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β-THALASSEMIA MAJOR PATIENTS PRESENTING IN DR. AKBER NAIZI TEACHING HOSPITAL ISLAMABAD

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Abstract

Consanguinity-related hemoglobin chain mutation is typically linked to the rising incidence and prevalence of thalassemia disorders. In Pakistan, consanguinity is directly linked to 70% of cases. The goal of this current research was to ascertain the incidence of various mutation types and how they related to age, gender, consanguinity, ethnicity/regional distribution, and B-thalassemia major patient presentation. Forty-two of the 60 patients who tested positive for beta-thalassemia major also had consanguinity. 15% of the mutations found in the study came from Rawalpindi, and 10% came from Islamabad. While IVS 1-5 (G-C) mutation was found in 3 out of 4 positive cases of Beta thalassemia major in D.G. Khan, Fr 41-42 (-TTCT) and IVS 1-5 (G-C) were more common in Rawalpindi. Of the 48 homozygous mutations, Fr 8-9 (+G) accounted for 33.3% of all observed mutations, while IVS 1-5 (G-C) accounted for 30%. With a percentage of 58.3% for Beta Thalassemia major, Fr 8-9 (+G) was the most commonly observed mutation out of the 12 heterozygous mutations in gene 1. In both males and females, the most commonly observed mutations were Fr 8-9 (+G) and IVS 1-5 (G-C).

Keywords: *Beta thalassemia major, β-globin gene, Frequency, Hemoglobin, Mutation*

INTRODUCTION

An autosomal recessive hematological disorder of the β-globin chains in the hemoglobin (Hb) molecule is known as beta-thalassemia (1). Thalassemia is often the cause of microcytic hypochromic anemia, which is caused by a decreased or absent integration of the hemoglobin chain. Thalassemia is caused by a quantitative error in the synthesis of hemoglobin. Conversely, hemoglobinopathies, such as sickle cell disease, are brought on by defects in the structure or function of hemoglobin. A genetic mutation in the beta-globin gene causes a reduced beta-globin chain of hemoglobin; this condition is referred to as beta-thalassemia (2). The prevalence of BT mutations is developed in individuals with Asian, Middle Eastern, and Mediterranean ancestry. The disease's extreme genotypic and phenotypic diversity is explained by the over 200 different thalassemia-causing mutations that have been identified in the beta-globin gene (3). Point mutations or deletions in the β-globin gene at chromosome number 11 are frequently the catalysts for it (4). The most common single-gene disorder worldwide, depending on the ethnicity and geographic distribution, is thalassemia. The molecular causes of beta thalassemia have been studied globally. The appearance of the red blood cells is abnormal because of this aberrant hemoglobin molecule. Anemia results from the spleen destroying these aberrant red blood cells. It is the most common monogenic hereditary

disease in the world. It started and spread at the same time as malaria in the Mediterranean, the Middle East, and Southeast Asia. Patients with thalassemia can now be found all over the world, and due to widespread migration, their incidence is steadily increasing. An estimated 3% of people on the planet carry beta thalassemia, and an average of 60000 thalassemia major babies are expected to be born each year worldwide (5). In Pakistan, the prevalence of beta thalassemia varies between 5 and 8%; there are over 10 million carriers of the trait. More than 5,000 kids there are also diagnosed with beta-thalassemia major each year. The main causes of Pakistan's high carrier ratio are illiteracy and cousin marriages, which account for 70% of the population. When the birth rate of infants with an illness exceeds 0.1/1000, according to the World Health Organization, a successful screening program should be initiated (6).

There are three primary subgroups of beta-thalassemia based on clinical manifestations: major, intermediate, and minor thalassemia. The major form of thalassemia necessitates regular, ongoing blood transfusions.

While thalassemia intermediate only needs occasional blood transfusions, thalassemia minor does not require any special care. Thalassemia can present clinically in a variety of ways in conjunction with other disorders involving aberrant globin, such as sickle cell disease (HbE and HbS) (7).

