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Original Article:

Inherited hemolytic disorders with high occurrence of ß-thalassemia in Sindhi community of Jabalpur town in Madhya Pradesh, India

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Abstract:
Hereditary hemolytic disorders such as hemoglobin disorders, β-thalassemia syndrome, G6PD deficiency, and ABO and Rhesus blood groups are the most common public health problems in India. Community genetic screening provides multifaceted information for finding prevalence, level of health education, preventive strategies such as genetic/marriage counseling to relieve the burden of vulnerable communities. However, such genetic screening studies are scanty in India. This study aims to find the prevalence of inherited hemolytic disorders in Sindhi community, identify the persons for genetic/marriage counseling and to suggest the relevant strategies for prevention and control to the affected families. A cross-sectional random study of 508 persons of Sindhi community belonging to all ages and both sexes was conducted for screening of hemoglobin disorders, G6PD deficiency and ABO and Rhesus (D) blood groups following the standard procedures and techniques from Jabalpur town in Central India. High frequency of β-thalassemia trait (20.5%), Hb D trait (2.2%) and hemoglobin D/β-thalassemia (0.2%), G6PD deficiency (0.8%), and a low prevalence of Rhesus negative (3.0%) blood group was observed in Sindhi community of Jabalpur town in Madhya Pradesh. A case of β-thalassemia major and Hb D-thalassemia were also encountered. Double heterozygosity of Hb D/β-thalassemia showed hypochromic and microcytic red cell morphology with mild anemia. Inherited hemolytic disorders are an important public health challenge in Sindhi community. Preventive genetics program needs to be vigorously taken up to ameliorate the sufferings of at risk communities in India.
Key Words: Public health, Blood groups, Hemoglobin disorders, β-thalassemia syndrome, G6PD deficiency, India

Introduction:

Hereditary hemolytic disorders are the preventable global and public health challenges. Recent estimates suggest that about 7% of the world population is carrier and 300,000–400,000 affected children are born every year in the world.(1) Sporadic cases of hemoglobin D ($\beta^{121Glu\rightarrow Gln}$) also occur in many parts of the Indian subcontinent.(2) Detrimental thalassemias result from genetic defects that cause reduced synthesis of polypeptide chains to form hemoglobin. With a 3-5% prevalence rate of β-thalassemia carriers, the estimated carrier population in India would be 30-50 million.(3) The community control of β-thalassemia syndrome and hemoglobin disorders consists of community awareness generation, screening, prevention, and genetic counseling. The huge burden of hemoglobinopathies and dismal health scenario in India place heavy emotional, social and financial burden on individual, family and the community and contribute significantly to high morbidity and mortality.(4)

The inherited erythrocytic deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is also an important metabolic, genetic and public health challenge in malaria-endemic areas of India.(5) It is a predisposing factor in the causation of drug-induced hemolytic anemia and congenital non-spherocytic hemolytic disease. The deficient fibroblasts in humans suffer growth retardation and premature cellular senescence. Major clinical manifestations are the drug-induced-hemolytic anemia, neonatal jaundice and hyper-bilirubinemia, darkening of urine, and chronic non-spherocytic hemolytic anemia.

The Sindhi community originally emigrated from Sindh region of Pakistan during partition in 1947 and settled in different parts of India mainly in major towns and cities for doing business and trade. Hospital based pathological/hematological studies have revealed a very high incidence of hemolytic disorders such as β-thalassemia, hemoglobin D (Hb D), and double heterozygosity of hemoglobinopathy/β-thalassemia or G6PD deficiency in this highly vulnerable community, which place a huge burden on public health care in India especially for the management/treatment/amelioration. It is highly essential that emphasis must shift from treatment to prevention in future of such births in at risk communities such as Sindhis of Jabalpur town in Madhya Pradesh. This study was designed with the aims to find the prevalence of hemolytic disorders in Sindhi community, identifying the persons for genetic/marriage counseling and to suggest the option of prenatal diagnosis to the affected/vulnerable Sindhi families.

