TYPES AND FREQUENCY OF HEMOGLOBINOPATHIES, DIAGNOSED BY HB ELECTROPHORESES IN THE LADY READING HOSPITAL PESHAWAR, PAKISTAN

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ABSTRACTS

To determine the frequencies of various Haemoglobinopathies in the suspected anemia patients, this study was carried out. A total of the 350 patients displayed symptoms of anemia were assessed for this study. Cellulose acetate electrophoresis at pH 8.4 was performed on Hemoglobin extracted from the red blood cells of the patients. Out of the total 350 patients, 115 (32.9 %) were diagnosed with beta thalassemia trait with 55 (15.7 %) males and 60 (17.1 %)female ratio.45(12.9 %) patients were diagnosed with beta thalassemia major with 15(4.3 %) males and 30(8.6%) females ratio. Sickle cell anemia was diagnosed in 3 (0.8 %) patients, all of them were females. Sickle beta thalassemia was diagnosed in 07(02 %) patients with 04 (1.1 %) males and 03 (0.9 %) females ration, while 01 male (0.2 %) was diagnosed with Hb D trait.

Most of the patients, 98 confirmed patients (28 %) of the total tested were from the age group < 2 and there was no diagnosis of any disorder beyond age 40 years.

The high prevalence of beta thalassemia warrants further investigation to unravel the causes of high prevalence of the disorder and other hemoglobinopathies. Before the curing of the various haemoglobinopathies with hematopoietic stem transplant and CRISPR-CAS9 gene editing technology can be actualized in future, heterozygous carrier testing should be carried out in general population especially premarital testing to reduce the incidence of the hemoglobinopathies in the population.

INTRODUCTION

Haemoglobinopathies are single gene inherited disorders. The disorders are due tomutations in coding regions of globin, the proteins component of haemoglobin (Hb) complex. Some Haemoglobinopathies particularly having mutations in regulatory regions such as promoters and enhancers alters the globin proteinproduction and leads to a well-known disorder thalassemia syndrome.[1]If mutations are in the regulatory regions of globin then although the globin proteins are synthesized normally but with altered rate. On the contrary if the mutations are in the coding regions then the proteins might be produced at the normal rate but the proteins will not be normal as amino acids sequence are altered and it leads to qualitative reduction in the globin proteins.Quantitative defects present as thalassemia, while the qualitative changes collectively known as Hb variants, that results in the the great health problems ranging from sickle cell disease to unstable Methemoglobinemiaencompassing many variants .[2]

According to World Health Organization(WHO) almost 07% of the world population are genetic carrier (heterozygous) of different hemoglobin disorders and consequentlythree hundred thousand to five hundred thousand children are born every year with variousHemoglobin disorders and majority of them are from the under developed countries.[3]Certain haemoglobinopathies such as sickle cell anemia remain in high frequency in certain African population due to the resistance of the carriers against Malaria Geographically ethnic populations of south East Asia e.g; e.g., India, Pakistan, Afghanistan, Bangladesh, Indonesia and Thailand are at an increased risk of thalassemia or sickle cell disorders why? Because of the mutations' prevalence?[4,5]

These disorders exert a huge burden on these patients and on their families, on entire health sector andthe entire community.[6,7]These haemoglobin disorders are a major health problem in almost all developing countriesincluding Pakistan.[8,9]They are generally not curable but hematopoietic stem transplant and CRISPR-CAS9 gene editing technology in future will cure the disorders.

Currently thedisorders should be properly managedby population screening, genetic counseling, and prenatal diagnosis.[10,11]

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Our main objective of the study was to find various types of Haemoglob disorders and their frequency of distribution in Peshawar, Northern part of Pakistan, which can be helpful in formulating various strategies for theeffective control and prevention of these inherited disorders in the general population of Khyber Pakhtoonkhwah..

MATERIAL AND METHOD

This retrospective cross-sectional study was carried out at pathology department of lady reading hospital Peshawar, a tertiary care hospital of Khyber pakhtoonkhwah province of Pakistan, from January. 2016 to December 2016. This study was conducted on350 patients having suspected diagnosis of Anemia after complete blood counts (CBC), were referred for the screening of Hemoglobin disorders. Current study was approved by the institutional review board. The blood sample from patients with low Hemoglobin, were collected who were suspected with hemoglobinopathies.