A group of hereditary blood disorders known as beta-thalassemia ranges in phenotype from severe anemia to conditions where the patient is scientifically asymptomatic. Their definition is based on defective hemoglobin beta chain synthesis. Treatment options for beta-thalassemia major (BTM) include hematopoietic stem cell transplantation, antioxidants, fetal hemoglobin induction, and blood transfusions (9). In any case, the most common form of treatment is routine blood transfusions because they provide the body with the normal levels of hemoglobin that these patients require. The risks associated with routine blood transfusions include iron overload and viral infections like hepatitis B and C which are transmitted through transfusions (10).

Globally, the regions with the highest prevalence rates of beta-thalassemia mutations are the Mediterranean Sea, the Middle East, and South and Central Asia. Worldwide, there are 68,000 infants innate with B-thalassemia. Worldwide, there are between 80 and 90 million carriers or roughly 1.5% of the global population. It is estimated that up to 15% of Cyprus' Greek and Turkish populations are carriers (11). The incidence also resembles malaria because a suggested viability advantage generates the selective pressure for the elevated carrier prevalence in these communities. For several reasons, including founder effects and gene drift, thalassemia is more common in the aforementioned regions (12).

The objectives of the study were: to determine the frequency of different types of Mutations in B-thalassemia major and to find an association of ethnicity/regional distribution, age, gender, and consanguinity with different types of mutation in patients presenting with B-Thalassemia major.

MATERIALS AND METHODS

STUDY DESIGN

This observational study was conducted at Department of Hematology in Dr. Akbar Niazi Teaching Hospital, Islamabad to evaluate the frequency of different types of mutations among β-Thalassemia major and to find association of ethnicity/regional distribution, age, gender and consanguinity with different types of mutation in B-Thalassemia major patients presenting in Dr. Akbar Niazi Teaching Hospital. Duration of the study was six months after the approval of the synopsis. Patients having β Thalassemia minor and those not willing to give consent were excluded from the study.

STUDY SUBJECTS AND ETHICAL APPROVAL

The research was approved by the Institutional Review Board (IRB) of Islamabad Medical & Dental College, Pakistan (82/IMDC/IRB-2022). To take approval of patients, a consent form was designed as it also fulfilled the requirement of the study as well as the ethical committee (annexure 1). The consent form was filled and signed by every participant and these signed forms were kept as a record. The subjects of the study were 34 males and 26 females with B- Thalassemia major. Demographic characters, for example, age;

race, family history, and consanguinity were interrogated from the patients. The clinical variable, Hb Electrophoresis result was also gathered from some of the patients.

SAMPLE COLLECTION

Sixty patients diagnosed with Beta Thalassemia major from Dr. Akbar Niazi Teaching Hospital (ANTH), Islamabad were consigned for the sample pool. With informed consent blood samples were drawn from different individuals ANTH molecular laboratory was used to extract DNA. The VIVANTIS® catalog number GF-BD-100 DNA extraction kit was utilized to isolate DNA from patient blood. From peripheral blood lymphocytes, DNA was extracted. The buffer was restored by standard protocols. Three different reaction mixtures were utilized in the ARMS-PCR process, which was used to detect mutations or deletions in B-thalassemia.

RESULTS

STUDY PARTICIPANTS

During the current study, 60 patients were diagnosed with Beta Thalassemia major in which 34 were male and 26 were females (Fig. 1a)

GEOGRAPHICAL DISTRIBUTION

This observational study was conducted to assess the different types of mutations in beta-thalassemia major patients in various geographical areas of Pakistan. According to the study, 15% of mutations were observed from Rawalpindi and 10% of mutations were from Islamabad as shown in Fig. 1b.

DISTRIBUTION OF CONSANGUINITY

Out of the 60 positive patients for Beta Thalassemia major, 42 patients showed consanguinity. The details are shown in the Fig. 1c.

TYPES OF MUTATIONS

A total of 60 patients with mutations were observed from different demographic areas of Pakistan. 48 were homozygous and only 12 appeared heterozygous as.

