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HEMOGLOBINOPATHIES IN VADODARA: A HOSPITAL-BASED STUDY

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Abstract
The inherited disorders of hemoglobin are responsible for an extremely complex series of clinical phenotypes. Sickle cell anemia and thalassemia can cause chronic ill-health and life-threatening situations. Present study was carried out at pipaiya, Vadodara, Gujarat state. The practice of consanguineous marriages is an accepted sociocultural Phenomenon in this region. This study was hospital-based and the pediatric cases of hemoglobinopathies were identified based on clinical data, family history, red blood cell indices and hemoglobin in electrophoresis. Sickle cell disorder has remained a neglected field of research in our country. The present study was started in rural population of Gujarat to find out the magnitude of sickle cell disorders. 46 subjects were studied. . Through this study, we created awareness among people about the disorders. The villagers were explained about its' hereditary nature and that no curative treatment is available, although it can be prevented through marriage counseling. It is necessary to continue with such activities since prevention appears to be the only solution in present circumstances.
Purpose & priorities goal: No Child birth with Sickle Disease by 2020, Prevention of death from Sickle Cell Crisis, To improve health status and quality of life of Sickle Cell Anemia patients. Hemoglobinopathies pose economical and psychosocial burden Extensive screening for hemoglobinopathies is very much essential, Socio-cultural aspects of a region need to be explored to evaluate their Interaction with genetic factors.

Key Words: Abnormal Hemoglobins, Compound Heterozygote, Family Studies, Hemoglobinopathies, Hemoglobin Electrophoresis, Pedigree, Red Cell Indices, Sicklecell trait, Thalassemias.

Introduction

The inherited disorders of hemoglobin are the commonest single-gene disorders in man. They fall into three overlapping groups : structural variants; thalassemias characterized by reduced rate of synthesis of one or more globin chains; and conditions in which fetal hemoglobin synthesis persists beyond the neonatal period, collectively known as hereditary persistence of fetal hemoglobin. Hemoglobin disorders are responsible for an extremely complex series of clinical phenotypes [1].

The World Health Organization (WHO) has suggested that about 5% of the world population are carriers for different inherited disorders of hemoglobin [2]. WHO reports also state that about 370,000 severely affected homozygotes or compound heterozygotes of thalassemia are born every year. The UNICEF in 1996 estimated that there were 29.7 million carriers of beta thalassemia trait in India and about 10,000 infants with homozygous beta thalassemia born every year [3]. The general incidence of thalassemia trait and sickle cell hemoglobinopathies in India.

varies between 3-17% and 1-44% respectively [4],[5],[6]. It is estimated that there are about 65,000-67,000 beta-thalassemia patients in India witharound 9,000-10,000 cases being added everyyear. The carrier rate for beta-thalassemia gene varies from 1 to 3% in Southern India to 3 to 15%in Northern India[7],[8],[9]. In developing countries, in which there is highMortality from infections and malnutrition in the first year of life, many of the hemoglobinopathies are unrecognized. Sickle cell anaemia and thalassemia major can cause life-threatening situation and chronic ill health. They pose economical and psychological burden on the affected individual and his/herfamily, and the society as a whole. Hence, the population needs to be screened for hemoglobindisorders so that appropriate measures for treatment and prevention can be taken. The present study was a pilot study carried out in DHIRAJ HOSPTIAL 1226 bedded and S.B.K.S MEDICAL COLLEGE AND RI , situated in pipariya rural area of Vadodara. To the best

of our knowledge, it is the first of its kind reported from central Gujarat. This region has unique socio-cultural practices among caste chavada, rathod, solanki and vasava. The practice of consanguineous marriages preferably with Maternal kindred's, is an accepted socio-cultural phenomenon respective of religion, caste and economic status. The study aimed to identify the children with hemoglobin disorders, using the available hematological and biochemical tests. We also attempted to make a detailed family study of thalassemia patients,among castes chavada,rathod, solanki and vasava to evaluate the inheritance patterns and to identify thalassemia carriers.

