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**Research Paper**

# Exploring the Distribution of ABO and Rhesus Blood Groups among Beta Thalassemia Patients in Punjab, Pakistan: A Cross-Sectional Study

Mahpara Anwar[1], Muhammad Waqas[1*], Razia Bashir[1, 2], Humera Ambreen[2], Khadija Munir[1], Maham Iqbal[1], Muhammad Arshad[3]

[1]*Department of Zoology, Division of Science and Technology, University of Education, Lahore, Punjab, Pakistan*

[2]*Department of Zoology, University of Sargodha, Sargodha, Punjab, Pakistan.*

[3]*Department of Life Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Punjab, Pakistan.*

*Corresponding Author: waqassiddique614@gmail.com; mtf2202199@ue.edu.pk

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Beta thalassemia is a genetic hematological disorder characterized by impaired or absent hemoglobin synthesis. It affects millions around the world, requiring lifelong blood transfusions. People have different blood types based on genetically determined antigens that play a vital role in transfusion safety. Blood group antigens show strong correlation with many diseases like diabetes, immune disorders, cancers etc. This study was designed to assess the distribution of ABO and Rh blood groups in thalassemic patients. We analyzed the impact of age, gender, and caste on the distribution of ABO and Rh blood groups in beta-thalassemia patients. This study included 188 thalassemic patients. It was a cross sectional, single centric and observational study. The ABO blood groups were screened by slide hemagglutination method after the permission from the ethics committee and with the consent from the patients. We observed that thalassemia is more common in blood group B. Gender wise and age wise distribution of thalassemia was in order of B>O>A>AB. Thalassemia was more common in males with Rh+ve blood groups than Rh –ve. It was found that the most affected blood group with beta thalassemia is B+ve. We concluded that males are more affected with thalassemia major than females. More thalassemic patients were found to be Rh+ve.

## Abstract

## Introduction

Thalassemia is a genetically inherited heterogeneous group of blood disorders (Brancaleoni et al., 2016). It is classified as an inheritable autosomal recessive disease (Badeli et al., 2019). This disorder is caused by the formation of abnormal hemoglobin. The condition primarily arises due to a weakened synthesis of one or more globin chains in hemoglobin (Bakr et al., 2014; Hammod et al., 2018). Hemoglobin is composed of four protein chains; two alpha and two beta units. A genetic defect in producing either alpha or beta chains leads to reduced hemoglobin concentration within red blood cells, resulting in lowered RBC production and pronounced anemia (Musallam et al., 2023). The underlying pathology of beta-thalassemia major involves three critical components: persistent anemia with hemolysis, faulty red blood cell formation, and excess iron accumulation (Steinberg et al., 2009; Bekhit et al., 2017). Symptoms may include failure to thrive, increasingly pale appearance, difficulty in feeding, frequent diarrhea, recurring fevers, stunted growth, weak musculature, and jaundice (Demosthenous et al., 2019). Additional indicators comprise dark-colored urine, long-term oxygen deficiency, enlargement of liver and spleen, and skeletal abnormalities (Waqas et al., 2024; Hattab, 2017; Sultan et al., 2016).

The clinical onset of thalassemia major happens between 6 to 24 months of age. Affected children fail to thrive and gradually become paler (Galanello & Origa, 2010). The disease can be diagnosed by complete blood count (CBC), hemoglobin (Hb) concentration, electrophoresis and deoxyribonucleic acid (DNA) test (Danjou et al., 2015). Prenatal diagnostic tests consist of chorionic villus sampling, amniocentesis, fetal blood sampling, and the analysis of fetal cells in maternal blood (Tahura et al., 2016; Sinha et al., 2017, Viprakasit & Ekwattanakit, 2018). Treatment comprises both curative and standard therapies. Curative treatment involves gene therapy and bone marrow transplantation. Conventional therapy includes the regular RBCs transfusions (Borgna-Pignatti et al., 2004; Mehmood et al., 2018; Tari et al., 2018).

Beta-thalassemia is commonly found in Mediterranean countries, the Middle East, India, Central Asia, southern China, as well as countries along Africa's northern coast and in South America (DeLoughery, 2014; Dede et al., 2016). The highest rates of carrier frequency are observed in Cyprus (14%) and Sardinia (10.3%), with an overall global carrier frequency of approximately 1.5% (Sarmi, 2012; Jha & Jha, 2014).

Numerous blood group systems have been recognized in humans, characterized by specific agents present on various blood cells. The ABO blood group was first recognized by Landsteiner

in 1901, while the Rhesus blood groups were identified later by Landsteiner and Wiener in 1940 (Landsteiner et al., 1940). Since that time, over 20 unique blood group systems have been established, with the ABO and Rhesus groups being the most clinically significant. Blood type is determined by the presence or absence of antigenic substances on the surface of red blood cells, identifiable through specific antibodies (Storry, 2003; Garg et al., 2014).

