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Family Study a tool to diagnose variant haemoglobinopathies and ambivalent cases.

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Abstract : *The Objective of this study is to diagnose variant haemoglobinopathies and ambivalent cases with the help of family studies in tertiary care hospital where genetic testing is not available.*
Methods. *Patient attending paediatric and ANC clinic were screened by doing Complete blood count and then screening was done on anaemic patient by doing nestroft and solubility test .Positive sample from these patient were taken for CE-HPLC. Graph of HPLC obtained and , abnormal haemoglobinopathies were provisionally diagnosed and confirmed by doing family study of such index cases..**Results:-** Family studies were done in 94 cases, and involved parents or first degree blood relatives like siblings, and conclusive diagnosis could be obtained in 86 cases. We got conclusive result just by doing family study in 91.5 percent cases.*
Conclusion. *Family study In centers, which do not have the facility for genetic analysis, family studies by HPLC can be equally useful.*
Keywords: *Family Study, HPLC and Variant Haemoglobinopathies.*

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I. Introduction

Haemoglobinopathies, which include thalassaemia and sickle-cell disease, are major public health problems especially in the Mediterranean area, Middle East, Indian subcontinent, Far East and tropical Africa.

It has been estimated that about 250 million people are heterozygous for these disorders and at least 300 000 lethally affected homozygotes are born annually throughout the world.[1] In India the presence of sickle cell gene (HbS) was first detected in Nilgiri hills of southern part.[2] In India HbS gene is mostly confined to tribes in central and south India and the frequency ranges from 5 to 35 %.11 In central India study on sickle cell anaemia has been carried out mostly on tribal groups and very few on castes and other population.[3] Sickle cell gene is widely spread in all districts of eastern Maharashtra i.e. Vidharbha, north Maharashtra i.e. Satpuda ranges, nandurbar and some parts of marathwada region.[4] Automated cation-exchange High Performance Liquid Chromatography (HPLC) has emerged as an excellent screening tool for diagnosing these abnormal haemoglobins/ thalassaemic states.[5]

Haemoglobin fraction analysis by cation-exchange HPLC has the advantage of quantifying HbF and HbA2 along with haemoglobin variant. Screening in a single, highly reproducible system, making it an excellent technology to screen for haemoglobin variants and haemoglobinopathies.[6]

II. Material and Methods

This is a study of screening for haemoglobinopathies in Paediatric patients by High Performance Liquid Chromatography (HPLC).The study was carried out in Department of Pathology of a tertiary care hospital, after the approval of the college ethical committee.Study population: Parents and siblings of index cases from paediatric opd, All pregnant women who came for ANC clinic and those who came voluntarilyfor testing. Exclusion criteria.1) Diagnosed cases of β-thalassaemia major, SCD or homozygous for other haemoglobinopathies.2) Neonatal patients, are to be deffered till age of 6 months.3)Patients received blood transfusion recently (with in 3 months)Study design: The present study is a prospective, cross-sectional, hospital based, descriptive study.Study period: Conducted from January 2013 to June 2014. Patients fulfilling the above mentioned inclusion and exclusion criterias are included in study as cases, after that detailed history was taken and complete clinical examination was done.

After consent from parents, 4 ml of venous blood was withdrawn from patients. 2 ml blood was collected in 4% K2 EDTA (Ethylene diamine tetra-acetic acid) anticoagulant bulb and from remaining 2 ml, serum was separated for further processing. All the Patients attending OPD, who were anaemic and/or presented with hepatosplenomegaly and showed sickling test and/or fetal fraction positive were evaluated further. Clinical findings were correlated with all other investigations. Radiological investigations like USG abdomen, X-ray chest and X-ray hip joints were done and other specific investigations as and when required. Hematological

profile of cases was done, which included PS, CBC including RBC Indices, reticulocyte count and if required, bone marrow.Beta Thalassaemia short program was used in the study. Reagents, wash solution and mini cartridges, were from Bio-Rad. With the help of CE-HPLC, exact percentage of HbS, HbF, HbA2 and HbA0 was estimated to classify the cases. Then study of family members accompanying the cases was done to confirm the diagnosis and to determine ethnic background.

III. Observation and Results

Of the 300 cases blood samples analyzed on CE-HPLC for haemoglobinopathies, 142 (43.33 %) were normal haemoglobin and 158 (52.67 %) were variant haemoglobins. Of the 158 cases, 67 (22.33%) were diagnosed as S-β double heterozygous, 39(13%) as sickle cell trait, 27 (9 %) as β-thalassaemia major, 10 (3.33 %) as sickle cell disease, 05 (1.66 %) were diagnosed as β-thalassaemia trait, 03 (1%) were unknown haemoglobins , 02 (0.66 %) as HbE-β-thalassaemia double heterozygous, 02 (0.66 %) as SCT + Hb D trait and 01 (0.3%) as Hb E trait. Of the 158 paediatric cases with variant haemoglobins, 59 (37.34 %) parents have history of consanguineous marriage. Of the 300, cases screened; we detected 27 cases of β-thalassaemia major, 10 cases of sickle cell anaemia,01 case of β thalassaemia intermedia , 67 cases of S β-thalassaemia, 02 cases of E β-thalassaemia,02 case of compound heterozygous sichle cell trait with hemoglobin D trait(HbSD) and lastly 03 cases of unknown haemoglobins, these cases warranted family studies.

