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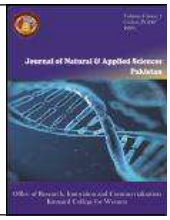

BETA THALASSEMIA: A NOT-SO-RARE GENETIC DISORDER IN PAKISTAN

Rhea Aqueel [1,*], Iram Anjum[2] ,
[1]Department of Biological Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan.
[2]Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan.

Article Info

*Corresponding Author
Tel: +92 332 4475851
Email Id: rheaaqueel@gmail.com

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Abstract

Autosomal recessive β-thalassemia is a hematological disorder in which the absence or reduction of β-globin chains in hemoglobin molecules cause anemia. It is characterized by pallor, splenomegaly, growth retardation and changes in the facial bones. Methods of treatment by blood transfusions cause iron overload in the body which can lead to cardiac failure in most patients affected with β-thalassemia major, to counter which, iron chelation therapies are provided. The mutations of β-globin gene have a variable frequency based on regional distribution and ethnicity. Mutational analysis is being done using the robust and speedy techniques like ARMS PCR, M-ARMS PCR and RFLP so that the mutation spectrum of β-thalassemia can be detected and genetic counseling can be offered to families especially where consanguineous marriages are a ritual and β-thalassemia has been identified. This review has been written by retrieving all the relevant information from recent research articles about β-thalassemia. The purpose of this review article is to compile the most relevant and recent information regarding β-thalassemia. It is believed that by mutation screening and prenatal diagnosis accompanied with genetic counseling, this disorder can be eventually eradicated from our population.

1.Introduction

Beta-thalassemia is grouped as a hereditary blood disorder in which the β-globin chains in the hemoglobin molecule are either absent or are produced in insufficient quantities. This leads to abnormal hemoglobin molecules making the red blood cells to appear abnormal, which are destroyed by spleen and cause anemia (Muncie & Campbell, 2009; Galanello & Origa, 2010). The patients appear pale showing signs of diarrhea, irritation, feeding problems with enlarged liver and spleen leading to an enlarged abdomen (Lin, Lin, & Chang, 1991).

The first disease ever to be studied in the field of molecular biology was β-thalassemia (Baig *et al.,* 2005; Hassan *et al.,* 2013).Thalassemia is the most common single gene disorder worldwide having an incidence of 1 in 100,000 annually (Vichinsky, 2005). Mutations are mostly specific to ethnicity or geographic distribution, while immigration has played a major role in genetic diversity. Thalassemia demands the need of a public health program creating awareness by genetic counseling (Ishaq *et al.,* 2012).

It is important to know the various methods that can help identify people who are at risk of β thalassemia. As it is an autosomal recessive disease, β-thalassemia can appear in its major form if two persons carrying a single copy of the defected β-globin gene give birth to an offspring. Carrier screening is therefore necessary prior to marriages especially in families where the history of β-thalassemia prevails (Rowley, 1976).

2.Literature Review

2.1Types Of Beta-Thalassemia

The severity of symptoms related to β-thalassemia may vary based on the type of β-thalassemia *i.e.* β-thalassemia major, β-thalassemia intermedia and β-thalassemia minor. Varying phenotypes and clinical features are related to all these three types. Thalassemia major patients exhibit severe anemia in the first 2 years after birth while those with intermedia show moderate anemia (Galanello & Origa, 2010).

Beta-Thalassemia Major also called as Mediterranean anemia/Cooley's anemia is the most severe form of this disorder, causing patients to become transfusion dependent from the first two years of life. There is poor muscle development and impaired growth. Splenomegaly is one of the major symptoms of beta thalassemia major which is the enlargement of the spleen caused due to the destruction of a large number of abnormal red blood cells. Changes in the cranial and facial bones such as depression of the nose-bridge and protrudent upper teeth are also observed (Galanello & Origa, 2010).

