ORIGINAL ARTICLE

INCIDENCE OF β-THALASSEMIA CARRIERS IN MUZAFFARABAD, AZAD JAMMU AND KASHMIR

AHMAD M.M.,[1] SALARIA M.S.,[2] QAMAR S.,[3] BUKHARI M.H.,[4] QURESHI A.H.[5] AND SOAZ M.A.[6]
[1,2,4]Departments of Pathology, AJK Medical College/ Combined Military Hospital Muzaffarabad and PMC, Faisalabad and [3,5]King Edward Medical University, Lahore – Pakistan

Abstract

Background and Objective: β-thalassemia is a common problem among the haemoglobinopathies in Pakistan, with a high carrier rate. Consangious marriages are increasing the problem every year. Awareness and counselling is essential to check the problem. The present study was designed to determine the frequency of β-thalassemia carriers in healthy population from Muzaffarabad Azad Jammu and Kashmir. In this cross – sectional population based study, people from Muzaffarabad were motivated to get themselves tested for beta thalassemia trait.

Methods: People found healthy on basis of a questionnaire and clinical examination were included in the study. Complete blood counts and peripheral smear reviews were performed on EDTA samples. Hemoglobin (Hb) electrophoresis was performed. Subjects with mean corpuscular volume (MCV), 76 fl, mean corpuscular Hb (MCH), 27 pg were subjected to HbA2 level estimation by elution method. Hemoglobin A2 of more than 3.5% was considered diagnostic for beta-thalassemia trait. Azad Jammu and Kashmir is a territory with considerable ethnic heterogeneity. This is basically a tribal society with a high proposition of consanguineous marriages. We created awareness among the general population.

Conclusion: Using strict criteria for healthy population, we conclude that incidence of thalassemia trait is 5.6% in our study population. β-thalassemia trait is present in almost all ethnic groups. To control thalassemia major, Screening program for thalassemia carriers must be initiated in the area.

Keywords: β-thalassemia trait, carriers, Azad Kashmir.

INTRODUCTION

Hemoglobinopathies are defined as disorders with abnormal hemoglobin and constitute a group of quailtative and quantitative disorders of hemoglobin while thalassemia is the term used to describe disorders with significant decrease in the rate of synthesis of one or more globin chains.[1]

Hereditary disorders of hemoglobin have a worldwide distribution. The frequency of abnormal hemoglobin varies considerably with geographical location and racial groups. It is estimated that approximately 7% of the world population carry a gene for clinically important hemoglobin disorder.[2]

Hemoglobinopathies are a major public health problem in Saudi Arabia. Important hemoglobinopathies in Saudi Arabia and other middle east countries are alpha thalassemia, beta thalassemia and sickle cell anemia. In a premarital screening program for thalassemia and sickle gene, incidence of these two genes was reported to be 7.75%.[3]

Similar figures are reported from other gulf countries. In a community based survey of under – five year age children in Sultanate of Oman, incidence of thalassemia and sickle gene was reported as 8.27%.[4]

In various countries there is considerable variation in the incidence of β-thalassemia in small geographical regions.[5,6]

Among hemoglobinopathies, β-thalassemia is a major problem in Pakistan with a carrier rate of 5 – 8%. First cousin marriages are 37.1% and total consanguineous marriages are 50.3% Using Hardy Weinberg equation, each year about 6,000 to 7,500 homozygous β-thalassemia are born in Pakistan. Regional incidence varies considerably depending on the gene prevalence and birth rate in the area[7-9] Large number is added annually to the already existing homozygote population. Most of the available studies about frequency of beta thalassemia are in small groups of hospital – based patients and/or population groups. We also understand that the incidence is higher in some groups.[10] Overall there is a stated lack of information about the true gene frequency in many parts of the world.[11]

Similarly incidence in various ethnic groups in Pakistan is not well known. More accurate assessment of gene frequency of βTT in the population provides solid data about the health burden posed by the disease and is of great help to plan control programs for β-thalassemia in the area.[12]

Corresponding Author: Dr. Malik Mahmood Ahmad
Department of Pathology AJK Medical College/ Combined Military Hospital Muzaffarabad and PMC Faisal Abad; Source of Support: AJK Health Department Muzaffarabd

The present study was undertaken to find out the incidence of beta thalassemia gene in the area. At the same time, data was collected on ethnic variation.