HOMOZYGOUS MUTATION

Out of 48 homozygous mutations**, Fr 8-9 (+G)** was the most frequently observed mutation with a percentage of 33.3% and **IVS 1-5 (G-C)** with 30% respectively as shown in Fig. 1d.

HETEROZYGOUS MUTATION (GENE 1)

Out of 12 heterozygous mutation in gene 1, **Fr 8-9 (+G)** was the most frequently observed mutation with a percentage of 58.3% and **Del 619**with a percentage of 16.7% as shown in the Fig. 1e.

HETEROZYGOUS MUTATION (GENE 2)

Out of 12 heterozygous mutations in gene 2 **IVS 1-5(G-C**) was the most frequently observed mutation with a percentage of 58.3% as shown in the Fig.1f.

CROSS-TABULATION BETWEEN GENDER AND HOMOZYGOUS MUTATION

The Table I show cross tabulation between gender and type of homozygous mutation in patients that were positive for Beta Thalassemia major. Fr 8-9 (+G) and IVS 1-5 (G-C) was the most frequently observed mutation in both males and females.

DEMOGRAPHIC AREA AND HOMOZYGOUS MUTATION

An observational study was conducted to assess the different types of mutations in beta thalassemia major patients in various geographical areas of Pakistan. According to the study 15% of mutations were observed from Rawalpindi and 10% of mutations were from Islamabad. Fr 41-42 (-TTCT) and IVS 1-5 (G-C)

occurred more frequently in Rawalpindi whereas 3 out of 4 positive cases of BT major had IVS 1-5 (G-C) mutation in D.G Khan (Fig. 2).

