

## Consanguinity ratio in $\beta$ -thalassemia major patients in District Bannu

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### Abstract

**Objective:** To assess the frequency of consanguinity in  $\beta$ -thalassemia major patients and its association with age, gender and hepatitis C virus antibody positivity.

**Methods:** The cross-sectional study was conducted from June 2013 to July 2014 at various hospitals of district Bannu in the North Western Khyber Pakhtunkhwa province of Pakistan. Data was recorded on a predesigned questionnaire.

**Results:** Out of 180 subjects, 133(74%) parents were cousins, while 47(26%) were unrelated. The frequency of anti-hepatitis C virus antibody positivity was 14(7.77%).

**Conclusion:** High prevalence of the disease in the study region was due to consanguineous marriages.

**Keywords:** Beta Thalassemia Major, Hepatitis C, Consanguinity, Bannu. (JPMA 65: 1161; 2015)

### Introduction

Thalassemia is an inherited disorder characterised by abnormal and low production of haemoglobin (Hb) and excessive destruction of red blood cells (RBCs). Thalassemia causes varying degrees of anaemia, which can range from significant to life-threatening. It is estimated that 1.5% of the world's population are carriers of beta-thalassemia i.e. at least there are 80 million to 90 million people with an estimated 60,000 new cases being born each year.<sup>1</sup> These hereditary anaemias are caused by mutations that decrease Hb synthesis and RBC survival. These hereditary anaemias are caused by decreased or absent production of one type of globin chain; either  $\alpha$  or  $\beta$ . Such haematological disorders range from asymptomatic to severe anaemia, which can cause significant morbidity and mortality. Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%) and southeast Asia.<sup>2</sup>

Thalassemia was and still is particularly prevailing in areas in which malaria is or was endemic, but the exact mechanism is still not known.<sup>3</sup> It is thought that in areas where malaria was prevalent, humans underwent a small genetic change or adjustment in their deoxyribonucleic acid (DNA) which gave them an advantage over those in whom this did not occur, i.e. this

change made them more resistant to the malaria infection. This is because important changes occurred in the RBC environment following this genetic change that did not allow the parasite to survive and multiply, causing illness and ultimately death.<sup>4</sup> In Pakistan, the number of registered thalassemia-hit children in different thalassemia centres is around 22,000 whereas a similar number of children are living in villages and are not registered with any thalassemia centre. The occurrence of thalassemia carriers is 5-8% in various racial groups which translate into 7 to 10 million individuals. The average life expectancy of  $\beta$ -thalassemia patients in Pakistan is 10 years. Prospective prevention, which includes population education, screening of couples in child-bearing age group, genetic counselling and pre-natal diagnosis, is an effective way to cope successfully with such a disease.<sup>5</sup> Studies have suggested that poverty, consanguinity and unawareness about the disease are the prominent factors in increasing the prevalence of this particular genetic disorder.<sup>6</sup>

Globally, efforts have been made for the prevention of thalassemia by mass education and various prevention strategies, like mass screening, pre-natal diagnosis and termination of pregnancy and pre-marital screening. Population screening, genetic counselling, pre-natal diagnosis and option of terminating affected pregnancies remain the mainstay of any strategy to devise a control programme, and investigating the underlying molecular defects in  $\beta$ -thalassemia is an important prerequisite for such programmes.<sup>7</sup>

The current study was conducted to collect information about consanguinity from  $\beta$ -thalassemia major patients.

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## Subjects and Methods

The cross-sectional study was conducted from June 2013 to July 2014 at various hospitals of district Bannu in the North Western Khyber Pakhtunkhwa (KPK) province of Pakistan. The participating hospitals were District Head Quarter (DHQ) Hospital, Bannu, Khalifa Gul Nawaz Teaching Hospital, Women and Children Teaching Hospital, and Haemophilia and Thalassemia Centre, Bannu. The study protocol was approved by the ethical committee of the University of Science and Technology, KPK.

A 2ml venous blood sample was taken with sterilised syringes in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tubes (K2 EDTA-BD Franklin Lakes, NJ, USA), which was then transferred to the thalassemia centre at Women and Children Hospital for screening. Other information was collected by interviewing the  $\beta$ -thalassemia major patients or their close relatives. Data was recorded on predesigned questionnaire, carrying all the information like name, age, gender, area, blood group, Hb, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), Hbalpha-1 (HBA1), Hbalpha-2 (HBA2), HbF (HBF), marital status, anti-hepatitis C virus (HCV) negativity or positivity etc.

Haematological parameters like Hb, MCV, MCH were measured (Sysmex, XP-100, Japan), and so was the case with HBA1, HBA2 and HBF values (Minicap Sibia. HB Electrophoresis). The sample size was based on HBF level. Patients with HBF level more than 2% were included in the study, while those below 2% were excluded. Subjects found to be thalassaemic were screened for HCV positivity, as thalassaemic children are at risk of getting infection due to repeated blood transfusions. For this purpose, a further 2ml blood was collected with sterilised syringes in BD Vacutainer serum tubes (K2 EDTA-BD Franklin Lakes, NJ, USA) and was allowed to clot. Serum was separated by centrifugation at 8000 rpm for 5 minutes at room temperature.