The ABO blood grouping system comprising of four types of blood determined by the existence or lack of A and B surface antigens (Denomme, 2011). The blood groups include A, B, O, and AB. The significance of the ABO blood group system lies in the strong antigenic properties of A and B. ABO antigens are oligosaccharides expressed on RBCs as glycoprotein and glycolipids (Yamamoto et al. 2014, Suresh et al. 2015). Blood group A has antigen A on the surface of RBCs and antibody anti-B in blood circulation, B blood group has antigen B on the surface of RBCs and antibody anti-A in blood circulation, AB blood group has antigens A and B co-expressed on the surface of RBCs while no antibodies in circulation, whereas O blood group has no antigens on the RBCs surface but contains antibodies anti-A and anti-B in circulation (Chandra and Gupta, 2005, Harmening et al. 2005).

There are a number of studies which have reported the association of blood groups with different human diseases (Lukens et al., 1998; Brecher & Hay, 2011). Some studies indicated that people with type O blood are more susceptible to peptic ulcers, for instance, and women with type A blood have higher incidence of ovarian and endometrial cancers compared with other blood types. Furthermore, blood type A has also been linked to greater risk for coronary heart disease (Marinaccio, 1995; Alkout et al., 2000; Wazirali et al., 2005). Conversely, persons with O type blood are less likely to have pancreatic cancer, whereas those with A, B, and AB blood types are at a higher risk (Wolpin et al., 2009).

The present research was carried out comparing the ABO blood groups of beta-thalassemia and normal populations to identify which populations are more prone to developing the disorder. An assessment was made on the development of beta-thalassemia among different age groups of both sexes and its relation to ABO system blood groups. Such a strategy could help indicate which sections of the population are likely to be susceptible or resistant to beta thalassemia, along with possible solutions for regional rare blood shortages. These findings may also help guide the staff responsible for blood collection or preparation in healthcare facilities where this prevalence needs

determination. The results of this research may enhance blood bank planning to ensure the availability of specific blood types, reducing the risk of shortages in healthcare services.

## Materials and Methods

### ABO and Rh blood typing

To determine the blood groups, the blood group detection kit (DIAGAST- DIA00104) was used. The ABO blood group was identified using the slide hemagglutination technique (Marbut et al., 2018). Red blood cells contain antigen A and antigen B on their surfaces, which agglutinate upon contact with reagents that have the corresponding antibodies. A labeled slide was prepared with drops of anti-A, anti-B, and anti-D serum. A drop of blood from a finger prick was added to each serum and mixed. The agglutination results were observed and recorded after mixing

### Data Analysis

The collected data were analyzed statistically using percentage methods. The Chi-square test was applied to compare categorical variables at https://www.icalcu.com/stat/chisqtest.html. A p-value of $\leq 0.05$ was regarded as significant statistically.

## Results

### Consanguinity in beta-thalassemia

Percentage distribution of consanguinity in beta-thalassemia is shown in Figure 1. Incidence of beta-thalassemia was the highest in the children of parents having cousin marriages 129 (69%) and less observed in distant relation marriages 59 (31%) respectively.

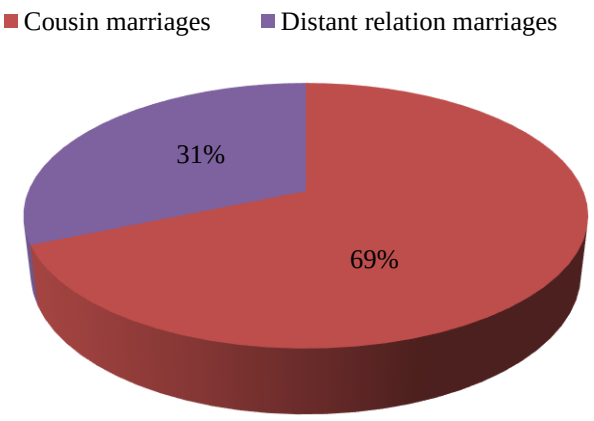


Figure 1. Frequency of Consanguineous Marriages Among Beta-Thalassemia Patients

## Caste Wise Distribution of Beta-thalassemia

Figure 2 showed the distribution of beta-thalassemia in different castes. Beta-Thalassemia was found to be more common in Arain with 28%, Rajpoot 20%, sheikh 12.2% and least common in Jutt 9% followed by Khokhar 4.8%. While the remaining belong to other minor ethnic groups.