Patients and Methods:

For the present cross-sectional study, out of five different settlements of Sindhi community, namely, Madar Tekri (132), Shanti Nagar (83), Laal mati or Dwarika Nagar (139), Bhant-

alaya (47) and Omti or Bhartipur (107) in Jabalpur Town of Madhya Pradesh, 508 blood samples were randomly collected for screening of hemoglobin disorders, G6PD deficiency and ABO and Rhesus (D) blood groups in Central India. A majority of these individuals had migrated from Sindh state in Pakistan to Jabalpur and are living here since over 60 years. Both the sexes irrespective of their age and morbidity pattern were included in the study, making the sample representative of the community. Sindhi community is an inbred population in India. Because of the practice of consanguinity resulting in inbreeding, the homozygosity of recessively inherited characters was apparent in the community.

About 2-3 ml. intravenous blood samples were collected using ethylene diamine tetra acetic acid (EDTA) as anticoagulant by disposable syringes and needles from each individual after obtaining the informed/written consent in the presence of community leaders. A doctor recorded all the signs and symptoms related to hemoglobinopathies after clinical examination on the pre-designed proforma. Any other ailment present was treated/referred to local health facilities. Blood samples so collected were transported to laboratory at Regional Medical Research Centre for Tribals, Jabalpur under ice-cold conditions within 24 h of collection. Laboratory investigations were carried out following the standard procedures after cross checking for quality control from time to time. Hematological parameters were studied by using an automated Blood Cell Counter (Model: MS_9, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France).

The sickling test was performed on red cells by using freshly prepared 2% sodium metabisulphite solution as reducing agent.(6) The routine hemoglobin electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-Borate buffer at pH 8.6 and quantification of A_2 fraction of hemoglobin by elution method (Weatherall 1983). The value more than 3.5% of hemoglobin was taken as cut off point for determining the ß-thalassemia trait. Estimation of fetal hemoglobin was done as described by Weatherall.(7) Confirmation for the presence of Hb D was done as described elsewhere.(8) Family studies were carried out to confirm the diagnosis, wherever it was felt necessary. However, the data presented here refer to probands only.

The G6PD enzyme deficiency was primarily detected by using Dichlorophenol Indophenol (DCIP) dye as described by Bernstein.(9) Females heterozygous for G6PD deficiency have two populations of cells, one with normal G6PD activity and the other deficient. This is the result of inactivation of one of the two X chromosomes (Lyon's hypothesis) in individual cells early in the development of the embryo. All progeny (somatic) cells in females will have the characteristics of only the active X chromosome. The total G6PD activity of blood in female will depend on the proportion of normal to deficient cells. In most cases, the activity will be between 20 and 80% of the normal. However, a few heterozygotes (about 1%) may have almost only normal or almost only G6PD deficient cells. The present study has not at all encountered any such ambiguity; therefore, there were either 60-80% of the cells normal or deficient in all cases. Subsequent confirmation was done by following the Beutler et al.,(10) and WHO procedures (11) in case any doubt arose for the detection of G6PD deficiency.

The typing of ABO and Rhesus (D) blood groups was done as per the instructions of the manufacturer (Tulip Diagnostics Private Limited, Panaji, Goa) in India.

Results:

Table 1 presents the distribution of hemolytic disorders studied in Sindhi community from different localities. It was apparent that the frequency of hemoglobin disorders (Hb D trait and β-thalassemia trait) varied from 18.6% to 26.5%, with an average being 22.9% in Sindhi community of Jabalpur town. The frequency of β-thalassemia trait was high in all the localities, ranging between 17.7-31.9%, with an overall frequency of 20.5%. A case of β-thalassemia major and Hb D-thalassemia were also encountered (not shown in table). Double heterozygosity of Hb D/β-thalassemia showed hypochromic and microcytic red cell morphology with mild anemia. However, no case of sickle cell or any other hemoglobinopathies was recorded.

Table 1. Distribution of hemolytic disorders in Sindhi community of Jabalpur Town of Madhya Pradesh, India.

