 months. The mean transfusion frequency of the studied population were 30.21 (±21.77) days

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with maximum transfusion frequency were 150 days. The mean Hb of the studied population were 6.731313 (±2.0306) g/dl with maximum Hb were 13.000 g/dl. The mean RBC of the studied population were 2.74273 (±1.17436) (m/ ul) with maximum RBC, s were 9.300 m/ ul. The mean HCT of the studied population were 18.791515 (±) % with maximum HCT were 43.600 %. The mean TLC of the studied population were 13.869091 (±36.323) /ul with maximum TLC were 362.0000 / ul. The mean Platelets of the studied population were 299.801010 (±206.076) /ul with maximum platelets were 1079.000 /ul (Figure 4). The mean Ferritin of the studied population were 3538.85(±2758.599) ng/mL with maximum ferritin were 15536 ng/mL. The mean HbA1 of the studied population were 20.442424 (±27.8775) % with maximum HbA1 were 97.0000 %. The mean HbA2 of the studied population were 3.310101 (±5.18157) % with maximum HbA2 were 51.0000 %. The mean HbF of the studied population were 76.111111 (±28.3777) % with maximum HbF were 99.0000 %. As shown in the given table.

Variables	**Mean± SD**	**Minimum**	**Maximum**	**Variance**
Age (years)	8.873±6.71	1	45.0	45.12
Weight (Kg)	22.58±8.88	8.0	50.0	79.02
Age at 1st transfusion (month)	19.04±23.96	1	144	574.202
Transfusion frequency(days)	30.21±21.77	3	150	474.06
Hb (g/dl)	6.731313±2.0306	2.9000	13.000	4.123
RBC (m/ ul)	2.794273±1.17436	1.0100	9.3000	1.379
HCT (%)	18.791515±	7.4000	43.6000	53.014
TLC (/ul)	13.869091±36.323	1.6000	362.0000	1319.37
Plts (/ul)	299.801010±206.076	2.2000	1079.000	42467.48
Ferritin(ng/mL)	3538.85±2758.599	307	15536	7609869.1
HbA1 (%)	20.442424±27.8775	.0000	97.0000	777.157
HbA2 (%)	3.310101±5.18157	.0000	51.0000	26.849

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HbF (%)	76.111111±28.3777	.0000	99.0000	805.298

Table 1: Descriptive analysis of the studied population

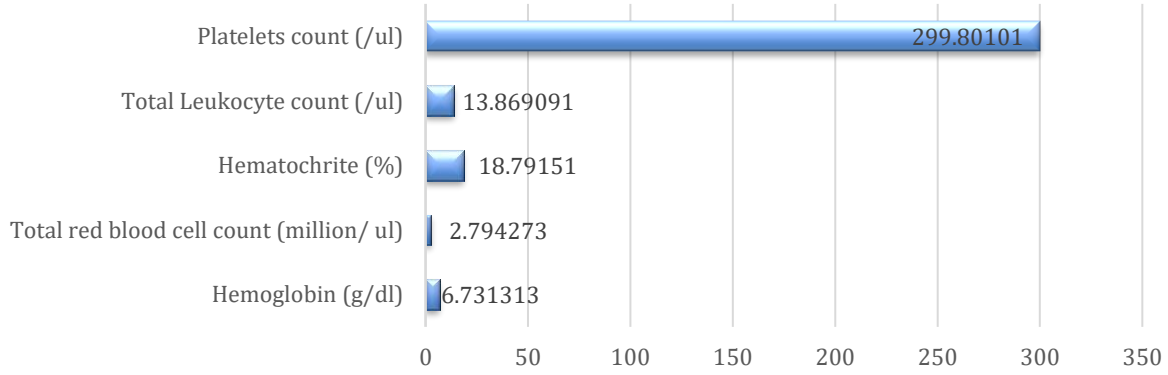

Figure 4: Graphical representation of complete blood count parameters with mean values

