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Study of Double Heterozygous Cases by Using Cation Exchange High Performance Liquid Chromatography in Nanded Region of Maharashtra

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ABSTRACT:*The double heterozygous hemoglobinopathy cases includes both the qualitative and/or quantitative disorders of haemoglobin present in an individual inherited from both the parents. The prospective study was carried out in Department of Pathology,DrShankarraoChavan Government Medical College,Nanded during the period of Jan-2013 to Dec-2013. We screened patients attending OPD and IPD at medical college, RH and PHC with anemia, positive screening tests and Hb-electrophoresis for hemoglobinopathies. The family members of positive HPLC samples for heterozygosity were also screened. In our study total 117 double heterozygous cases of Sβ thalassemia were found.The average age of presentation was found to be 13.4 years. The history of consanguineous marriage was found in 65 cases (55.55%). The most commonly Sβ thalassemia affected ethnic group is Buddha–SC i.e 29 cases (24.78%) followed by Muslims i.e 25 cases (21.37%) may be due to consanguinity which is commonly practiced in SC and Muslim communities.The early detection of double heterozygous cases is important so as to reduce the rise in new cases which can be done by proper counselling and avoidance of consanguineous marriage.*

KEYWORDS:*Consanguineous,double heterozygous, HPLC, Sβ thalassemia*

I. INTRODUCTION

The study of compound heterozygous hemoglobinopathies are all clinically significant, as these combinations present with different manifestations and degrees of severity, making precise identification important**.** Correct identification of the subtype would be useful in defining the clinical course of the disease and also in genetic counseling. Early institution of supportive care, such as infection prophylaxis, rehydration and avoidance of stress situation in patients, would reduce morbidity and mortality associated with the complications of these haemoglobinopathies.Definitive diagnosis can only be made by family studies or DNA analysis.[1]In our region, with paucity of funds and inaccessibility to facilities like DNA analysis, confirmation of double heterozygosity by family studies is cost effective and an equally efficacious method in early diagnosis and control of the rise in new cases of hemoglobinopathies .[1]

II. MATERIALS AND METHODS

We conducted prospective study from Jan 2013 toDec 2013

2.1 Inclusion criteria:

[1] All the patients of anemia showing sickling test, solubility test and NESTROFT positive.
[2] Patients with clinical suspicion of hemolytic anemia.
[3] Family members of these patients.
[4] Samples received from RH and PHCs which have positive picture of Hemoglobinopathies on Hb Electrophoresis.

2.2 Exclusion criteria:

Patients who received blood transfusion in the last 6 months were excluded from the study.

After screening cases as per the above mentioned criteria, detailed history was taken, complete clinical examination was done and blood samples were obtained in 4% K2 EDTA vacutainers. The haematological tests were performed on Mythic cell counter and HPLC was performed on BIO RAD variant HPLC machine.Clinical aspect and lab investigations were correlated.Positive cases were further called for family screening.

2.3 Analyte Identification Windows:[2]

Analyte Name	Retention time (minutes)	Band (minutes)	Window (minutes)
F	1.15	0.15	1.00-1.30
P2	1.45	0.15	1.30-1.60
P3	1.75	0.15	1.60-1.90
A0	2.60	0.40	2.20-3.30
A2	3.83	0.15	3.68-3.98
D-window	4.05	0.15	3.98-4.12
S-window	4.27	0.15	4.12-4.42
C-window	5.03	0.15	4.88-5.18

P2 and P3 are minor peaks associate with glycosylated HbA.

III. RESULTS

The retrospective study was conducted in Department of Pathology, Dr.S.C.G.M.C.,Nanded during Jan 2013 to Dec 2013.

Incidence

Total 1043 cases of hemoglobinopathies were screened on HPLC out of which 250 cases of sickle disorder were found.

Total number of double heterozygous cases were 117 out of 250 sickle disorder patients which contributed to around 46.8%.

Table1. Age wise distribution:

Age (years)	**Double heterozyous cases n=117**			
	M	F	T	%
0 to 10	20	15	35	29.91
11 to 20	33	20	53	45.30
21 to 30	12	8	20	17.09
31 to 40	3	3	6	5.12
41 to 50	2	1	3	2.58

Maximum number of cases were found in the age group 11-20 years. Average age of presentation of patients with double heterozygosity was 13.4 years.

Tab.2 : History of consanguineous marriage:

	Total n=117	**%**
H/O consanguineous marriage	65	55.55

History of consanguinity was seen in majority of the cases ie.65 (55.55%).