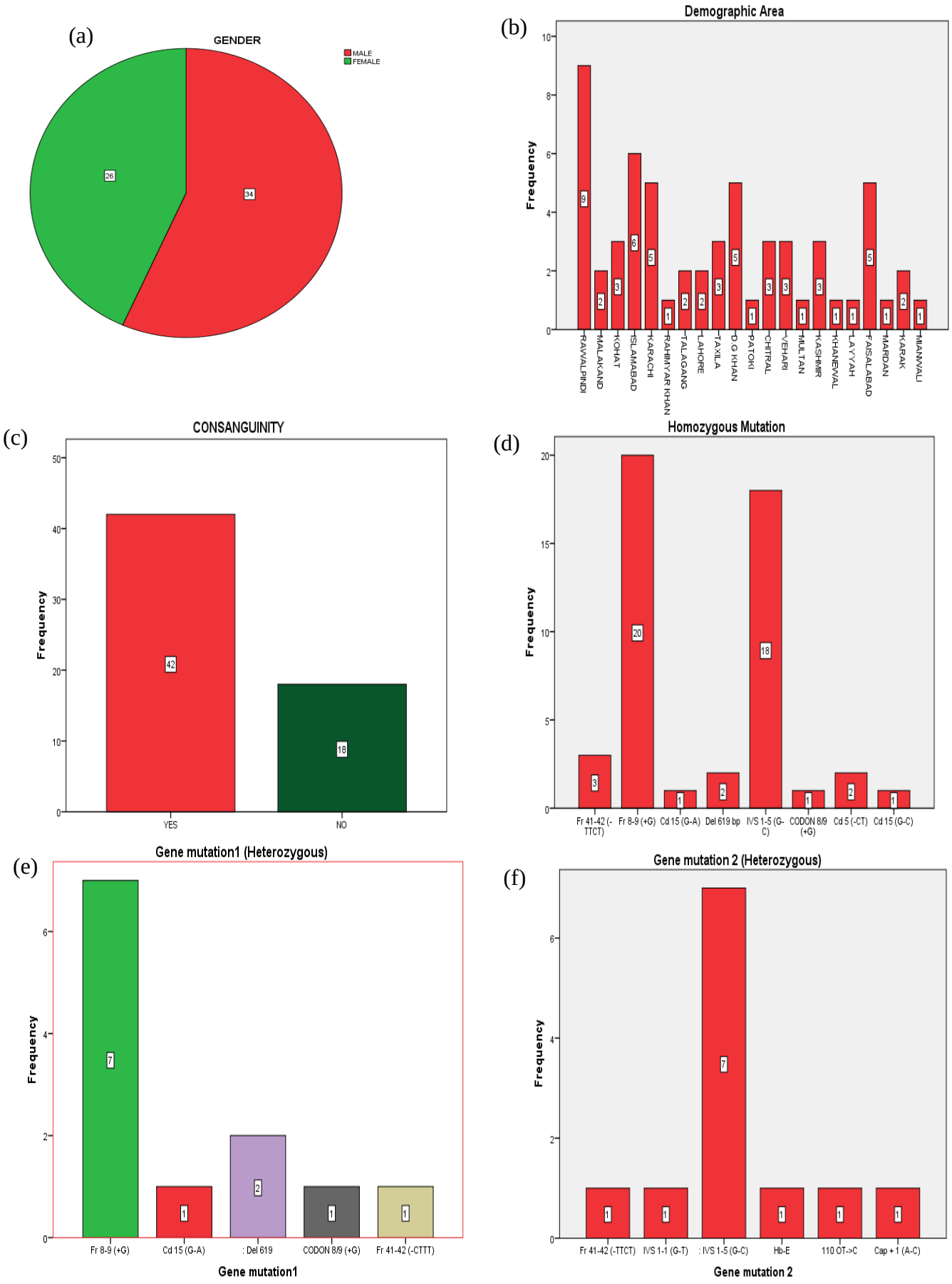

Fig. 1 (a). Gender distribution of study samples **(b).** Frequency of geographical distribution in beta thallasemia major study **(c).** Distribution of consanguinity among patients **(d).** Frequency of homozygous mutation in sample population **(e).** Frequency of heterozygous mutation in Gene 1 of sample population **(f).** Frequency of heterozygous mutation in Gene 2 of sample population

Table I. Different types of mutation according to gender

Gender	Types of mutation							
	Fr 41-42 (-TTCT)	Fr 8-9 (+G)	Cd 15 (G-A)	Del 619 bp	IVS 1-5 (G-C)	CODON 8/9 (+G)	Cd 5 (-CT)	Cd 15 (G-C)
Male	0	12	1	2	11	1	1	1
Female	3	8	0	0	7	0	1	0
Total	3	20	1	2	18	1	2	1

*Cross tabulation between gender and type of homozygous mutation in patients that were positive for Beta Thalassemia major. Fr 8-9 (+G) and IVS 1-5 (G-C) was the most frequently observed mutation in both males and females