Out of 101 cases of β thalassemia syndrome 55.5% are male and 46.5% are female. Trend in transfusion of these patients is increasing over time were 21.2%, decreasing over time were 15.2%. The patients whose has transfusion frequency same from start were 13.3% and the patients whose transfusion frequency are variable were 32.3%. Mostly β-thalassemia major patients have severe splenomegaly. Out of 101 patients 60.6% are mild, moderate are 31.3% and severe splenomegaly were 8.1% of total patients. In severe splenomegaly, splenectomy is performed. Among 101 cases 5.1% done splenectomy and 94.9% did not do splenectomy. Thalassemic patient are mostly anemic depend upon the Hb level they are mild, moderate, severe. Among 101 patients 6.1% have mild anemia,60.6% have moderate and 33.3% have severe anemia, transfusion is mostly done for thalassemia major patients they take regular transfusion and rare cases of thalassemia intermedia patients also required RBC, s transfusion. The frequency of RBC, s transfusion among 101 cases is 86.9% done regular transfusion and 13.1% did not done RBC, s transfusion. The major problem associated with regular transfusion in BTM is iron overload. These patients take iron chelation therapy among 101 cases 36.4% done iron chelation therapy and 63.4% did not done iron chelation therapy.

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Proper treatment is done by the 39 and 60 did not take proper treatment Among HbF induction 25 patients responsive to hydroxyurea,11 is non-responsive and 3 did not take hydroxyurea.24 are responsive to Thalidomide, 14 are non-responsive and 1 did not take Thalidomide. 14 are responsive to combinatorial treatment,7 is non-responsive and 18 did not take combinatorial treatment.

Characteristics			**Frequencies**	
			N	%
Gender		Male	54	54.5
		Female	45	45.5
Clinical Severity	Trend in transfusion	Increasing with time	21	21.2
		Decreasing with time	15	15.2
		Same from start	31	31.3
		Variable	32	32.3
	Anemia	Mild	6	6.1
		Moderate	60	60.6
		Severe	33	33.3
	Splenomegaly	Mild	60	60.6
		Moderate	31	31.3
		Severe	8	8.1
	Splenectomy	Yes	5	5.1
		No	94	94.9
	Bone pain	Yes	53	53.5
		No	46	46.5
	Iron overload	Mild	16	16.2
		Moderate	12	12.2
		Severe	71	71.7
Supportive Treatment	RBCs transfusion only	Yes	86	86.9
		No	13	13.1
		Yes	36	36.4

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	RBCs transfusion with iron chelation		No	63	63.6
Curative Treatment	Proper treatment		Yes	49	49.4
			No	60	60.6
	HSC Transplant		Yes	0	0
			No	99	99
	Gene therapy/Genome editing		Yes	0	0
			No	99	99
	HbF Induction	Hydroxyurea	Responsive	25	64.1
			Hydroxyurea not taken	3	7.7
			Non-Responsive	11	88.2
		Thalidomide	Responsive	24	61.5
			Thalidomide not taken	1	2.6
			Non-Responsive	14	35.9
		Combinatorial Treatment	Responsive	14	35.9
			Combinatorial not Taken	18	46.2
			Non-Responsive	7	17.9
Post-treatment Complaint			No complaint	42	42.4
			Bone pain	57	42.4

Table 2: Different characteristics analysis of the studied population.

The HbF correlation with different hematological parameters shows that there was no correlation found between Hb (r= -0.133,P=0.19),RBC (r=-0.072,P=0.293) ,ferritin (r= - 0.024, P =0.721), TLC (r = 0.43 ,P =0.672) ,platelets(r =0.013,P = 0.849) and HCT (r = -0.62 ,P = 0.545) with HbF , while the correlation between HbA2 and HbF (r = -0.285, P = 0.000), HbA1 and HbF (r = 0.882 , P = 0.000) was found significant as shown in the given table (3).

Table 3: HbF correlation with Hematological Parameters

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Parameters	co-efficient of correlation	Significance (2 Tail)
HB	-0.133	0.19
RBC, S	-0.072	0.293
Ferritin	-0.024	0.721
HbA2	-0.285	0.000
TLC	0.43	0.672
Platelets	0.013	0.849
Hct	-0.62	0.545
HbA1	0.882	0.000

HbA2 correlation with different hematological parameters shows that there was no correlation found between Hb (r= - 1.67,P= 0.99),RBC (r= 0.001,P=0.99) ,ferritin (r= 0.073, P =0.294), HbA2 (r = 1 , P = nil), TLC (r = 0.044 ,P =0.526) ,platelets(r = -0.007,P = 0.923) and HCT (r = 0.022 ,P = 0.757) with HbA2 , while the correlation between HbA1 and HbA2 (r = 0.177 , P = 0.012) was found significant as shown in the given table (4).

