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ORIGINAL ARTICLE

Spectrum of Inherited Hemoglobin Disorder in patients presenting for Hb Electrophoresis: A Single Center Study in District Dera Ismail Khan

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ABSTRACT

Background: Hemoglobinopathies remains an important public health Problem for developing countries like Pakistan as it has been associated with high morbidity and mortality rates. The objective of this study was to know the frequency distribution of various hemoglobinopathies in ethnically and geographically diverse population of Dera Ismail Khan of Pakistan referred for Hemoglobin electrophoresis.

Methods: This cross sectional study was conducted at Pathology department of Gomal Medical College, Dera Ismail Khan, from January 2017 to December 2021. About 3 ml of venous anticoagulatedblood specimen were collected from suspects for Complete Blood Count, Sickling Test and hemoglobin electrophoresis.

Result: Of the 538 cases, 219(40.7%) were found normal and 319(59.3%) were found positive for abnormal hemoglobin disorder. Among positive samples 317(58.9%) were male and 221(41.1%) were female. BetaThalassemia was the most common hemoglobinopathyobserved in 194(60.8%) followed by sickle cell 105(32.9%), hemoglobin E, D and C were found in 9 (2.8%), 4(1.2) and 2(0.6%) respectively. Frequency of Beta Thalassemia is quite high in Balochtribe followed by Mehsud and Marwat. Sickle gene was more prevalent in Sherani followed byBhatni and Ustrana.

Conclusion: Beta Thalassemia and Sickle cell are the two most common hemoglobin disorders found in our set up. Mass screening with genetic counselingand education should be initiated to reduce disease burden.

Keywords: Electrophoresis, Hemoglobinopathies, Dera Ismail Khan.

INTRODUCTION

Hemoglobinopathies are the heterogamous group of hereditary disorder characterized by structural alterations of hemoglobin molecule, result from either production of abnormal hemoglobin chain, such as substitution of one amino acid as seen with Sickling disorders or underproduction of a given globin chain resulting in the thalassemia[1].

Hemoglobinopathies are the global problem and associated with numerous morbidities and mortalities in low and middle class income countries, World Health Organization (WHO) recognized about 7% of the world populations are carrier for different hemoglobin disorder and spreads in more than 70% countries of the world[2].

Annually 0.3-0.5 million children are born with severe hemoglobin disorder; 80% of these children are from developing and underdeveloped countries especially from Asia and Africa.[3-5] Among them 83% are born with Sickle Cell Disease (SCD) and the remaining 17% with Beta Thalassemia Major (BTM).[6]

Earlier, it was thought that North America and Northern Europe were non-endemic regions for hemoglobinopathies, but now these regions also have high incidence of hemoglobinopathies due to immigration of people from hemoglobinopathies-endemic regions[2].

In Pakistan among in all hemoglobinopathies Beta (B)-Thalassemia is one of the most prevalent inherited disorders of the country and about 5% of Pakistanis carry the heterozygous B-Thalassemia gene. [3, 7]It is difficult to predict trend of prevalence of other hemoglobinopathies in Pakistan, because relatively limited information's are available regarding prevalence in Pakistan. Dera Ismail Khan is the northern area of Khyber Pakhtunkhwa, Pakistan with considerable racial heterogeneity and tribal society with high tendency of consanguineous marriages.

This study was therefore designed to check the frequency of different hemoglobin disorders in patients referred for hemoglobin electrophoresis to Pathology department of Gomal Medical College Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

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MATERIALS AND METHODS

This cross-sectional study was conducted and designed in Pathology Department of Gomal Medical College Dera Ismail Khan from January 2017 to December 2021. Ethical committee of Gomal Medical College approved the study protocol.

Dera Ismail Khanis situated on the west bank of River Indus. It issurrounded by Bannu, Lucky Marwat cities of Khyber Pakhtun Khwa, MianwaliandBhakkarcity of Punjab (through Chashma Barrage) and Zhob in city of Batochistan and South Waziristan through district Tank. Patients visited to different government and private hospital referred to pathology lab of Gomal Medical College for hemoglobin electrophoresis studies were registered in the study. Patients having blood transfusion in last four weeks, critically ill and those who refused were excluded from the study.