Those with *Beta-Thalassemia Intermedia* and *Beta-Thalassemia Minor* show less severity in symptoms or no symptoms at all because only one copy of the β-globin gene is present and leads to reduction in β-globin chains (Thein, 2013). These patients are known as beta-thalassemia carriers or heterozygous beta-thalassemics and are moderately transfusion dependent.

2.2Mode Of Inheritance

Beta-thalassemia is a monogenic hematological disorder that exhibits autosomal recessive mendelian inheritance pattern (Baig *et al.,* 2005). If a child is affected with beta thalassemia it is obligatory for the parents to be carriers of the defective β-globin gene. During each pregnancy there is 25% possibility of the child to be normal, 50% possibility to be a carrier, and a 25% possibility to be affected (Galanello & Origa, 2010).

2.3Molecular Genetics

The β-globin locus on chromosome 11 contains four genes *i.e. ε (epsilon), γ (gamma), δ (delta)* and *β* (*beta)* whose onset of expression is in the same sequence too (Figure 1). The *ε-globin* gene is expressed in early stages of embryonic development whereas *β-globin* gene is expressed at the adult stage. The *γ-globin* gene has two copies on the same chromosome while all the others are present as a single copy. Therefore there are two copies of the *β-globin* gene in one cell to balance the proteins produced by the four *α-globin* genes (Bunn & Forget, 1986).

The prime gene of interest for beta-thalassemia is the *β-globin* gene coding for β-globin chains present in hemoglobin. Mutations in this gene cause complete absence of β-globin chains leading to beta zero thalassemia or reduced production of β-globin chains causing beta + thalassemia.

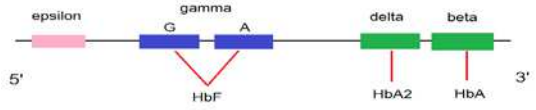

Figure 1: Beta globin gene cluster on chromosome 11 showing all five beta globin genes

Small nucleotide deletions, insertions or point mutations in the *β-globin* gene are the main cause

of β-thalassemia. Some may be cis-acting and some may be trans-acting. In cis-acting mutations the effect on the β-globin gene is due to some mutations present inside the *β-globin* gene locus. In trans-acting mutations an element outside the *β-globin* gene locus is responsible for the altered expression of the *β-globin* gene itself (Galanello & Origa, 2010; Thein, 2013; Boonyawat, Monsereenusorn, & Traivaree, 2014).

2.4Mutational Analysis

More than 800 mutations have been identified in the β-globin gene according to the database of haemoglobin variants and thalassemia mutations (Hassan *et al.,* 2013). Polymerase chain reaction, dot-blot hybridization and sequencing the genomic DNA directly have been the preferred methods for the diagnosis of β-thalassemia (EL-Fadaly *et al.,* 2016).

Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) is the most popular, cost-effective and speedy method used for Single Nucleotide Polymorphism (SNP) genotyping for analyzing gene mutations in complex diseases. In ARMS PCR two reactions are set parallel in which the first one has an oligonucleotide primer having the sequence complementary to a specific mutation at its end with a common primer, and the second reaction has a normal primer coupled with a common primer. If an amplified product is achieved in the first reaction, it means that the mutation is present. No product means that the DNA sequence is normal. In the second reaction, no amplified product suggests a mutation while if the product is there, it indicates the presence of a normal DNA sequence. PCR products can then be visualized using gel electrophoresis techniques. Point mutations, small nucleotide deletions or insertions can easily be detected using ARMS-PCR. Its application for carrier screening and prenatal diagnosis has been of great value in controlling β-thalassemia (Old *et al.* 1992; Galanello & Origa, 2010).

Multiplex amplification refractory mutation system (M-ARMS) PCR can also be used for the detection of more than one mutation at the same time in a reaction mixture provided all ARMS primers are amplified with same common primer (Newton *et al.,* 1989). Most mutation screening of β-thalassemia all over the world is being carried out using M-ARMS PCR as it is robust, accurate, and speedy technique and detect single base changes/ deletions in alleles (Mirasena *et al.,* 2008; Sheth *et al.,* 2008; Hassan *et al.,* 2013).