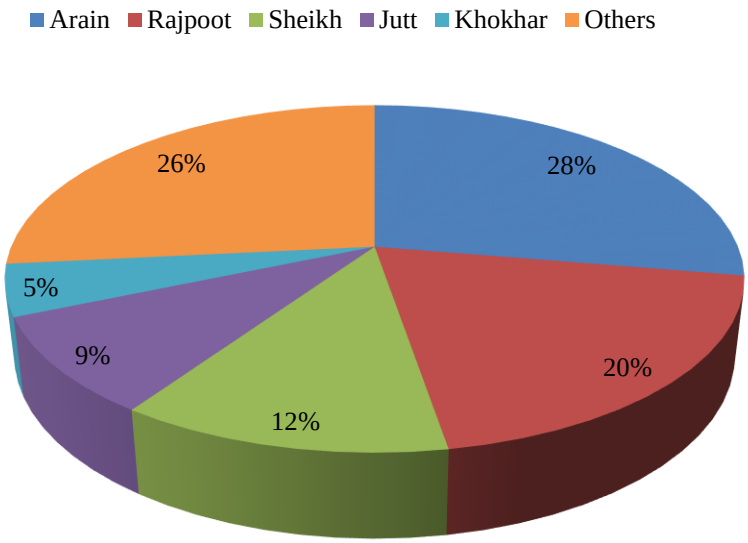


Figure 2. Distribution of Beta-Thalassemia in Different Castes

## ABO Blood Groups Distribution in Beta-Thalassemia

Table 1 presents the ABO blood groups distribution alongside the Rh blood group among thalassemia patients, categorized by gender. The study included a total of 188 patients, comprising 53% males (n=100) and 47% females (n=88). The ABO blood group frequency was in order of B +ve > O +ve >A +ve >AB +ve >B –ve >A –ve >AB–ve >O –ve in male patients with (29%) > (28%) > (22%) > (11%) > (7%) > (2%) > (1%) respectively. In contrast, among female patients frequency of ABO and Rh blood group was B +ve> O +ve>A +ve >AB +ve>A–ve, AB–ve, O–ve> B–ve with 45(51%)>21(23.8%)>15(17%)>4(4.5%)>1(1.3%) respectively. It also revealed that male patients are highly affected by the disease than females.

**Table 1. Overall Distribution of ABO Blood Groups in Beta-thalassemia**

| Blood Groups | A | | B | | AB | | O | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | |
| Male | 22(11) | 02(1.06) | 29(15.3) | 07(3.7) | 11(5.8) | 01(0.53) | 28(15) | 0 | 100(53) |
| Female | 15(8) | 01(0.53) | 45(24) | 0 | 04(2.1) | 01(0.53) | 21(11.1) | 01(0.5) | 88(47) |
| Total | 37(19) | 03(1.5) | 74(39.3) | 07(3.7) | 15(7.9) | 02(1.06) | 49(26) | 01(0.5) | 188(100) |

**Age Wise Distribution of ABO Blood Groups**

The age wise distribution of ABO blood group in thalassemia patients is shown in Table 2. As for the effect of age wise distribution of ABO blood groups in beta-thalassemia, patients were categorized into two groups: <15years comprise 95 (50.5%) and >15years consist of 93 (49.5%) patients. It showed that among <15years B +ve 19.6% is highest in distribution while O +ve 14.3% is 2nd highest followed by A+ve 9.5%, AB +ve 3.1%, B –ve 2.1% and O –ve 0.53%. Similarly, among >15 years B +ve 19.6%, O +ve 11.7% and A +ve 10.1% is highly distributed followed by AB +ve 4.8%, B –ve 1.6%, and AB -ve 1.06% while no patient was found to be O-ve.

When patients of two age categories 1-15Y and >15Y were compared by using chi-square. The observed p-value were A +ve (0.86), A –ve (0.56), B +ve (0.7), B-ve (0.7), AB +ve (0.4), AB –ve (0.15), O+ve (0.47) and O–ve (0.31). However, no statistically significant difference was noted among ABO blood groups with respect to age.

**Table 2. Age Wise Distribution of Blood Groups in Beta-Thalassemia**

| Blood groups | A | | B | | AB | | O | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | |
| Age <15Y | 18(9.5) | 02(1.06) | 37(19.6) | 04(2.1) | 06(3.1) | 0 | 27(14.3) | 01(0.53) | 95(50.5) |
| Age >15Y | 19(10.1) | 01(0.53) | 37(19.6) | 03(1.6) | 09(4.8) | 02(1.06) | 22(11.7) | 0 | 93(49.5) |
| Total | 37(19.6) | 03(1.6) | 74(25) | 07(3.7) | 15(7.9) | 02(1.06) | 49(26) | 01(0.53) | 188(100) |
| *$X^2$-value* | 0.02 | 0.33 | 0.14 | 0.14 | 0.6 | 2 | 0.51 | 1 | 0.02 |
| *P-value* | 0.86 | 0.56 | 0.7 | 0.7 | 0.4 | 0.15 | 0.47 | 0.31 | 0.88 |

## Discussion

### Gender-Based Distribution

Our findings reveal that beta-thalassemia was more prevalent among male patients (53%) than females (47%). This is consistent with several previous studies. For instance, Bejaoui and Guirat (2013) reported that in Faisalabad, the proportion of affected males was 65.66% compared to 34.33% for females. Similarly, Khan (2015) in Bannu, Pakistan, found 56.95% male and 43.05% female thalassemia patients. Qurat-ul-Ain et al. (2011) also reported a male-to-female ratio of 65.66% to 34.36%. These repeated observations across regions suggest a possible gender-related predisposition, which may be due to cultural, genetic, or reporting biases in healthcare access and diagnosis.