Tab.3: Caste wise distribution of cases in present study:

Caste	**Total N=117**
Buddha (Mahar)	29(24.78%)
Muslim	25 (21.37%)
Banjara	12 (10.25%)
Matang	12 (10.25%)
Chambar	10 (8.55%)
Teli	9 (7.70%)
Kunbi	7(5.99%)
Others	13 (11.11%)

Maximum number of cases were found in Boudhaie. 29 cases (24.78%) followed by muslims 25 (21.37%).

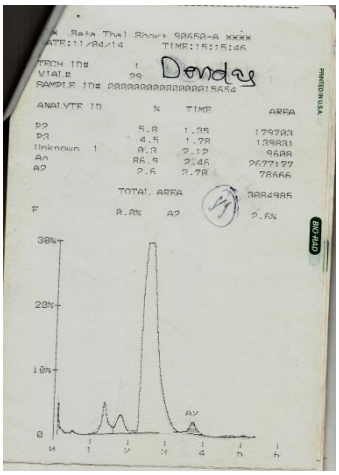

Fig.1: Normal HPLC report

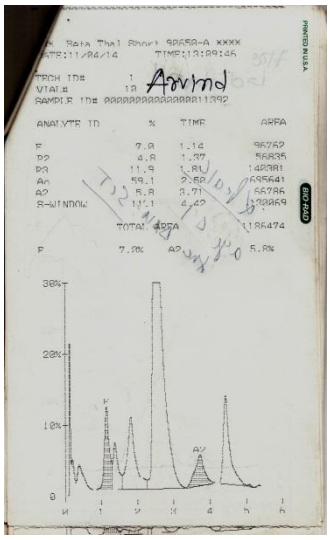

Fig.2: Double Heterozygous pattern: Sickle cell trait with Thalassemia trait.

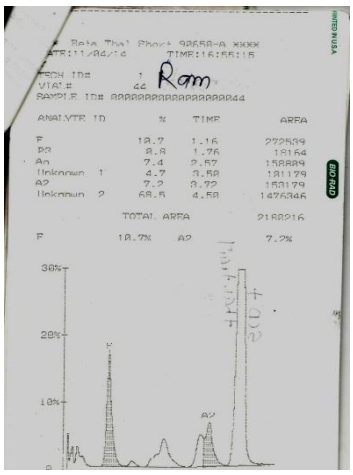

Fig.3: Double Heterozygous pattern: Sickle cell disease with Thalassemia trait.

IV. DISCUSSION

4.1 Incidence of double heterozygous cases in present study is 46.8% which is comparable to the study conducted by Dangiet al[3] (45.7%) in 2010 and Tyagi et al[4] (34.04%) in 2003. R.S Balgir[5] (2010) reported the incidence rate of 32.11% of double heterozygous cases amongst the sickle disorder patients. This difference is may be due to regional variation.

4.2 Average age of presentation in present study was found to be 13.4 years which is comparable to the study conducted by Balgir et al (13.5 years) in 2010 and Tyagi et al (14.2 years) in 2003.

4.3 In the present study history of consanguineous marriage was the predominant cause for the occurrence of double heterozygous cases. It was found in 65 cases (55.55%). Patel D.K et al[6] and Tariq H.A et al[7] also stated that the main reason for increased incidence of double heterozygous cases in particular communities like SC and Muslims is consanguinity which is comparable to the findings of present study.

4.4 The caste wise distribution was carried out in Nanded and the nearby zone. It was found that the maximum number of double heterozygous cases were found in Boudha community i.e 29 cases (24.78%) followed by Muslims 25 cases (21.37%). Banjara contributed to 12 cases (10.25%), Matang 12 cases (10.25%), Chambar 10 cases (8.55%), Teli9 cases (7.70%), kunbi 7 cases (5.99%) and others 13 (11.11%).

V. CONCLUSION

5.1 Double heterozygous cases are known to have notorious presentation. Due to the mimicking nature of presentation in these cases, family studies have become an important part of the investigation protocol.

5.2 The double heterozygous cases in Nanded region were studied so as to classify them and particularly target the areas and communities which have the maximum prevalence.

5.3 The sickle cell disease control programme conducted by NRHM has been a revolution in detecting the hidden iceberg cases. More studies should be undertaken in the high prevalence areas so as to enable to screen more and more number of cases and to help in detecting indeterminate and hidden cases.

5.4 Early institution of supportive care have reduced morbidity and mortality associated with the complications of these haemoglobinopathies and ultimately have been of crucial importance in better outcome of diseased patients.

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