2.5Genetic Epidemiology

Consanguinity has played a major role in the inheritance of autosomal recessive disorders like β-thalassemia. Being an autosomal recessive disorder, β-thalassemia is most prevalent in Asia and also some parts of South America and Africa owing to higher rates of consanguinity (Flint *et al.,* 1998).

In Cyprus, the carrier state is 14% which is the highest in the world followed by Sardinia which has a carrier frequency of 10.3% (Flint *et al.,* 1998). This disease has travelled to European countries especially due to population migration and intermarriages. It has been found that 1.5% of the world's total population are beta-thalassemia carriers, out of which 60,000 born annually are symptomatic mostly from developing countries.

The spectrum of β-thalassemia mutations varies among various ethnic groups and populations. Kattamis *et al.,* (1990) found a wide variation of β-thalassemia mutations by conducting a study on 174 thalassemia major patients in Greece, revealing 17 different β-globin gene mutations. Many mutations which had not been identified before were also identified, proving beneficial in prenatal diagnosis of β-thalassemia in the Greek population.

In another study carried out by El-Hazmi *et al.,* (1995), it was found that IVS-ll-1 and IVS-l-110 are the most prevalent mutations in Arab population. In Jordan, Syria and Egypt, IVS-ll-745 and IVS-l-1 mutations have been found to be more prevalent (Zahed, 2001). Only the people of Saudi Arabia had codon 39, Cap +1, codon 6, IVS-l-5 and IVS-I, 3′ end (-25 bp) mutations whereas the populations of Lebanon and Syria only have codon 39 mutation (Zahed, 2001). Other mutations have not been found to prevalent or specifically linked to any of the ethnic groups in the Arabian countries.

Comparison of the β-thalassemia alleles between the people of United Arab Emirates (UAE) and people from other countries living in UAE was done by El-Kalla *et al.* (1993) revealing 13 as compared to 17 mutations in expatriates. However, IVS-I-5 (G→C) was found to be the most common mutation in both study groups.

2.6Beta Thalassemia In Pakistan

Pakistan is a country where consanguinity is very common. The carrier frequency in the population of Pakistan is 6% which is significantly high than the overall global frequency, though lower than Cyprus and Sardinia (Usman *et al.,* 2009). Many invasions

took place in the past and to date consanguineous marriages are culturally preferred. Both of these factors are a major reason for the greater genetic diversity that is observed in β-thalassemics today (Usman *et al.,* 2009). Recently, it has been reported that carrier frequency in Pakistan is almost 5-7% with over 5000 children with β-thalassemia major being born each year (Ahmed *et al.,* 2010; Ishaq *et al.,* 2012). The main goal for determining the frequency of β-thalassemia mutations in a specific geographical area is, to have a solid background not only for genetic counseling but also for prenatal diagnosis wherever opportunities arise. A number of studies have been carried out for the detection of β-thalassemia mutations in various ethnic groups of Pakistan on the basis of which prenatal diagnosis and genetic counseling are currently being offered.

A study conducted in Azad Kashmir by Ahmed *et al.,* (2016) reported the carrier rate of β-thalassemia to be 5.6%. In Islamabad and Rawalpindi, the carrier frequency was 4% (Iqbal *et al.,* 2012) while in Karachi the prevalence rate was reported to be 5.5% (Ali *et al.,* 2012). In Lahore, β-thalassemia prevalence was reported to be 52% (Majeed *et al.,* 2013) while in Faisalabad a study conducted on a large family reported the carrier rate to be 44.4% (Baig *et al.,* 2008) There was a high prevalence of β-thalassemia in Dera Ismail Khan where the frequency was 18.5% (Hussain *et al.,* 2013) and even higher in Abbotabad where prevalence reported was 58.2% (Nosheen *et al.,* 2015).