Materials and Methods

Source of Data:

1. Children with anaemia, generalized weakness, fever and splenomegaly, visiting the Paediatrics O.P.D. of the Dhiraj hospital; 2. Childern with severe hemolytic anaemia, hepatosplenomegaly and history of blood transfusion, and admitted to the Paediatrics ward of the hospital. Above children were aged between 3 months to 18 years (n =46), and were referred to the Departments of Biochemistry and Pathology for laboratory investigations of hemoglobinopathies.Our research protocol was approved by the Ethical Committee of the institute of sumandeep vidypeeth university. Voluntary consent was obtained from the subjects of the study. Detailed family history was collected from the patients / guardians regarding previous history of hemolytic anaemia and blood transfusions, any medications taken, consanguinity in marriages, and clinical signs and symptoms.Reproductive history of the parents with regard to abortions, death of any child, still birth was collected.
2. Laboratory investigations: 5 ml. of blood was collected in vacutainer tubes having EDTA asanticoagulant. a) Hematological parameters: Hb, PCV, MCV, MCH, MCHC, RBC count andWBC count was measured using Sysmex cell counter [10]. Peripheral smear was evaluated for features of red cell morphology [10] b) NESTROFT (Naked Eye single tube red cell osmotic fragility test) was performed to screen the cases of beta-thalassemia trait [9,10]. c) Hb electrophoresis : The red cell hemolysate was diluted to have Hb concentration of 10µg/10µl.

   Electrophoresis was carried out at alkaline pH of 8.6, on cellulose acetate strips, at 450V for 40 minutesthe strips were stained in Ponceau S. we had fully automated electrophoresis Genio-s interlab

   Interpretation of the migration pattern of the test samples was undertaken by comparing them to those of known controls obtained from Genio interlabBioscienecs,Italy fully automated electrophoresis provided with interpreting softare attached desktop.. Location of HbF was confirmed by performing electrophoresis with cord blood [10], [11].

   d) Estimation of HbA2 : After the run of electrophoresis, the HbA2 and HbA bands were eluted from the cellulose strip and their absorbances were read at 415 nm,using which HbA2 % was calculated [10].

   e) Estimation of HbF : HbF% was measured by alkali denaturation method of Betke[10,11].f) Test for unstable hemoglobins : Stability of Hb in isopropanol was tested [11] .

   g) Test for HbS : Solubility of deoxygenated Hb in saponin-phosphate buffer was tested and then HbS% was calculated [10],[11].

   FAMILY STUDIES: Family studies of four patients with hemoglobinopathies were done. Out of them, two were cases of beta thalassemia major,one of beta thalassemia trait, and one case of a compound heterozygote of HbS/beta thalassemia .The blood samples of available members of the family tree were collected and subjected to hematological and biochemical investigations of hemoglobinopathies.

   ANALYSIS OF DATA: Diagnosis of hemoglobinopathies was made based on the hematologic parameters, laboratory tests and clinical findings, as mentioned in standard literature [4, 10, 11] . The values of hematologic parameters were expressed as mean. Pedigree diagrams were drawn based on the family studies [12].

Results

The results of the present study are children aged between 3 months and 18 years, were the subjects of our study. They were suspected cases of hemoglobinopathies, and referred for laboratory investigations of hemoglobinopathies. Therewere 2 children with beta thalassemia major, 2 with beta thalassemia trait one case of a compound heterozygote of HbS/beta thalassemia trait. Out of the fourty six children, twenty three were males and twenty three were females. Out of the four cases of thalassemia major, three (75%) were males and only three (25%) were females. We found one case thalsemia trait were female.

Thirteen children showed normal patterns of hemoglobin electrophoresis .In five cases, the laboratory and clinical data were insufficient to confirm the diagnosis. Beta-thalasemia trait was characterized by Hb of 8.4 g%, MCV of 53.1fl, MCH of 17.7pg, HbA2 value of 5%, and positive NESTROFT. In beta-thalassemia major cases, Hb was 5.5g%, MCV was 62fl, MCH was 18pg, HbA2 was 3%, and HbF was 55%. Beta-thalassemia major cases were also characterized by severe microcytic hypochromicanaemia, anisocytosis, poikilocytosis and high percentage of target cells, as demonstrated byperipheral smear study. Sickle cell trait cases showed positive solubility test and HbS value of 25%.