Screening for HCV was performed by Immunochromatographic test (ICT).

The HCV one-step test device is a lateral flow chromatographic immunoassay based on the principle of the double sandwich technique. The membrane is coated with recombinant viral antigen on the test line region of the device. During testing, the serum specimen reacts with the antigen coated particles. The mixture migrates upward on

the membrane chromatographically by capillary action to react with the recombinant antigen on the membrane and generate a coloured line. Presence of this coloured line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear at the control line region, indicating that the correct volume of specimen has been added.

The test device was placed on a clean and level surface, and 10ul of serum was transferred. One drop of sample buffer was added immediately. After five minutes, the coloured line(s) in the device was observed. The result was considered Positive if two lines appeared; one coloured line in the control line region, and another in the test line region. The result was negative if only one line appeared in the control line region.

## Results

Out of 180 subjects 110 (161%) were males and 70(39%) were females. Overall, 133(74%) parents of the affected children were cousins, while 47(26%) were unrelated. The frequency of anti-hepatitis C virus antibody positivity was 14(7.77%) (Table).

Area-wise incidence was most frequent in Frontier Region 39(21.66%), followed by Ghoriwala 28(15.55%), Nurur 25(13.88%), Bazar Ahmad Khan 19(10.55%), Surani 17(9.44%), Mandan 14(7.77%), Mameshkhel & Sukarhi 13(7.22%) each and then least in the city (6.66%) (Figure).

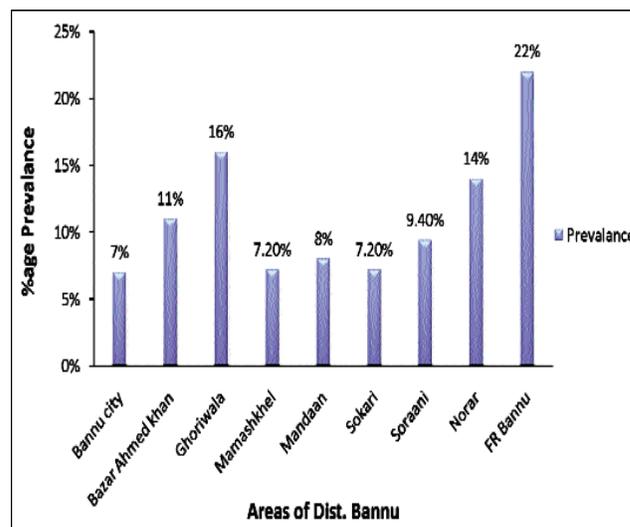


Figure: Area-wise distribution of the disease.

Table: Distribution of thalassaemic patients in relation to consanguinity and hepatitis C virus (HCV) antibody positivity.

Total Patients	Male	Female	HCV	%	Consg;	Frequency	Non-Consg;	Frequency
180	110	70	14	7.77	133	73.80%	47	26.11%

## Discussion

Thalassemia is recognised as the most prevalent genetic blood disorder in the world. However,  $\beta$ -thalassemia, the most common autosomal single-gene disorder worldwide, is found in about 60 nations with a carrier frequency of about 150 million.<sup>8</sup> In our study, the number of affected males (61.11%) was comparatively higher than the affected females (38.89%). The large number of thalassemia patients were due to parents' lack of knowledge about the disease and higher consanguinity ratio. The high frequency of the disease in Frontier Regions (21.66%) was due to low literacy rate, unawareness about the disease and poor health facilities. The prevalence of anti-HCV antibodies was 7.77% among all  $\beta$ -thalassemic patients, and this is the least value recorded so far in the region.

In the current study, incidence of  $\beta$ -thalassemia was highest among consanguineous marriages (73.80%) compared to un-related ones (26.2%). The findings are in line with a study<sup>9</sup> that reported the rate of first-cousin marriages among the parents of affected children to be 63% in Faisalabad.

The proportion of male patients was higher than the female. There were 56.95% male while females were 43.05%. Similar results are reported by different authors. A study<sup>10</sup> from Turkey reported that 56.7% patients were male and 43.3% were female. Another study<sup>11</sup> reported higher prevalence of male (64%) than (36%) female patients. Male-to-female ratio was 1.8:1.0. This gender-ratio difference in thalassemic patients (males more affected than females) is noteworthy and deserves further investigation considering thalassemia as a single-gene disease transmitted by a recessive mode of inheritance. The preponderance of males over females in the present study is difficult to explain. One possible reason is the fact that the people are more concerned with the health of the male offspring and, hence, are more likely to seek medical care for them.

One study<sup>11</sup> done in Faisalabad noticed higher consanguinity (75.32%). In our study, the prevalence of HCV positivity was 7.77%. A study performed in KPK<sup>12</sup> found that the prevalence of HCV in thalassemia patients was 15%.

The prevalence of anti-HCV antibodies might have been caused either due to unscreened blood transfusions, or due to unavailability of health facilities in the region.

## Conclusion

Consanguineous marriages showed high incidence of the disease than the unrelated couples. Males were more affected than females. Special centres for thalassemia should be encouraged.

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