Diagnostic Categories	Sex	Madar Tekri N=132 No. (%)	Shanti Nagar N=83 No. (%)	Laalmati N=139 No. (%)	Bhan Talaya N=47 No. (%)	Omti N=107 No. (%)	Total N=508 No. (%)
Normal (HbAA)	Male	49 (37.1)	24 (28.9)	50 (36.0)	16 (34.0)	42 (39.2)	181 (35.6)
	Female	57 (43.2)	37 (44.6)	57 (41.0)	15 (31.9)	45 (42.1)	211 (41.5)
	Total	106 (80.3)	61 (73.5)	107 (77.0)	31 (65.9)	87 (81.3)	392 (77.1)
Hemoglobin D (HbAD)	Male	1 (0.8)	0 (0.0)	4 (2.9)	0 (0.0)	0 (0.0)	5 (1.0)
	Female	1 (0.8)	0 (0.0)	5 (3.6)	0 (0.0)	1 (0.9)	7 (1.4)
	Total	2 (1.5)	0 (0.0)	9 (6.5)	0 (0.0)	1 (0.9)	12 (2.4)
β-Thalassemia Trait	Male	10 (7.6)	9 (10.8)	13 (9.3)	8 (17.0)	10 (9.3)	50 (9.8)
	Female	14 (10.6)	13 (15.7)	11 (7.9)	7 (14.9)	9 (8.4)	54 (10.6)
	Total	24 (18.2)	22 (26.5)	24 (17.2)	15 (31.9)	19 (17.7)	104 (20.5)
G6PD Deficiency	Male	3 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.6)
	Female	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	1 (0.2)
	Total	3 (2.3)	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	4 (0.8)
Rhesus (D) blood group (-ve)	Both Sexes	7 (5.3)	1 (1.2)	0 (0.0)	2 (4.2)	5 (4.7)	15 (3.0)
ABO Blood Groups:							
A	Both Sexes	14 (10.6)	13 (15.7)	9 (6.5)	3 (6.4)	13 (12.2)	52 (10.2)
B		67 (50.8)	32 (38.6)	71 (51.0)	26 (55.4)	44 (41.1)	240 (47.2)
AB		15 (11.3)	4 (4.8)	12 (8.6)	2 (4.2)	16 (14.9)	49 (9.6)
O		36 (27.3)	34 (41.0)	47 (33.8)	16 (34.0)	34 (31.8)	167 (32.9)

The G6PD deficiency was detected in two localities with a frequency range of 2.1-2.3%, the average being 0.8% in the Sindhi community.

The frequency of Rhesus negative blood group was high in three localities (Table 1), with an average of 3.0%. B blood group predominated over other blood groups in all the localities of Sindhi community except one (Shanti Nagar), where O blood group was the highest (Table 1). The frequency of O blood group varied between 27.3-41.0%. On the whole, the highest frequency of B blood group (47.2%) was recorded, followed by O (32.9%), A (10.2%), and AB (9.6%) blood groups in Sindhi community.

Discussion:

The most salient finding emerges out from the present cross-sectional community study of the Sindhi community is the highest frequency of β-thalassemia trait (20.5%) in Jabalpur town of Madhya Pradesh. High frequencies of β-thalassemia trait in Sindhi community were reported based on hospital studies/referral services by many earlier investigators as high as 12.2% (12), 5.6% (13), and 7.7% (14) from different parts of India. A screening study done among 446 Sindhi individuals belonging to the age group between 10 years and above of Nagpur city in Maharashtra state showed the frequency of β-thalassemia trait to be 16.8% (15), which can not be considered as the representative of Sindhi community due to bias in age and selection of samples. Hence, the study does not provide a fair estimate of prevalence of the trait. Similarly, Jawahirani et al.(16) showed extremely variable frequency of β-thalassemia trait based on the heterogeneity of Sindhi community as well as of territorial endogamy in subcaste of Larkhana Sindhi (17.0%) and Dadu Sindhi (8.0%). Both these studies lacked the proper sampling procedure for finding the exact prevalence of β-thalassemia in the Sindhi community. However, the present cross-sectional study was carefully designed after overcoming the above cited lacunae and is authentic one.

It was seen that the Sindhi community of Jabalpur town showed a comparatively higher prevalence of heterozygous Hb D disease (2.2%) and Hb D/β-thalassemia (0.2%) in the population. However, Kate et al.(17) have shown the overall incidence of 4.62% for Hb D (three persons from two families had homozygous Hb D) in a study carried out on Sindhi population (238 screened) in and around Pune city, Maharashtra state. In the present study, out of 508 individuals screened, 11 were heterozygous for Hb D disease. One case of double heterozygosity of Hb D/β-thalassemia was also encountered. None of these individuals had any evidence of severe hemolytic anemia. They were in general asymptomatic and healthy individuals.