SUBJECTS AND METHODS

This cross sectional population based study was carried out, with approval and support of AJK health department, at Combined Military Hospital Muzaffarabad. After obtaining verbal consent, each individual was given a number and a detailed proforma was filled. Proforma included details of age, sex, and cast, family history of hemoglobinopathies and blood transfusion history. Questionnaire was developed on basis of literature. Clinical examination included evidence of anemia, splenomegaly, lymphadenopathy. All healthy people were included. People with family history of hemoglobinopathies and history of blood transfusion or showing evidence of anemia, splenomegaly, lymphadenopathy were excluded. A random sample of five hundred persons was selected from residents of municipal area on door to door basis.

Three milliliter blood was collected under standardized conditions in potassium EDTA for complete blood count (CBC) and hemoglobin electrophoresis. Smear prepared immediately and blood poured in EDTA bulb. Sample was gently mixed soon after collection.

EDTA sample was processed on Sysmex K-1000 hematology analyzer. Equipment was calibrated by the vendors. Weekly and monthly maintenance performed as per recommendations. Quality control was performed by running three controls daily morning and then after every 50 samples. Analyzer performed hemoglobin estimation by modified Drabkin's method. Instrument measures the number and size of particles suspended in an electronically conductive fluid. Particles are forced to flow through a small aperture having an emerged electrode on either side. With passage of particle through aperture, there is a change in resistance between electrodes that produces a voltage pulse of short duration and magnitude of this pulse is proportional to the particle size. Series of pulses are then electronically computed and red cell indices are derived. Dried smears were stained with Leishman's stain in a batch of ten slides each. Mainstay of the project was cellulose acetate electrophoresis using Tris-EDTA – Borate buffer at a pH of 8.9.

Hemolysate was prepared from packed cells obtained after removing plasma from centrifuged blood and then washing by centrifugation in 3 – 5 volumes of saline three times. Packed cells were mixed with equal volume of distilled water and shaken vigorously for two minutes. To this mixture, half the amount of carbon tetrachloride was added, mixed for five minutes. Then it was centrifuged for 20 minutes at 3000 rpm. Top layer of lysate was removed and one drop of 2 % potassium cyanide was added to the lysate. Hemoglobin content of lysate was adjusted between 3 and 4 g/dl as instructed by the manufacturer of the electrophoresis strips.

Whole process of electrophoresis was carried out as per procedure manual of the electrophoresis apparatus and kit insert of the electrophoresis strip. Tris-EDTA – Borate buffer at a pH of 8.9 was used and same was used for Hb A2 quantitation. Elevation of Hb A2 presents the best practical approach for the diagnosis of beta thalassemia. Hemgolbin A2 estimation was done where red cell morphology or indices indicated the need to do so. Elution technique using cellulose acetate and tris-EDTA buffer pH 8.9 was used because of non-availability of column chromatography and densitometry. Quality control was established by A2 reference preparation.

Red cell parameters/indices including Hemoglobin, Red blood cell counts, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, were assessed. Stained peripheral smear for all the people were examined for abnormal findings.

Iron deficiency was excluded on complete blood picture and peripheral smear findings. Twenty five parents and siblings of children found to have β-thalassemia were called for investigation for βTT and follow-up for counseling as required. Simple percentages were used for statistical purposes.

RESULTS

A total of 500 cases were studied. 292 male and 208 males comprised this study with an overall male to female ratio of 2.43: 1.73 (Table 1).