While B blood group was more prevalent among both sexes, our data indicated that female patients with B blood group appeared more susceptible than their male counterparts. Furthermore, Rh-positive cases were significantly more frequent in both sexes, with slightly higher representation in males, which aligns with global trends showing Rh-positive as the dominant phenotype.

### Caste-Wise Prevalence

In terms of ethnic background, beta-thalassemia was most common among individuals belonging to the Arain caste (28%), followed by Rajpoot (20%), Sheikh (12.2%), Jutt (9%), and Khokhar (4.8%). These trends align partially with findings from Hafeez et al. (2007), who observed high prevalence among Rajpoots, followed by Jutts, Arain, Sheikhs, and Pathans. Qurat-ul-Ain et al. (2011) also reported Rajpoots as a highly affected group. The predominance of thalassemia in specific castes may reflect patterns of consanguineous marriages, which are culturally prevalent within these communities.

Our study recorded a high incidence of consanguineous marriages (69%) among thalassemic families, mostly among first cousins, which strongly contributes to the transmission of this autosomal recessive condition. This finding is supported by Baig et al. (2005) who reported a consanguinity rate of 63% in Faisalabad, and Al-Riyami and Ebrahim (2005) who found a 58% rate in Oman.

### Age-Wise Distribution

Our analysis of age-wise distribution showed that both age groups (<15 years and >15 years) had almost equal representation (50.5% and 49.5%, respectively). The prevalence of ABO and Rh blood groups was consistent across age groups, with no statistically significant difference. Rh-positive individuals were more common in both groups, in line with worldwide trends. This suggests that age is not a determining factor in ABO/Rh distribution among thalassemia patients.

### Comparison with Global and National Studies

In our study the ABO blood group distribution of thalassemia patients was as follows: B > O > A > AB. The predominant blood group was B-positive, followed by O-positive, A-positive, and AB-positive–representing 39.3%, 26%, 19.6%, and 7.9%, respectively. These results are consistent with Khan et al. (2014), who reported comparable trends among blood donors in Lahore. However, some of the international studies do not align with these findings. For example, Salih (2016) reported that the O blood group was the most common among thalassemic patients in Iraq (49.4%).

Furthermore, according to Mohssin et al. (2015), the same group was common in Baghdad (59.1%). Our findings, on the other hand, are consistent with some regional studies that contend that the B blood group is the most common among donors, such as Ilyas et al. (2013) and Iqbal et al. (2009). In our analysis, the proportion of Rh-positive (93%) to Rh-negative (7%) people is consistent with worldwide trends. Similarly, Khan et al. (2014) also reported 93% Rh-positive prevalence among blood donors in Lahore.

### Social Impact and Mortality Trends

Patients with thalassemia have historically had an extremely high death rate because of a lack of public awareness, difficulties with diagnosis, and treatment availability. However, many organizations such as the Sundas Foundation, Fatimid Foundation, Al-Khidmat Foundation, and Kashif Iqbal Foundation have all made significant contributions to manage this disease in recent years. Through various initiatives, these organizations have raised public awareness, facilitated genetic screening, and enhanced access to blood transfusions through reliable blood banks. As a result, thalassemia patients' life expectancy and quality of life have considerably enhanced in Pakistan.

## Conclusion

The findings of this study showed that the persons having B +ve blood group had the highest risk of developing beta-thalassemia and hence required intervention through education and early screening. The study indicated that the male population was more vulnerable to beta-thalassemia compared to the female population, wherein a high incidence of Rh-positive blood existed among the cases. The findings of this research can be helpful in reassessing the policies of public health, particularly in the distribution of blood bank material and the coordination of transfusion operations. The information regarding the association of ABO and Rh blood group patterns with thalassemia cases enables medical practitioners to control blood donations and stocks more effectively. Additionally, better therapeutic interventions and higher public awareness improved the patients' life expectancy. It also highlights the importance of genetic counseling and outreach programs within vulnerable groups.

**Ethical Approval and Consent**: The proposal and protocol for this study was approved by the Advance Study and Research Board at the University of Education, Lahore. Ethical approval was also granted by the Ethical Committee of the University. Informed consent was secured from all participants involved in the study.

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