CBCs was performed by Sysmex XN 1000 analyzer and blood smears were stained with Giemsa .Assessment of grading of hypochromia , microcytosis, macrocytosis, polychromasia was performed using microscopic morphology. Cellulose acetate Electrophoresis at a pH of 8.4 was run on Hemoglobin extracted from the red blood cells. *Cellulose acetate membranes*. (Plastic-backed membranes) (7.6 × 6.0 cm) were used. HbA2 value higher then 3.5% was kept as a cut-off point for the diagnosis of beta thalassemia trait.

RESULTS

A total 350 patients were included in this study including all age groups and both the genders. Among 350 suspected cases 171(48.9 %) were diagnosed with hemoglobin disorders while the rest 179 patients (51.1%) were reported normal after Hb electrophoresis.

Among the total 350 patients assessed, 171 (48.9 %) were males while 179 (51.1%) were females with Male /Female ratio 0.9 : 1 (Table 1).

Out of the total 350 patients, 115 (32.8 %) were diagnosed with Beta thalassemia trait,45(12.9 %) patients were diagnosed with beta thalassemia major. Sickle cell anemia was found in 3 (0.8 %) patients, sickle beta thalassemia in 07(02 %) patients while 01 (0.2 %) patient was diagnosed withHb D trait. The table 2 and the pie chart 1summarizes the data.

The gender-wise stratification is shown in Barchart 1. Among all age groups included in the study, age was divided into various age groups:group 1: < 2 years; group 2: 2-5 years;group 3: 6-10 year;group 4:11-15 years;group5:16-20 years;group 6:21-25 years;group 7: 26-30 years; group 8:31-35 years and group 9:36 -40 years.Minimum age group was <2 years while maximum age group was up to 40 year. Age-wise stratification is shown in table3.

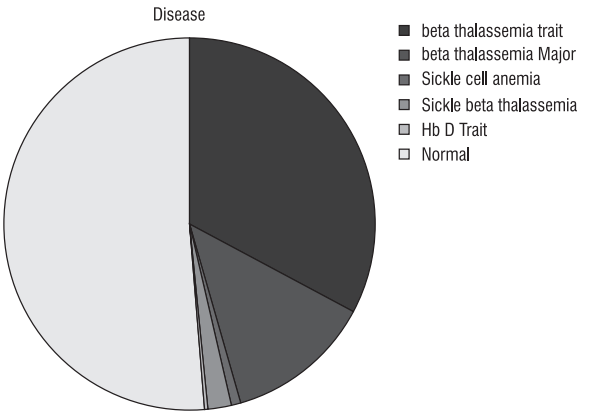

Figure 1: disease frequency distribution(n=350)

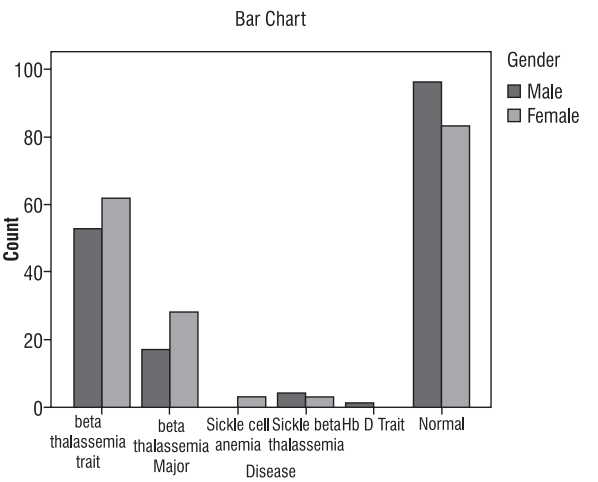

Figure 2: gender-wise disease stratification (n=350)

Table 1; Gender distribution (n= 350)

Gender	Frequency	Percentage
Male	171	48.9
Female	179	51.1
Total	350	100.0

Table 2; Disease distribution frequency (n=350)

Types of Haemoglobinopathies	Frequency	Percentage(%)
Beta thalassemia trait	115	32.9
Beta thalassemia Major	45	12.9
Sickle cell anemia	3	0.9
Sickle beta thalassemia	7	2.0
Hb D Trait	1	0.3
Normal	179	51.1
Total	350	100.0

Table 3; Age-wise Disease stratification (n=350)