Parameters	Co-efficient correlation	Significance (2 Tail)
Hb	-1.67	.099
RBC, S	0.001	0.99
Ferritin	0.073	0.294

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HbA2	1	NO Value
TLC	0.044	0.526
Pletlets	-0.007	0.923
HCt	0.022	0.757
HbA1	0.177	0.012

Table 4: HbA2 correlation with hematological parameters

Parameters	**co-efficient correlation**	**Significant**
Trend in Transfusion	-.105	.300
Splenomegaly	-.077	.449
splenectomy	0.039	0.637
Iron overload	-0.033	0.685
Anemia	0.082	0.312
Bone pain	-.019	.855

The HbF correlation with different clinical parameters shows that there was no correlation found between trend in transfusion (r= - 0.105,P=0.300), splenomegaly (r=-0.77, P=0.449) ,splenectomy (r= 0.039, P =0.637), iron overload (r = -0.033 ,P =0.685) , anemia (r =0.082,P = 0.312) and bone pain (r = -0.019,P = 0.855) with HbF , as shown in the given table (5).

Table 5: HbF correlation with different clinical parameters

The HbA2 correlation with different clinical parameters shows that there was no correlation found between trend in transfusion (r= 0.113,P=0.114), splenomegaly (r=-0.077, P=0.449) ,splenectomy (r= 0.028, P =0.737), iron overload (r = 0 ,P =0.997) , anemia (r = -0.065,P = 0.433) and bone pain (r = 0.130,P = 0.198) with HbA2 , as shown in the given table (6).

Table 6: HbA2 with different clinical parameters

Parameters	**Co-efficient correlation**	**Significant**
Trend in Transfusion	0.113	0.114
Splenomegaly	0.077	0.449

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Splenectomy	0.028	0.737
Iron overload	0	0.997
Anemia	-0.065	0.433
Bone Pain	0.130	0.198

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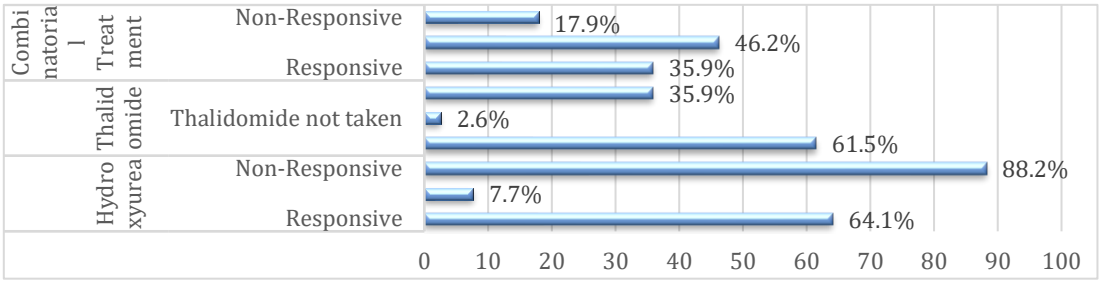
Figure 5: Treatment responsive and non-responsive trend in beta Thalassemia Major patients



DISCUSSION

According to our research the proportion of male (54.5%) patients was higher than the female (45.5%). There were 56.95% male while females were 43.05% reported by Muhammad Sadiq Khan et al. Almost similar to our finding This gender-ratio difference in thalassemic patients (males more affected than females)it is difficult to explain but one possible reason is that possible reason is the fact that the people are more concerned with the health of the male offspring and, hence, aremore likely to seek medical care for them(21)

Our result not agreed with study conducted by Renzo Galanello et al. Transfusion frequency in patient with confirmed β thalassemia major with (hb level<7g/dl) more than 14 days. However in patients with (hb level>7g/dl) more then 360days because according to our result transfusion frequency of patients with β thalassemia major was 3 days with (HbF level<7g/dl) was30 days and the patients with (hb level>7g/dl) was 150days(22)

The mean HCT of our studied population were 18.791515 (±) % with maximum HCT were 43.600 %.Our research nearly agrees with Md. Fazlul Karim et al, (2015), who found mean of HCT (21.5±5.3)% in β thalassemia major patients.(23)