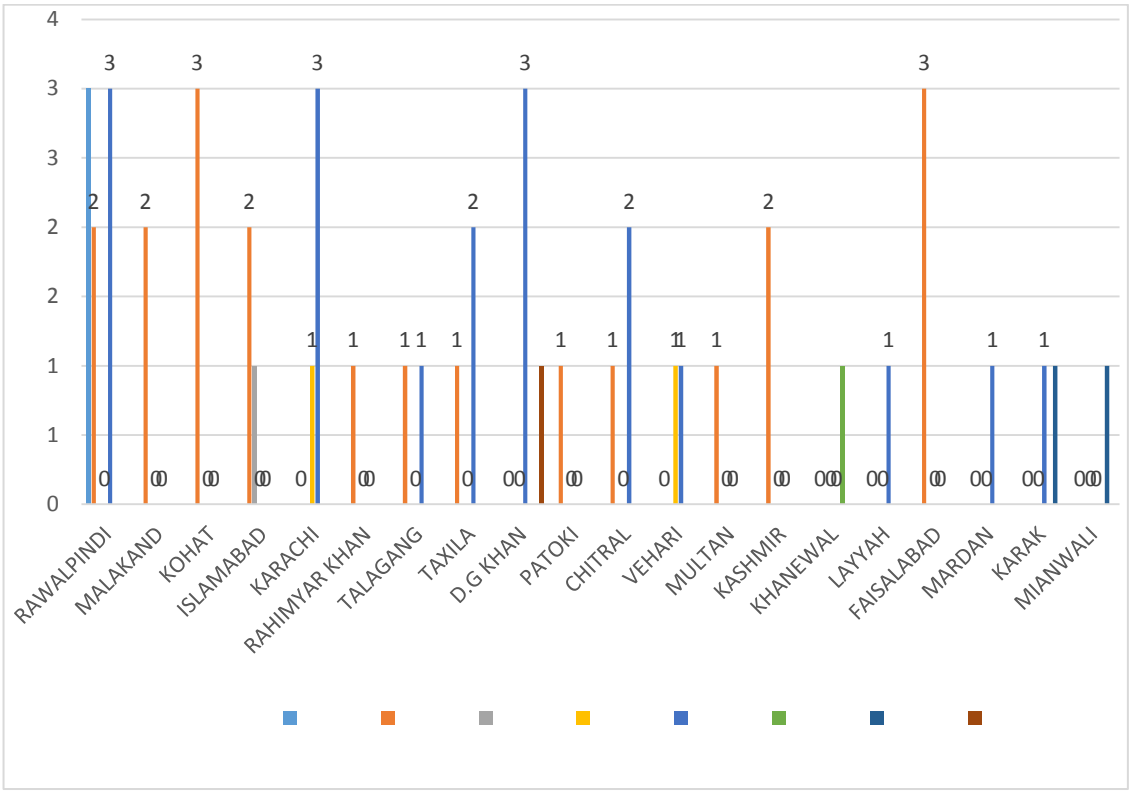

Fig. 2. Crosstabulation between geographical area and types of Mutation

DEMOGRAPHIC AREA AND HETEROZYGOUS MUTATION

Out of 60 observed mutations in patients positive for Beta Thalassemia major only 12 showed heterozygous mutation. The heterozygous mutations Fr 8-9 (+G) and IVS 1-1 (G-T) were the most observed mutations in Islamabad and Lahore respectively. While a single case of Codon 8/9 (+G) was seen from Vehari.

Table II. Cross tabulation between demographic area and heterozygous mutation

Region	Types of mutated genes									
	Gene mutation 1				Gene mutation 2					
	Fr 8-9 (+G)	Cd 15 (G-A)	: Del 619	CODON 8/9 (+G)	Fr 41-42 (-CTTT)	Fr 41-42 (-TTCT)	IVS 1-1 (G-T)	: IVS 1-5 (G-C)	Hb-E	110 OT->C
Rawalpindi	1	0	0	0	0	0	1	0	0	
Islamabad	3	0	0	0	1	0	2	0	0	
Karachi	0	0	1	0	0	1	0	0	0	
Lahore	2	0	0	0	0	0	2	0	0	
Dera Ghazi Khan	0	1	0	0	0	0	0	1	0	
Vehari	0	0	0	1	0	0	0	0	1	
Kashmir	1	0	0	0	0	0	1	0	0	
Faisalabad	0	0	1	0	0	0	1	0	0	
Total	7	1	2	1	1	1	1	7	1	1

CHI SQUARE TEST FOR SIGNIFICANCE OF MUTATION

Chi Square test was performed for association between gender of the patients, consanguinity, and type gene mutation. A strong association was seen between male and homozygous mutation with p value 0.001 and strong association was observed between female and consanguinity with p value 0.002.

Table III. Test Statistics Chi square test for significance of mutation

Gender		Homozygous Mutation	Consanguinity	Gene Mutation 1 (heterozygous)	Gene Mutation 2 (heterozygous)
Male	Chi-Square	36.897[a]	1.882[b]	0.000[c]	0.600[d]
	df	6	1	4	3
	Asymp. Sig.	0.0001	0.170	1.000	.896
Female	Chi-Square	6.895[e]	9.846[f]	3.571[g]	4.571[h]
	df	3	1	1	2
	Asymp. Sig.	0.075	0.002	0.059	0.102