The five most common mutations in Pakistan are IVS-1-5 (G–C), FSC-8/9 (+G), Cd 41/42 (–TTCT), 619 bp deletion and IVS-I-1 (G→T) as shown with their relative prevalence in **Table 1.** IVS-1-5 (G–C) is the most prevalent mutation followed by Cd 41/42 (–TTCT) and FSC-8/9 (+G) that cause beta zero thalassemia. Beta thalassemia major patients with 619 bp deletion do not exhibit mild symptoms like the IVS-I-1 (G→T) mutation (Khan & Riazuddin, 1998; Sachdeva *et al.,* 2006). These five mutations account for 90% of all the underlying mutations of β-thalassemia in not only Pakistani population but in our neighboring country India too. The reason could be because India and Pakistan were one country before partition in 1947 and the concept of intermarriages has been a common cultural norm (Usman *et al.,* 2009).

The differences in frequencies observed in Table 1 are based on ethnicity as well as provincial boundaries.

	Beta-Thalassemia Mutations				
Ethnic Groups	**IVS 1-5 (G–C)**	**Fr 41/42 (–TTCT)**	**Fr 8/9 (+G)**	**IVS-I-1 (G→T)**	**619 bp deletion**
Punjabis	48.6%	24%	17.1%	6.8%	3.4%
Pathans	47.3%	28.4%	18.9%	3.1%	2.1%
Sindhis	36.5%	13%	34.8%	4.3%	11.3%
Balochis	36.8%	27.4%	7.4%	21%	7.4%
Urdu Speaking Migrants	61.6%	12.5%	9.2%	8.3%	8.3%

Table 1. Frequency of β-thalassemia mutation in the five major ethnic groups in Pakistan (Usman *et al.,* 2009).

Furthermore, it has been found that IVS-I-5 (G-->C) is the most common mutation in all ethnicities of Pakistan, followed by Fr 8/9 (+G) (Ahmed *et al.,* 1996; Baig *et al.,* 2006a; Hafeez *et al.,* 2007; Ansari *et al.,* 2012).

To make matters worse, consanguineous marriages play a major role in making β-thalassemia a highly prevalent disease in Pakistan resulting in a high incidence of this trait among siblings up to 61% (Majeed *et al.,* 2013). Pre-natal diagnosis is available only in the major cities of Pakistan and people living in the rural areas do not have access or otherwise are illiterate and have low income to benefit from these facilities. Studies conducted on carrier screening (Baig *et al.,* 2006; Baig *et al.,* 2008; Manghrio *et al.,* 2014; Asif & Hassan, 2014) prove to be highly helpful in providing genetic counseling and prenatal diagnosis to minimize affected births in a developing country like Pakistan.

Costs involved with prenatal diagnosis are a major problem in prevention of the disease. National Institute for Biotechnology and Genetic Engineering (NIBGE) and Multan Institute of Nuclear Medicine and Radiotherapy (MINAR)

started free chorionic villus sampling (CVS) in areas near Multan. More than 90% of the people admitted to not get involved in prenatal diagnosis because of the expenses involved. This free initiative helped them in terminating the fetus incase it turned out to be thalassemia major (Baig *et al.,* 2012). Pakistan requires providing free facilities and there is a major need to create awareness among the people to benefit from these facilities.

3.Conclusion

As consanguinity still prevails in Pakistani population with more than 60% population practicing cousin marriages, the inheritance of this hematological disorder is highly likely with rather increased frequency. Molecular characterization and genetic counseling are vital tools for long-term control and prevention of β-Thalassemia. On the basis of mutation screening, genetic counseling and prenatal diagnosis can be offered to the affected families which will eventually help to reduce prevalence of this lifelong tormenting disorder especially in Pakistan (Hafeez *et al.,* 2007; Raza *et al.,* 2016). It would be a cost-effective method for the management of this problem in our country (Yatim *et al.,* 2014). Genetic counseling regarding the consequences of frequent cousin marriages should also be provided. More research need to be conducted for this disorder to understand the full mutational spectrum of this disorder in Pakistan and the National Thalassemia Prevention Program should be made more effective to help eliminate this life tormenting disorder from Pakistan.

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