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Original Research Article

A Study of Haemoglobinopathies by Various Methods

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Abstract: Haemoglobinopathies constitute a very important causative factor for anaemias of childhood. The two common haemoglobinopathies widely distributed across our country are the beta thalassemic syndromes and sickling disorders. The present study is carried out with a view to assess magnitude of these disorders in our area by haemoglobin electrophoresis and to evaluate the utility of NESTROFT as screening test of Thalassemia minor, to evaluate HbA_2 level by electrophoresis with clinical and hematological parameters, and to find out other haemoglobinopathies associated with thalassemia. The present study was carried out during the period of 2 years from September 2014 to September 2016. During this total 200 cases were studied. NESTROF carried 94.28% sensitivity, 92.72% specificity, 73.33% positive predictive value (PPV) and 98.70% negative predictive value (NPV) in detecting β thalassemia trait in present study. MENTZER Index < 12 carried 88.57% sensitivity, 97.57% specificity, 88.57% PPV and 97.57% NPV in detecting β thalassemia trait in present study. NESTROF is a simple, cheap, easy and rapid method of mass screening programme of thalassemia detection has quite acceptable sensitivity and specificity. But negative test in high risk community must be repeated and confirmation must be done by other confirmatory tests. Thalassemia is associated with many other haemoglobinopathies. In this study, common association with Sickle cell disease was found. For marriages, instead of matching HOROSCOPE of Brides and Groom, thalassemia test should be done. It will be a great help to mankind by preventing the "THALASSEMIA MAJOR" and giving birth to healthy baby and healthy society.
Keywords: NESTROF, Meintzer Index, Thalassemia, Sickle cell disease, Haemoglobinopathies

INTRODUCTION:

Haemoglobinopathies constitute a very important causative factor for anaemias of childhood. They mimic nutritional anaemias and then prove deceptive and refractory to the usual corrective measures. The two common haemoglobinopathies widely distributed across our country are the beta thalassemic syndromes and sickling disorders [1]. The former is more common in certain non-tribal ethnic groups and later amongst the tribal population. β-Thalassemias are the most important types of thalassemia because they are so common and produce severe anaemia in their homozygous and compound heterozygous state [2]. It is also a major health problem in Gujarat State, in India (prevalence 7.48%) especially in some communities such as Sindhis, Bhanushalis, Parsi, Muslims, etc. In Gujarat, its highest incidence was found in Surat followed by Rajkot and Jamnagar in 2009. Sickling disorders are prevalent in Africa, Mediterranean countries and India. In India, the disease is seen amongst tribals and certain ethnic groups in Central India mainly in Madhya Pradesh, Orissa, Andhra Pradesh and Vidharbha region of Maharashtra [3]. Sometimes, there is double heterozygous state of sickling disorders and β thalassemia. This is called HbS-Thalassemia syndrome. In this disorder, prognosis is better than that of thalassemia major or sickle cell anaemia. The present study is carried out with a view to assess magnitude of these disorders in this part of our country by haemoglobin electrophoresis. Electrophoresis refers to movement of charged molecule in an electrical field. Migration of Haemoglobin molecule in electrical field depends upon its net charge which in turn depends upon amino acids present in globin chain. In electrophoresis technique, different stabilizing media such as filter paper, cellulose acetate, starch gel, agar gel, acrylamide etc. are used [4, 5].

MATERIAL AND METHODS:

The Present Study comprises of total 200 cases. Cases of all ages and both sex were included.

Inclusion criteria:

- All the cases of hemolytic disorders.
- NESTROFT positive patients.
- Beneficiaries of Thalassemia camp.

Exclusion criteria:

- Diagnosed cases of thalassemia major of thalassemia ward.
- Diagnosed cases of Iron deficiency anaemia (patients with Mentzer's index > 12).

Total cases are divided in following groups

Group A: This group includes patients attending Pediatrics and Medicine OPDs for severe anaemia. Detailed clinical history, examination, family and/or past history and investigations were recorded in the electrophoresis requestion form. They were investigated by various laboratory tests as follows:-

- Complete blood count (to measure MENTZER index)
- Peripheral smear examination
- NESTROF test
- Hemoglobin electrophoresis (Hb electrophoresis)

Group B: This group included screening of population at high risk of thalassemia. In this study, camp was done to screen "AAHIR" community people. Their blood sample was taken for NESTROF test and Hb electrophoresis.

RESULTS AND DISCUSSIONS:

In our study we found the following results:

Out of total 100 patients in group A, 63 patients showed haemoglobinopathy and 37 patients did not show any haemoglobinopathy.

Table 1: Different Types of Haemoglobinopathies in Group A (N=63)

Type of Haemoglobinopathy	No. of cases	Percentage (%)
β thalassemia major	11	17.46
β thalassemia intermedia	2	3.17
β thalassemia trait	28	44.44
Sickle cell anaemia	2	3.17
Sickle cell trait	11	17.46
HbS/HPFH	4	6.37
HbS/β thalassemia	5	7.93
Total	**63**	**100.00**

Most common haemoglobinopathy detected was β thalassemia trait (44.44%) in group A. Out of 100 persons of Aahir community, 15 showed haemoglobinopathy while 85 were normal. Most common haemoglobinopathy was β thalassemia trait (46.66%) in group B.