Standard questionnaire designed was used to collect patient information like sex, age, cast, blood transfusion history, family history of hemoglobin disorder and other clinical findings like anemia, jaundice, splenomegaly etc. About 3 ml of Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated venous blood was collected from each referral subject aseptically after taking proper informed consent from patients/guardian. Peripheral blood film was made and stained by using 10% Giemsa; each sample was primarily subjected for Sickling test by using sodium metabisulfite method as previously suggested by Ullah[8]. An automated haematology analyser (Nihon Kohden, Tokyo) was used to estimate hemoglobin level and other red cell parameters like Mean Cell Volume (MCV), Mean Cell Hemoglobin Concentration (MCHC), Mean Cell Hemoglobin (MCH), Packed Cell Volume (PCV) and Red cell Distribution width (RDW). Hemolysate was prepared from whole blood by using normal saline, distilled water and carbon tetrachloride. Hemoglobin electrophoresis was performed at alkaline pH using methodology as per instruction provided by manufacturer's instructions (Clever Scientific, UK). For B-Thalassemia Trait (BTT) diagnosis HbA2 value >3.5% was considered as cut-off point. The collected data on preformatted questionnaire and results obtained after investigation were analyzed using SPSS version 17. A Chi-square test was also used to check the association between age groups and gender.

RESULTS

A total of 538 participated in the present study were referred from government and private hospital to Gomal Medical College pathology department for hemoglobin electrophoresis. Of the 538 blood samples 219(40.7%) were found with normal hemoglobin and 319(59.3%) had detected for hemoglobinopathies. Out of 319, male were 194(60.8%) and female were 125(39.2%). Among analyzed blood samples B-Thalassemia was the most common hemoglobin disorder 194(60.8%) followed by sickle cell disease 105 (32.9%). Other variants hemoglobin like Hb E, Hb D and Hb C were also noted in our population as shown in Figure 1.

Dera Ismail Khan become densely populated district with approximately 1.6 million populations after Soviet invasion of Afghanistan and internally displaced persons that are forced to flee from Federally Administered Tribal Areas (FATA) a tribal region in the northwest of Pakistan. This district is hub of different ethnic groups (twenty eight different castes) which shows showed various hemoglobinopathies in present study. Incidence of different hemoglobin disorders were studied in different ethnic groups living in Dera Ismail Khan. Among these groups, Baloch showed highest incidence of hemoglobinopathies (14.4%) followed by Mehsud (7.5%), Marwat (7.2%), Bhetani (5%), Gandpur (4.3%), Wazir (3.4%) and Kundi (3.4%). Sickle was most common in Sherani (17%) subsequently in Bhetani (9%), Ustrana (3.8%) and Kakar (1.5%) as depicted in table 1.

Figure 1: Frequency of overall Hemoglobinopathies detected by Hb electrophoresis in our study (n=319).

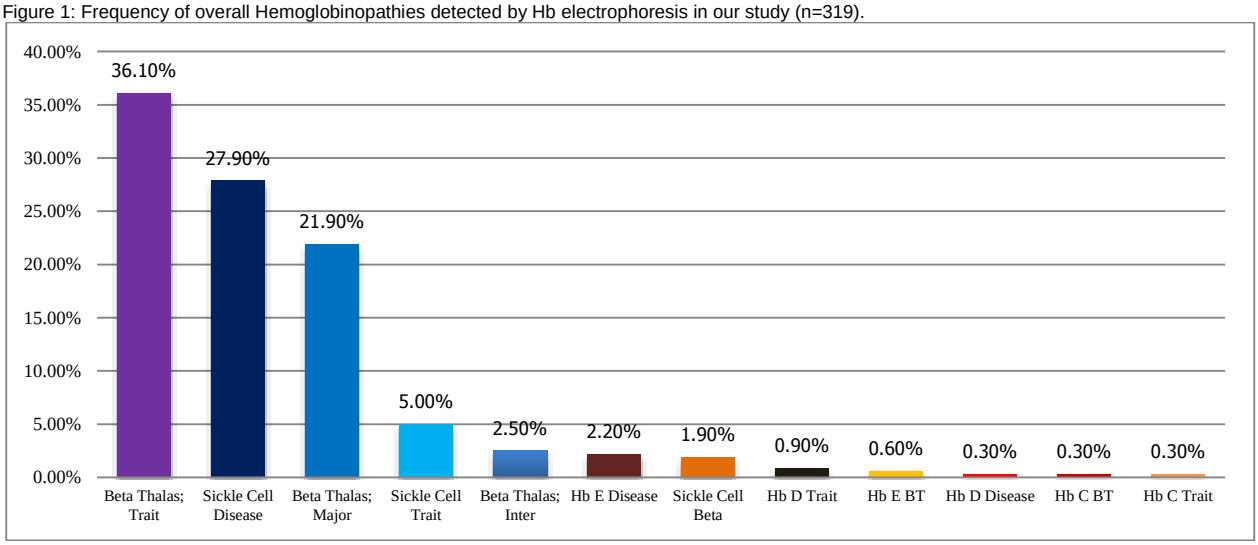

Table1: Distribution of different Hemoglobin disorder in our study. (n=319)