In India, the allele frequency of Hb D is relatively low (1%) with a tendency to cluster in the Northwestern part of the country.(8,18) Although, geographic spread shows regional variations of Hb D distribution with about 3% prevalence in Northwestern India especially in undivided greater Punjab, 2% in Uttar Pradesh and about 1% in Gujarat and Maharashtra states of India.(8,19) The prevalence of Hb D in India has been documented with variable allele frequency from Punjab, Gujarat, Jammu & Kashmiir, Uttar Pradesh, Maharashtra, Karnataka, Orissa, West Bengal, Assam, and Goa in homozygous, heterozygous state, concurrence with β-thalassemia or sickle cell/Hb E hemoglobinopathies.(2,8)

Although the frequency of G6PD deficiency is low (0.8%) in the present study yet it can cause severe hemolytic anemia/complications with concurrent occurrence of β-thalassemia or other common hemoglobinopathies prevalent in this part of the country.(20) G6PD deficiency is the most common defect in hexose monophosphate (HMP) shunt pathway resulting in oxidative damage to red blood cell (RBC) membrane and causes hemolysis. The combined effect of HMP shunt is to metabolize glutathione (GSH) responsible for protecting intracellular proteins from oxidative stress. In the absence of reduced glutathione (GSH), oxidative stress can lead to hemolysis of erythrocytes resulting in hemolytic anemia. A host of agents like antibiotics, antimalarials, analgesics; various infections, broad (flat) fava beans (vicia faba) and acute illnesses are associated with hemolysis in G6PD deficiency. The interaction of oxygen with heme in the presence of these offending agents results in production of oxidants (toxic products) and, if not effectively neutralized due to deficiency of G6PD enzyme result in cellular damage (death of RBCs) leading to hemolytic anemia.

Low frequency of Rhesus negative blood group in Sindhi community does not pose any major hemolytic threat in causing the newborns disease of erythroblastosis of fetalis. Moreover, the technology has now advanced enough to tackle this problem in the pregnant women.

Table 2. Distribution of hemolytic disorders in three generations in Sindhi community of Jabalpur Town, Madhya Pradesh

Generation	Sex	β-Thalassemia Trait	Hemoglobin D	G6PD Deficiency	Rhesus Negative
		N=104	N=12	N=4	N=15
		No. (%)	No. (%)	No. (%)	No. (%)
New (1-25 years)	Male	23 (22.1)	4 (33.3)	2 (50.0)	
	Female	18 (17.3)	5 (41.7)	0 (0.0)	
	Total	41 (39.4)	9 (75.0)	2 (50.0)	5 (33.4)
Middle (26-50 years)	Male	28 (26.9)	1 (8.3)	1 (25.0)	
	Female	31 (29.8)	2 (16.7)	0 (0.0)	
	Total	59 (56.7)	3 (25.0)	1 (25.0)	8 (53.3)
Old (51+ years)	Male	2 (1.9)	0 (0.0)	0 (0.0)	
	Female	2 (1.9)	0 (0.0)	1 (25.0)	
	Total	4 (3.8)	0 (0.0)	1 (25.0)	2 (13.3)

In order to know, whether the cases with hemolytic genetic disorders are increasing or decreasing with respect to generation gap at population level in the Sindhi community of Jabalpur town in Madhya Pradesh, all the cases detected positive for the hemolytic disorders (β-thalassemia trait, hemoglobin D trait, G6PD deficiency, and Rhesus negative) in the Sindhi community were classified into three groups based on the age of persons (Table 2), comprising the new (3rd) generation (age 1-25 years), followed by middle (2nd) generation (age 26-50 years), and old (1st) generation (age 51 years and above), irrespective of the reproductive wastage and mortality in each generation. It was assumed that normally at the age of 25 years each human being becomes mature enough to reproduce actively. It was noted that the 3rd generation (age 1-25 years) had the higher number of cases of hemolytic disorders, followed by 2nd generation (age 26-50 years), and 1st

generation (age 51 years and above), respectively (Table 2), showing that there is no impact of awareness generation, health education, and genetic/marriage counseling program on the reproductive behaviour of the Sindhi community. Such preventive genetics programs including imparting of genetic/marriage counseling to affected persons/couples need to be vigorously taken up at grass root level to ameliorate the sufferings of affected or at risk communities like Sindhi community of Jabalpur town of Madhya Pradesh in Central India.

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