Table 2 Different Types of Haemoglobinopathies in Group B (N=15)

Type	No. of cases	Percentage (%)
β thalassemia trait	7	46.66
Sickle cell trait	4	26.67
HPFH	4	26.67
Total	**15**	**100.00**

Table 3: Nestrof Test in β Thalassemia Trait in Both Groups (N=200)

Group		NESTROF +ve	NESTROF -ve	Total
A	$HbA_2 \geq 4\%$	26 (True +ve)	2 (False -ve)	28
	HbA2 < 4%	9 (False +ve)	63 (True -ve)	72
	Total of Group A	35	65	100
B	HbA2 ≥ 4%	7 (True +ve)	0 (False -ve)	7
	HbA2 < 4%	3 (False +ve)	90 (True -ve)	93
	Total of Group B	10	90	100
Grand Total		45	155	200

As per table, in group A, 2 cases were NESTROF negative with Mentzer's index < 12 and so their Hb electrophoresis showed HbA_2 > 4. So the NESTROF test was considered false negative. Statistics calculated as below:-

Sensitivity: - (TP X100)/ (TP + FN) = (33 x 100)/ (33 + 2) = 94.28

Specificity: - (TN X 100)/ (TN + FP) = (153 x 100)/ (153 + 12) = 92.72

Positive predictive value :- (TP X 100)/ (TP + FP) = (33 x 100)/ (35 + 10) = 73.33

Negative predictive value :- (TN X 100)/ (TN + FN) = (153 x 100)/ (65 + 90) = 98.70

TP = True Positive, FP = False Positive, TN = True Negative, FN = False Negative

Table 4: Mentzer's Index (MI) in β Thalassemia Trait in Both Groups (N=200)

Group		MI ≤ 12	MI > 12	Total
A	$HbA_2 \geq 4\%$	25 (True +ve)	3 (False -ve)	28
	HbA2 < 4%	3 (False +ve)	69 (True -ve)	72
	Total of Group A	28	72	100
B	HbA2 ≥ 4%	6 (True +ve)	1 (False -ve)	7
	HbA2 < 4%	1 (False +ve)	92 (True -ve)	93
	Total of Group B	7	93	100
Grand Total		35	165	200

Statistics calculated as below:-

Sensitivity: - (TP X100)/ (TP + FN) = (31 x 100)/ (31 + 4) = 88.57

Specificity: - (TN X 100)/ (TN + FP) = (161 X 100)/ (161 + 4) = 97.57

Positive predictive value :- (TP X 100)/ (TP + FP) = (31 x 100)/ (31 + 4) = 88.57

Negative predictive value :- (TN X 100)/ (TN + FN) = (161 X 100)/ (161 + 4) = 97.57

Table 5: Comparison of NESTROF Test and MENTZER'S Index for β thalassemia trait

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
NESTROF Test	94.28	92.72	**73.33**	98.70
MENTZER'S Index ≤ 12	88.57	97.57	**88.57**	97.57

As per table, Mentzer's Index is better than NESTROF test for screening of β thalassemia trait. Sometimes, NESTROF test can give false positive or false negative results.

Most of thalassemia cases were detected in Lohana community (19.50%) while most of Sickle cell disease cases were detected in Tribal, Bharwad and Patel community (12.10% for each 3). In thalassemia major cases pallor, weakness and infections were commonest symptoms and anaemia and hepatosplenomegaly with mongoloid facies were commonest signs. In sickle cell disease cases, patients were commonly presented with acute chest pain, bone pain and/or joint pain. Moderate to severe anaemia with marked anisopoikilocytosis and normoblastosis seems to be commonest haematological profile of thalassemia major. Most of cases of thalassemia major and intermedia showed moderate to severe degree of anaemia while most cases of thalassemia trait were either not anaemic or mildly anaemic. Most of the sickle cell disease cases were mild to moderately anaemic.

CONCLUSION:

As a consequence to the aim of the present study, the following conclusions are made:

1. NESTROF test is a simple, cheap, easy and rapid method of mass screening programme of thalassemia detection. NESTROF test has quite

acceptable sensitivity and specificity, but NESTROF negative test in high risk community must be repeated and further confirmation must be done by other confirmatory tests.

2. MENTZER's Index (MI) was useful to rule out iron deficiency anaemia cases from NESTROF positive cases and also found better than NESTROF test for detecting β thalassemia trait.
3. Cellulose acetate electrophoresis is a reliable method for haemoglobin electrophoresis and is very useful for screening and diagnostic purposes.
4. Screening of thalassemia is very essential particularly for high risk community, where consanguineous marriages are common and for screening of students of colleges and this screening saves the society and parent by preventing birth of thalassemic child.
5. Most of thalassemia cases were detected in Lohana community while most of Sickle cell disease cases were detected in Tribal, Bharwad and Patel community in present study.
6. Thalassemia major commonly manifests below and around age of one year.
7. Percentage of HbF (Fetal Haemoglobin) when measured first time in thalassemia major patient, it is definitively raised but when measured in patient who has received repeated transfusions, it might be decreased.
8. Thalassemia is associated with many other haemoglobinopathies. In this study, common association with Sickle cell disease was found.
9. For marriages, instead of matching HOROSCOPE of Brides and Groom, certificate of thalassemia test should be matched. It will be a great help to mankind by preventing the Devil's disease – "THALASSEMIA MAJOR" and giving birth to healthy baby and healthy society.

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