In compound heterozygote of HbS/betathalasemia, HbS was 60% and this case was

confirmed by electrophoresis, HbF and HbA2 estimations. Characteristic electrophoretic

patterns were observed in all the confirmed cases of hemoglobinopathies. As per the family history collected, 10 out of the 46 children of our study were the products of consanguineous marriages. Out of these, one were

beta thalassemia major-children, one were beta thalassemia carriers and one was a compound heterozygote of HbS/beta thalassmia, and one was an unconfirmed case of hemoglobinopathy.. two thalassemic children had history of blood transfusions.
We noticed sickle cell trait predominantly prevails in castes chavada, Rathod, solanki and vasava. out of 46 childrens study 63% were sickle cell trait belongs to rathod and 25% were sickle cell trait belongs to vasava. We also noticed sickle cell trait was not found in chauhan,Gohel, joshi,parmar,patel,prajapati,patel communities in central Gujarat .

Discussion

Hemoglobinopathies are of world-wide occurrence, though some geographical areas have high prevalence of these disorders. In India, average frequency of sickle cell gene is around 5%. The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat (6.4%) [8-10]. The distribution of beta thalassemia is not uniform in Indian subcontinent.The highest frequency of beta thalassemia trait is reported in Gujarat (10-15%), followed by Sindhi (10%), Punjab (6.5%), Tamil Nadu (8.4%) and Maharashtra [4], [5], [6], [13], [14], [15], [16], [17].
In India, the problem of hemoglobinopathies is compounded by the heterogeneity of population.
The different regions of India have different gene frequencies for the various hemoglobinopathies.
The fertility rates, the literacy rates, and the rates of consanguineous marriages are also diverse.
Central part of Gujarat comprising the district of Vadodara, near places Tilkwada Naswada, Bodeli, Waghodia , Anand , has unique socio-cultural practices. Consanguinity in marriages is a well-accepted social norm irrespective of religion, caste, educational status and economical back ground. Our's was a pilot study carried out in pipariya ,vadodara and it was hospital-based. For the family studies of four cases, we visited the houses of the patients. Diagnosis of hemoglobinopathies was made using the criteria mentioned in standard literature. 8.6% of the children (subjects of the study) were victims of beta thalassemia, 2.5 % were carriers of beta thalassemia, 39.1% (2) were cases of sickle cell trait, and one was a compound heterozygote of HbS/beta thalassemia. 23.9% were cases of sickle cell disease . 28.26% were normal healthy subjects In five cases, the diagnosis could not be confirmed as the available clinical and laboratory data were insufficient [Table/Fig 2]. These could be the cases of alpha-thalassemia or fusion chains such as delta-beta thalassemia, this conclusion being based on hematologic findings given in previous literature [1],[4] however, diagnosis needs to be confirmed with genetic tests and globin chain analysis.Few reports from India are available regarding the prevalence of compound heterozygotes of thalassemia [18],[19],[20] .
According to Garewal and Das [21], most patients with beta thalassemia are compound heterozygote's having inherited two different mutations, one from each parent. Consanguinity is known to play an important role in hereditary diseases, particularly in autosomal recessive traits. 20% (10 out of 46) of the cases in our study were offspring's of consanguineous marriages. Five children were offspring's of third-degree consanguineous mating (marriage between uncle and niece, which is very common in central Gujarat), and five children were offspring's of fourth degree consanguineous mating (marriage between first cousins) [22]. Since closely related individuals have a higher chance of carrying the same alleles than less closely related individuals, the children from consanguineous marriages are more frequently homozygous for various alleles than are children from non-consanguineous marriages [22]. Due to the strict practice of caste endogamy and consanguinity in marriages, association of thalassemia with structural variants of Hb such as E, C and S, is highly prevalent in India [20].
We made an attempt to make a detailed study of thalassemia carriers, inheritance patterns of hemoglobinopathies and further with an aim to help in genetic counseling. We could visit only four families of thalassemia patients due to non-availability of family members during the course of the study, migration of some of the families, hesitation of the socio-economically backward families to participate in the study, and certain misconceptions of illiterate people regarding blood disorders and marriages.

Conclusions

The population of vadodara appears to be a repository of thalassemia. We plan to undertake extensive screening of the population for hemoglobin disorders, with further aim of carrying out regular new born screening and prenatal diagnosis of hemoglobinopathies, which would go a long way in helping in genetic counseling. The unique socio-cultural practices of west Gujarat need to be explored for their interaction with genetic factors and their role in human health.

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