According to our study, the mean TLC of the studied population were 13.869091 (±36.323) /ul with maximum TLC were 362.0000 / ul, as it nearly agreed with study conducted by Kochar Kh. Saleh et al, (2021) who found mean TLC with standard deviation (11.8 ± 4.6) in β thalassemia major patients.(24)

According to our research study the mean serum ferritin of the studied population were 3538.85(±2758.599)ng/ml with maximum ferritin were 15536ng/ml in which 71.7% have high ferritin level which shows sever iron overload ,12.2% moderate and 16.2% have mild iron overload .but according to Amit Kumar Mishra et al. β thalassemia major patients showed 87.4% very high ferritin level .the mean serum ferritin level was found to be 2767 ng/ml in

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which 44.4% patients have moderate iron overload ,43.05%patientshad severe iron overload and 12.5% had mild iron overload .our results were not in agreement with a study in India conducted in 2013 among school of biotechnology by Amit Kumar Mishra et al.(2013).(25)

The mean HbA2 of the studied population were 3.310101 (±5.18157) % with maximum HbA2 were 51.0000 %.The mean value with standard deviation of HbA2 (3.65±6.73) was found in a study conducted by Tazeen Majeed et al. (2013), which is approximately equal to our study.(26)

According to the study conducted by Tazeen Majeed et al. The mean value with the standard deviation of HbF in β thalassemia major patients were 68.22(±32.56). In our study the mean of hbF with standard deviation was 76.111111(±28.3777). which is not agreed with Tazeen Majeed et al.(2013).(26)

Clinical severity

In our research out of 99 cases 60.6% were mild, 31.3% were moderate and 8.1% were severe splenomegaly but according to Sabih salih mehdi et al.(2009) there were 105 patients with thalassaemia major attending the centre for follow up and blood transfusion .out of 52/105 (49.5%)patients were found to have significant splenomegaly and 58/105 (55.23%)patients were found to have mild or no splenomegaly.our results are nearly similar with Sabih salih et al.(2009).(27)

According to the study finding by Antonio Piga et al. (32.6%) had been splenectomized, at a mean age of 10.0 ± 5.1 (range 1.7–31.1) and according to our study (5.1%) at mean age of 8.873±6.7175 done splenectomy which is totally different from our result and were not in agreement with our study.(28)

According to our results the frequency of regular RBCs transfusion among 99 cases were 86(86.9%) and 13 (13.1%) were not under transfused. Recently, study showed that 76/142 (53.5%) of the β thalassemia major patients are under transfused (Neeraj Shah et al.2010).so our results had no similarity with these findings.(29)

The inevitable consequence of regular lifesaving transfusion in β thalassemia major is the accumulation of excess iron within tissue. This causes progressive organ damage. In our study for such patients require regular blood transfusion out of 99 patients 36.4%were taking some form of chelation therapy and 63.4%were not taken iron chelation therapy. Our findings not

agreed with Neeraj Shah *et al*, 2010) who found 96/142 (67%)patients were taking some form of iron chelation therapy. (29)

Our hydroxyurea treatment result agreed with the study conducted by Khaled M. Musallam et al.almost (60-64.1%) patients were responding to hydroxyurea in our study population 25 patients(64.1%)are responded to hydroxyurea and 11(28.2) are non-responded(14)

Among HbF induction 24 (61.5%) patients are responsive to Thalidomide, 14(35.9%) were non-responsive and 1(2.6%) were not taken Thalidomide therapy.

According to our research study out of 39 patients 14(35.9%) were responsive ,7(17.9%) was non-responsive and 18(56.2%) not taken combinatorial treatment. But according to Akanksha Garg at al .A total of 87 patients were started on thalidomide and HU combination out of these 63(71.2%) patients were respondent and 24(29.8%) were non-responsive. Our results are not in agreement with Akanksha Garg et al (2021).(20).

CONCLUSION

In the present study, concluded that in patients with β thalassemia major, there is significant correlation between HbF and HbA2 and also HbA1 in hematological parameters so there is increasement of severity with level of HbF and HbA2 and also there is significant correlation between HbA2 and HbA1.But no correlation was found between HbF and HbA2 in clinical parameter

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