Family studies were done in 94 cases, and involved parents or first degree blood relatives like siblings, and conclusive diagnosis could be obtained in 86 cases.

Table no 1 shows a case in which parent were asymptomatic and the children required frequent blood transfusion with HbA0 9.7 %, HbA2 30.2% and HbF61.2%. The diagnosis of E beta thalassaemia was made after doing family study and counselling of parents were done , adviced about antenatal diagnosis of fetus in future pregnancy.The index case along with his brother registered to our blood bank and both were immunized and followed up regularly by social worker of our hospital.

Table no 2 shows In this case we found the index case showing all the features of thalassaemia major, need frequent blood transfusion. On HPLC the case shows sickle cell peak along with HbD Punjab peak, Family study was done and asymptomatic Father was found HbD Punjab haemoglobin carrier and Asymptomatic mother was found Sickle cell trait.Also sister showed both the peak. It was found and confirmed that our index case suffered from compound heterozygous Sickle cell trait with Haemoglobin D Punjab (SCT + HbD).This is a very rare compound heterozygous state of haemoglobinopathies, easily diagnosed just because of family study. .

Table no 3 This is case of an anxious couple whose first child suffered Thalassaemia major.

The couple was advised Prenatal Diagnostic Test and the case was referred to an Immunohaematology centre.The Chorionic Villus Tissue DNA Analysis was performed at 11 weeks 5 days . Unfortumately It suggested an β -Thalassaemia Major in the fetus. Family HPLC screening helps to identify such Thalassaemia carrier couples and such couples can be offered Prenatal Diagnostic Test, to avoid birth of an affected child in future pregnancies.

IV. Figures And Tables

Table no. 1 – Family study in case 1

	HbA0	HbA2	HbF	Presentation	Diagnosis
Index case	9.7	30.2	61.2	Severe anaemia	E-beta thalassaemia
Father	63.9	24.8	1.1	Asymptomatic	Hb E trait
Mother	82.1	6.5	1.8	Asymptomatic	Beta thalassaemia trait
Brother	29.1	28.8	40.6	Severe anaemia	E-beta thalassaemia

Table no. 2 – Family study in case 2

	HbA0	HbA2	HbF	HbS	HbD	Presentation	Diagnosis
Index case	1.1	1.8	10.5	42.4	43.8	Severe anaemia	SCT + HbD trait
Father	51.6	1.6	0	-	39.2	Asymptomatic	HbD trait
Mother	52.2	3.5	0.8	37.2	0	Asymptomatic	Sickel cell trait
Sister	1.2	1.8	10.5	42.6	43.8	Severe anaemia	SCT + HbD trait

Table no. 3 - Family study in case 3

	HbA0	HbA2	HbF	Diagnosis
Index case	**5.6**	**2.9**	**99.3**	**Thalassaemia Major**
Father	**85.5**	**5**	**0.6**	**Thalassaemia Trait**
Mother	**85.5**	**5.1**	**0.7**	**Thalassaemia Trait**

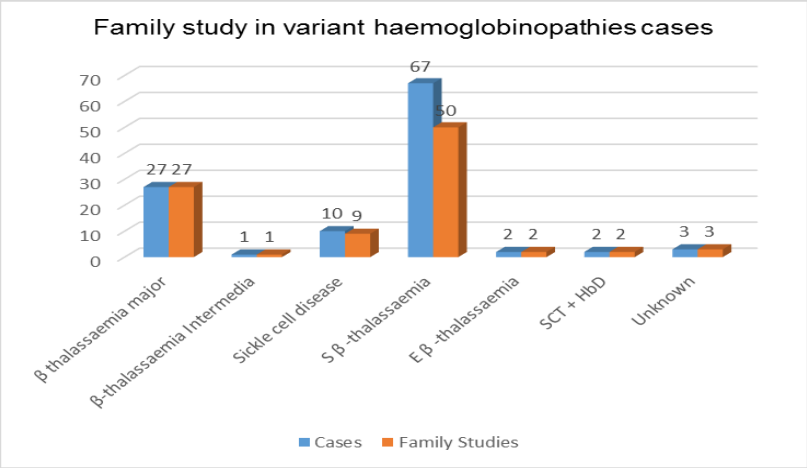
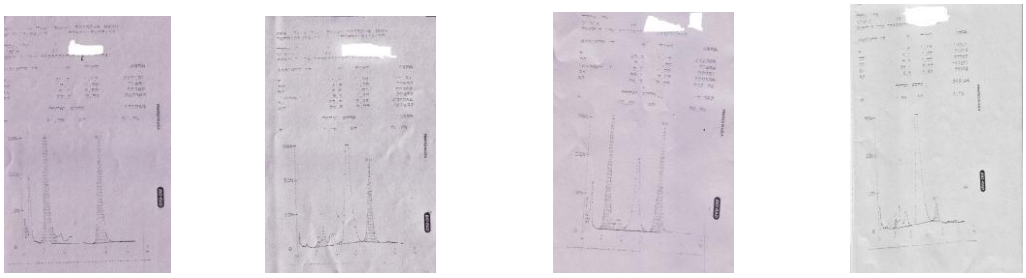