Table 1: *Age and Sex wise distribution of sample.*

Age	*Male No*	*Male %*	*Female No*	*Female %*	*Total No*	*Total %*
Less than 5 years	7	15.6	29	5.6	107	21.4
More than 5 to 15 years	45	9.0	31	6.2	76	15.2
More than 15 to 35 years	118	23.6	116	23.2	234	46.8
More than 35 to 50 years	35	7.0	23	4.6	58	11.6
Above 50 years	25	3.2	9	1.6	25	5.0
Total	292		208		500	

All the people studied were residents of Muzaffarabad. 93.8% had their origin from Muzaffarabad, 3.4% from Poonch, 1% from NWFP, 1% from Afghanistan and 0.8% from Punjab and Baluchistan. Almost all the races residing in the area were included (Table 2).

Table 2: *Race wise distribution of sample.*

Cast	*#*	*%*
Raja	110	22.0
Awan	101	20.2
Khawaja	59	11.8
Syed	52	10.4
Choudhary	47	9.4
Mughal	43	8.6
Pathan	31	6.2
Qureshi	29	5.8
Abbasi	21	4.2
Sheikh	5	1
Qazi	2	0.4

Out of 500 people studied, a total of 28 cases were diagnosed as having β-thalassemia trait. Age wise distribution of β-thalassemia carriers is depicted in Table 3.

Table 3: *β-thalassemia carriers age wise.*

Age in Years	*β-thal Carriers*
Up to 5 years	7
> 5 to 15 years	1
> 15 to 35 year	15
> 35 to 50 years	4
Above 50 years	1

Hb A2 was quantitated in cases where RBC counts or RBC morphology or A2 prominence on CAE indicated to do so. In total cases studied it had mean, standard deviation, variance, maximum and minimum of 3.90%, 1.2%, 1.5%, 6.3% and 1% respectively. Range of Hb A2 in thalassemia carriers was 3.9% to 6.3% with mean, standard deviation and variance, 4.9%, 0.5%, 0.3% respectively. Absence of HbA2 was not noted in any case.

DISCUSSION

The current study confirmed that beta thalassemia gene is prevalent in the area. The overall rate of β-thalassemia carriers was 5.6%.

Table 4: *Race wise incidence of β-thalassemia trait.*

Cast	*#*	*Number of Carriers*
Raja	110	6
Awan	101	5
Khawaja	59	2
Syed	52	4
Choudhary	47	2
Mughal	43	1
Pathan	31	3
Qureshi	29	2
Abbasi	21	2
Sheikh	5	0
Qazi	2	1

Strength of our study was that we approached general population on door to door basis. Proforma was set up to exclude possible confounding factors. We looked into various tribes/casts staying in the area. We did not have the sources for iron profiling to exclude iron deficiency but excluded iron deficiency by meticulous study of red cell indices and hematologist review of peripheral blood smears.[13]

Our findings are closer to most of these findings and indicate that gene frequency is significantly high in our population also. A very high frequency of β-thalassemia trait has been found in referred cases for hemoglobin electrophoresis to various hospitals in Pakistan like in Dera Ismail Khan this frequency was 18.5%.[14]

Families of thalassemia patients also show very high gene frequency. Studies show that in Pakistan carrier rate in these families vary between 31 – 68% in comparison to 5% in general population. In a study conducted in Lahore, 52% of samples from families of patients suffering from beta thalassemia major were carriers of gene on hemoglobin electrophoresis.[15]

Azad Kashmir is ethnically diverse territory with approximate population of 4 million with per capita income of 847 US dollars.[16] Azad Kashmir has its own health department with four medical colleges. With gradual control of malnutrition and communicable disease, β-thalassemia major patients would die at a young age are now surviving enough to seek medical attention.[17-18]

It is ***concluded*** that if we start prevention today, we will be able to focus and provide better care to the existing thalassemia patients otherwise the burden of disease is continuing and quality of care of thalassemia

will continue to deteriorate. People with thalassemia trait are well and usually only detected through routine blood testing.

Conflicts of Interest: None.

ACKNOWLEDGEMENTS
The authors are thankful to the families who participated in the study. We also acknowledge the help and cooperation of the health authorities and CMH Muzaffarabad.

Author's Contribution
All authors contributed equally in the project.

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