Cast	BT	SC	Compound Heterozyosity/ Others	Total
Afghani	1	--	--	1(0.3%)
Alizai	1	--	--	1(0.3%)
Awan	5	--	--	5(1.5%)
Babar	1	--	--	1(0.3%)
Baloch	46	1	2 (HBED)	49(15.3%)
Banochi	1	-	--	1(0.3%)
Bhatni	16	29	4(SC/BT)	49(15.3%)
Bilkhail	--	--	1 (HBDT)	1(0.3%)
Dial	--	--	2 (HBED)	2(0.6%)
Dohtar	3	--	--	3(0.9%)
Gandpur	14	3	1 (HBS/BT)1 (HBE/BT)	19(5.9%)
Gazar		--	1 (HBDD)	1(0.3%)
Kakar	6	5	--	11(0.3%)
Khokar	3	--	--	3(0.9%)
Kundi	11	1	--	12(3.7%)
Mandukhel	--	1	--	1(0.3%)
Marwat	23	--	--	23(7.2%)
Mehsud	24	--	--	24(7.5%)
Miani	1	--	1 (HBE/BT)	2(0.6%)
Panjabi	2	--	1(HBC/BT), 1 (HBCT)	4(1.2%)
Qazi	2	--	--	2(0.6%)
Rajpot	4	--	--	4(1.2%)
Rao	1	--	--	1(0.3%)
Sadozai	1	--	--	1(0.3%)
Sherani	7	54	--	61(19.1%)
Syed	2	--	--	2(0.6%)
Ustrana	4	12	--	16(5.0%)
Wazir	11	--	2 (HBED)	13(4.0%)

BT = Beta Thalassemia, **SC**= Sickle Cell, **SC/BT** = Sickle Cell /Beta Thalassemia, **HBDD** = Hemoglobin D Disease **HBDT**= Hemoglobin D Trait, **HBED** = Hemoglobin E Disease, **HBE/BT** = Hemoglobin E/Beta Thalassemia **HBC/BT** = Hemoglobin C/Beta Thalassemia, **HBCT** = Hemoglobin C Trait

DISCUSSION

Inherited disorders having an autosomal recessive pattern, thalassemia and hemoglobinopathy principally affecting the globin molecule of the hemoglobin. These hemoglobinopathies are restricted to certain castes, tribes, areas and even to some religions. Pakistan has a long invasions history and with strong culture, traditional norms for consanguineous marriage is 62% in which 80% were first cousin marriage that is highest among consanguinity belt leads to substantially high genetic diversity. About 29 million populations are suffering from different genetic disorder attributed by first cousin marriages. Intermarriages ratio is highest in Khyber Pakhtunkhwa province which makes it more prone for genetic disorders. Keeping in view this fact it is not possible to completely discourage risk marriages due to strong family traditions, norms regardless of the fact that genetic disorder is quite high in Pakistan. Among these hemoglobinopathies; Alpha (α)-Thalassemia, B-Thalassemia, Sickle cell disease and hemoglobin E were with relatively high prevalence. Every year, about 5000-9000 children born with B-Thalassemia major, 9.8 million (5-7%) cases of trait and unregistered patients are even many folds more[9].

The overall hemoglobin disorder in patients presenting for hemoglobin electrophoresis in our set up is 319/538 (59.3%). Similarly a report from Dera Ismail Khan revealed 75.6%[10]. The higher frequency by us is contrasts with some others. A study conducted in Islamabad revealed (28.4 %) by Waheed et al[11], while Saleem et al[12] from Northern Areas of KP (25 %) and about 34.2% from Karachi[13]. This may be attributed due to the patient distribution, because we analysed the samples size from single centre and did not represented the whole population.

In present study hemoglobin disorder was predominantly found in male with 60.8% and 39.2% females. Many researchers noted that hemoglobin disorder is more prevalent in male than female. A study done on population of northern areas of Pakistan to find the hemoglobinopathies pattern of distribution resulted in 2.2:1 male to female ratio compared to this study 1.5:1 ratio[12]. A study by Balgir in

India also notes that Hemoglobinopathies are more prevalent in males than females. Out of the 667 positive cases, 52.3% were males and 47.7% were females[14]. A study constitute of 300 cases was conducted in Dera Ismail Khan, Pakistan. All the 300 cases were checked for hemoglobinopathies by hemoglobin electrophoresis. Out of 300 patients 227(75.6%) were diagnosed with different hemoglobinopathies. Out of 227 hemoglobinopathic patients 150(66%) were males and 77(34%) were females relating to this study of higher hemoglobinopathic male ratio 60.8% compared to females39.2%[10]. The possible reason of higher ratio in male as compare to female is probably due to low access of females to the health care centers and hospitals. Another reason may be awareness about health is more in males as compare to females.