Age (year)	Beta thal-assemia trait	Beta thal-assemia Major	Sickle cell anemia	Sickle- beta thalas-semia	Hb D Trait	Normal	Total
< 2	20	40	0	1	0	37	98
2-5	12	5	1	1	0	18	37
6-10	18	0	1	0	0	41	60
11-15	18	0	1	2	1	23	45
16-20	16	0	0	1	0	22	39
21-25	23	0	0	0	0	21	44
26-30	7	0	0	2	0	13	22
31-35	0	0	0	0	0	4	4
36-40	1	0	0	0	0	0	1
	115	45	3	7	1	179	350

DISCUSSION

The overall frequency of various hemoglobinopathieswas 48% out of 100 suspected cases of anemia in our study. This prevalence is significantly higher than the prevalence of the disorders in the study conducted previously at Islamabad on 504 patients by Waheed U et al. which revealed a frequency of 28.4% of various hemoglobinopathies. However, this study supports the previous finding that thalassemia is highest in its frequency among all hemoglobin disorders in Paskistan..[9]Furtherstudy is warranted to find causes of this high frequency in Pakistan; research should be focused on the genetic causes such as genetic drift which might be a contributing factor in such high frequency of thalassemia. Out of the total 350 patients, 115 (32.9 %) were diagnosed withbetathalassemia trait with 55 (15.7 %)males and 60 (17.1 %)femalesratio; 45(12.9 %) patients were diagnosed with beta thalassemia major with 15(4.3 %) males and 30(8.6%) females ratio. Sickle cell anemia was diagnosed in 3 (0.8 %) patients, all of them were females.Sickle beta thalassemia in 07(02 %) patientswith 04 (1.1 %) males and 03 (0.9 %)females ration, while 01 male (0.2 %)case diagnosed withHb D trait.*(table 2).*

Most of the patients, 98 confirmed patients (28 %) of the totaltested were from the age group < 2years, followed by age group 6-10 years which had 17.1 % (60 cases) confirmed patients. Among the total, 115 patients of beta thalassemia trait were falling in the age group from 20 -25 years with 23 confirmed diagnosis, followed by 20 patients from age < 2 years. Patients diagnosed with beta thalassemia major were predominantly falling in age below 06 years particularly less than year 2 group which included 40 confirmed diagnosis and 05 confirmed patients from age 2-5 years group.

A study conducted by Shabbir et al.,(2016) found that 51,8% of the total 935 patients with various hemoglobin disorders had diagnosed with β-thalassemia minor while β-thalassemia major were 24,1% among them, which are higher frequencies as compared to our study. Similarly, higher frequencies were reported of HbD minor / trait, which was 6.7%, sickke/beta thalassemia was with frequency of 4.5%, sickle cell disease with 3.9%, 1.9% cases diagnosed with Hb E , and 1.7% patients were diagnosed with sickle cell trait.[7]

Another study conducted by Rao S, Kar R et al in 2010 showed the frequency of β-Thalassemia trait was 17%, sickle cell trait was the next commonest with frequency 2.3%. Frequency of sickle cell disease was calculated as 1.7%, Hb D trait was 1%, Hb E trait frequency was 0.8%, frequencies of sickle – β thalassemia, Hb-E disease, E – β thalassemia (0.6% each) and 0.4 % cases were of thalassemia major .[10]

An estimated figure of the children born with β-thalassemia each year is 5000–9000 , although no available registered documentary of the data in Pakistan[5]but according to a study conducted by Ahmed, Saleem, Modell, &Petrou, 2010,the estimated rate of carrier state is 5–7%, with 9.8 million carriers in the total population.[8]

Awareness regarding prevention of Thalassemia is on a rise in Pakistan and Khyber pakhtoonkhwah assembly has passed a bill regarding thalassemia control and prevention almost two years back but still awaited for implementation. .

This study was aimed to find the types and frequency of various hemoglobin disorders in Khyber pakhtoonkhwah region of Pakistan that may be effective in taking steps towards prevention as well as management of hemoglobin disorders. Furthermore, this may be very useful in the formulation of transfusion policies in hospital blood bank.

CONCLUSIONS

This study revealed a significantly high frequency

of Haemoglobinopathies in patients with suspected anemia disorders.Keeping the high frequency of thalassemia disorders, it demands prompt measures in the form of screening of population, especially pre-marriage testing , to detect the carrier status. Screening is affordable and it is an easily available way to filter the carrier state , and that can be easily made accessible in a variety of settings in various parts of the community.

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