DISCUSSION

In Pakistan, beta thalassemia poses a significant health risk. Every year, about 5,000 new children with transfusion-dependent TI or TM are added to the registry despite preventive measures. There is currently no permanent treatment available, except stem cell transplantation, which is too expensive. Iron chelation therapy and routine blood transfusions are the only options available to the majority of patients with transfusion-dependent thalassemia. The use of HU therapy to increase hemoglobin F offers yet another avenue for preventing blood transfusions and their related consequences. Predicting phenotype from genotype is crucial because, depending on their genetic makeup, patients differ in terms of their clinical severity and reaction to HU treatment. At the tertiary care hospital KPK Peshawar, a retrospective study was carried out. It revealed that of the 350 patients evaluated for hemoglobinopathies frequency and diagnosed by HB Electrophoresis, 171 (48.9%) were male, and 179 (51.1%) were female. Beta Thalassemia was identified in 60 participants participating in the current investigation. There were 34 men and 26 women who had positive diagnoses in terms of gender distribution. The study found that 10% of mutations were found in Islamabad and 15% were found in Rawalpindi. Out of a total 42 of the 60 individuals who tested positive for Beta Thalassemia major indicated consanguinity. Fr 8-9 (+G) and IVS 1-5 (G-C) were the two most often seen variants, with a frequency of 33.3% and 30%, respectively, out of the 48 homozygous mutations. The most often reported mutation, with a proportion of 58.3%, was Fr 8-9 (+G), one of 12 heterozygous mutations in gene 1. In both males and females, the most often found mutation was Fr 8-9 (+G) and IVS 1-5 (G-C). Similar to our study was carried out in KPK, Pakistan in which prominent mutations were Fr 8-9 (+G), CD 5 (-CT) Fr 41-42 (-TTCT), and IVS 1-5 (G-C). (15). In our study 15% of the mutations were found from Rawalpindi, and 10% came from Islamabad. While IVS 1-5 (G-C) mutation was found in 3 out of 4 positive cases of Beta thalassemia major in D.G. Khan, Fr 41-42 (-TTCT) and IVS 1-5 (G-C) were more common in Rawalpindi. According to a different study, out of 112 patients with β-thalassemia carriers in the Pakistani population, Fr 8-9 (+G) is the most common mutation (38.2%). This is followed by IVS1-5 (G-C) with 82 (28%), Fr41-42 with 24 (8.2%), Cd 5 with 20 (6.8%), and other mutations with 55 (18. 8%). Our results, which indicate that the five most common mutations in Pakistan are IVS1-5 (G-C), IVS1-1 (G-T), Fr 41-42 (-TTCT), Fr 8-9 (+G), and deletion 619 bp, are corroborated by the results of another study. (16) With 53.5% of the population being Malaysian, it has been found that 4.5% of them are heterozygous carriers of β-thalassemia. These results corroborate our findings that only 12 were homozygous and 48 appeared. The heterozygous showed a lower frequency than the homozygous in both studies. (17). Of the 100 patients diagnosed in a different study, 46 percent were men and 54 percent were women. This is also consistent with our study, which included 60 patients with major beta thalassemia, of whom 34 were men and 26 were women. (18). Our results are corroborated by another study, which found that of the 12 heterozygous mutations in gene 1, Fr 8-9 (+G) was the most frequently observed mutation with a percentage of 58.3%, followed by Del 619 with a percentage of 16.7%. IVS1-5 was found in 15 cases (17%), and Fr 41-42 was found

in 10 cases (11.4%) (19). In both males and females, the most commonly observed mutations were Fr 8-9 (+G) and IVS 1-5 (G-C) another study also supported in which Fr 8-9 (+G) was the most frequently observed mutation (20).

CONCLUSION

Out of 48 homozygous mutations, Fr 8-9 (+G) was the most frequently observed mutation with a percentage of 33.3% and IVS 1-5 (G-C) with 30% respectively 12 heterozygous mutations in gene 1, Fr 8-9 (+G) was the most frequently observed mutation with a percentage of 58.3% and Del 619 with a percentage of 16.7%. Thalassemia screening among young Pakistan population are recommended there are more than 10 million carriers in the country; and every year, around 5000 children are diagnosed to carry β-thal major (β-TM) in Pakistan population.

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