B-Thalassemia was the most common hemoglobin disorder found in our study 60.8%. In a study done in Dera Ismail Khan reported 62.23% for B-Thalassemia[10]. In Karachi a very high frequency also noted 75.9% for B-Thalassemia by Shabbir et al, 2016[13]. While in Islamabad 27% cases also detected for B-Thalassemia by Waheed et al, 2012[11].

In case of sickle cell disorder 32.9% patients were found with Hb S hemoglobin contrary to findings reported 1.92%, by clinical laboratory of The Aga Khan University suspects during 2005- 2006, which serves as referral lab for country[15] while a report from Karachi revealed 5.6% of sickle cell[13]. Our findings suggest that Dera Ismail Khanhas high incidence of sickle cell disease from the rest of the country, because Hb S gene predominantly exists in area of Dera Ismail Khan,Darazinda. Dera Ismail Khan connects via Darazinda to Zhob district of Province Balochistan. Sherani and Ustrana (Storyani) tribes are living in this region. While Bettani tribes also harbor the Sickle gene. Bettani are the residents of area Tank district in Dera Ismail Khan Division of KPK. In a study conducted by Hussain from Dera Ismail Khanalso noted that HBS (34.81%) was the second common hemoglobinopathy after B – Thalassemia, which supports our result[10]. Hussain also observed that sickle gene is present in Sherani, Ustrana and Bettani tribes. But we also noted the Sickle gene in Kakar, Gandpur, Baloch and Kundi castes because of immigration.

The occurrence of Sickle gene is not a surprise, many researchers hypothesized that this the Arab-Indian haplotype, Because Middle East, especially Iraq, Oman, Saudi Arabia, Yemen and Iraq were the trade routes between India and Europe. The flood of people of Balochistan in Oman took place between the twentieth and eighteen centuries, because the Arab – Indian haplotype is found in these immigrants[15-18]. The second evidence is that the HbS allele risen to high frequencies in malaria exposed population.[19] Because malaria is very frequent in this area, a study at the hilly region district Zhob of Blaochistan Province with adjoining to Dera Ismail Khan also reported the high incidence with 41.8% of malarial cases.[20] Another study conducted in Dera Ismail Khanrevealed 41.1% cases positive for malaria[21].

Among other variants hemoglobin like HbE, HbD and HbC are less than 1 % in our study. These findings are similar to those in past studies conducted in Pakistan[10 11, 13, 22].

CONCLUSION AND RECOMMENDATION

Hemoglobinopathies are among the most common monogenic diseases associated with consanguineous marriages and from infected immigrants. In Dera Ismail Khan, endemicity of hemoglobinopathies is might be due to inter-cousin marriages, displacement of people from militancy affected Tribal districts and Afghani population. That makes this area hotspot for such inherited disorders as local and migrated populations are strongly rooted with inter-cousin marriages. Even a new bill on thalassemia prevention and control has been passed recently Khyber Pakhtunkhwa assembly not yet implemented in true spirit which has contributed a lot in rise of such inherited disorders which can only be diluted out from population through awareness. Dera Ismail Khan has become endemic for these inherited disorders, preventive strategies like awareness among general population, premarital screenings general population, prenatal diagnosis and genetic counseling programs can help in reducing the burden of such endemic disease. Federal government should formulate such policies in which they should make mandatory the screening of such disorders before any sort of admission to educational institute, employment, antenatal clinics, especially before marriage and should allocate designated budget for such this endemic disorder.

Limitation: Pathology department of Gomal Medical College is serving as referral laboratory for diagnosis of hemoglobinopathies. As this department managing hemoglobinopathies in this area increase their experience, more and more families with this condition come forward and cascade screening and counselling such families could provide further suspects and carrier cases, and which could become a potentially good research center subjected to availability of funds and resources. With the need of upgradation of this pathology center, multi-molecular studies laboratories are required in this area in order to figure out the exact burden of hemoglobinopathies disorder in Dera Ismail Khan and in its neighboring areas.

Conflict of Interest: None

Source of funding: None

Author's Contribution: ZU and RR designed the study, conducted data analysis and drafted the manuscript. **ZU and NA** carried out the laboratory experiments and Specimen collection. **RB and AAK** provided guidance and coordination for design laboratory analysis, edited and revised the manuscript. **SB and SR** helped in samples and information collection from patients.

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