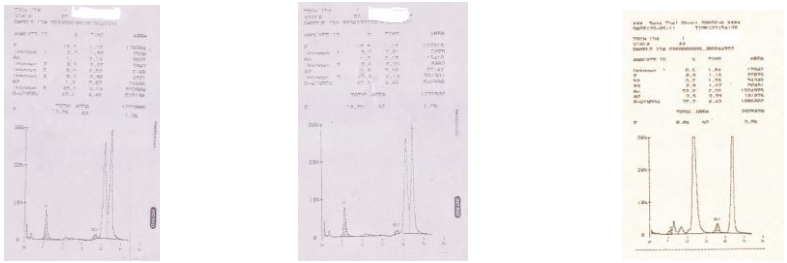
Figure 1:-Family Study in variant haemoglobinopathies



(A) (B) (C) (D)

Fig 2. HPLC chart of case 1

(A) Index case, (B)Brother, (C) Father and (D) Mother



(A) (B) (C)

Fig 3 HPLC chart of case 2

(A) Index case, (B)Sister, (C) Mother

V. Conclusion

4. Family studies and Counselling

CE-HPLC has emerged as a gold standard tool for the screening of haemoglobinopathies, but some cases remain undefined for the want of a diagnosis when HPLC results are ambivalent. The identity of hemoglobin variant is generally inferred from its electrophoretic mobility, its quantity, and the patient's ethnic

background. Family studies can be of considerable importance in elucidating the nature of disorders of hemoglobin synthesis, but definite identification can be achieved only by DNA analysis or amino acid sequencing.[6]

Of the 300, cases screened; we detected 27 cases of β-thalassaemia major children ,the parents of all 100% children were tested and all the parents were diagnosed as thalassaemia trait (carrier) on HPLC .These parents were specially counselled about chances of having various spectrum of thalassaemia gene effect in there next child , regarding prenatal diagnostic tests ,regarding how to take care of their thalassaemia major (sufferer) child, like immunization ,safe packed cell transfusion, chelation therapy e.t.c .Out of these 27 parents, all were adviced and counselled regarding prenatal diagnoses in next pregnancy. Out of 10 cases of sickle cell Disease and 67 cases of S β-thalassaemia ,09 (90%) and 50 (74.62%) family were studied and counselled regarding self care , hygiene , early identification of symptom, immunization ,blood transfusion services , prenatal diagnosis , premarital testing of spouse , about sickle cell disease control program in Maharashtra. Shah Sejal J et al (Vadodara in 2012) [7]-Studied 35 cases out of which Beta Thalassemia Major 16(40.0%) ,Thalassemia Intermedia 02 (5.7%) , Homozygous sickle cell disease 07 (20.0%) ,Sickle cell trait 04 (11.4%) ,Sickle cell Beta thalassemia 04 (11.4%) and HBE - Beta thalassemia 02 (5.7%).Dr. Mauchumi Saikia Pathak et al (Assam-2014) [8]-Abnormal haemoglobin fractions on HPLC were seen in 522 cases (65.25%) out of the 800 cases displayed. Hb E heterozygous is the most common form of Hb Variants (23.5%), followed by β thalassaemia trait (18.12%), Compound Hb E - β thalassaemia trait(9%),HbEhomozygous (6.5%), Hb Strait (3.25%), β thalassaemia major (2.13%), Hb S disease (2%), α -thalassaemia (0.63%) and Compound Hb S - β thalassaemia(0.12%). Some rare haemoglobinopathies were also diagnosed and confirmed by doing family study like 02 cases of E β-thalassaemia,02 case of compound heterozygous sickle cell trait with hemoglobin D trait(HbSD), 01 case of β thalassaemia intermedia and lastly 03 cases of unknown haemoglobins, these cases warranted family studies. Mukherjee M.B. et al in 2005 [9] reported this rare case in 10 year old female.

Family studies were done in 94 cases, and involved parents or first degree blood relatives like siblings, and conclusive diagnosis could be obtained in 86 cases. We got conclusive result just by doing family study in 91.5 percent cases. Study done by Rangan et al in 2009 shows after correctly diagnose index cases by performing HPLC on parental samples was helpful in coming to a meaningful conclusion in all our cases. In 100% of cases, we noted that the diagnosis obtained by family studies was commensurate with that obtained by DNA analysis and was in no way inferior to the latter procedure.[5]

In the Indian scenario, this study might be useful as there is a paucity of funds, and facilities for DNA analysis are not readily available. Thus, in centers, which do not have the facility for genetic analysis, family studies by HPLC can be equally useful. Although DNA analysis and genetic studies are diagnostic, but inaccessibility and prohibitant costs make it an unfeasible option and in such cases family studies using CE-HPLC can help at arriving at a proper diagnosis.

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