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VALIDITY OF NAKED EYE SINGLE TUBE RED CELL OSMOTIC FRAGILITY TEST FOR DETECTION OF B-THALASSEMIA TRAIT AGAINST HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

Medicine

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ABSTRACT

This manuscript is focussed to establish the validity of Naked Eye Single Tube Red Cell Osmotic Fragility test (NESTROFT) against gold standard method i.e., High-Performance Liquid Chromatography (HPLC) method for detection of B-Thalassemia Trait. NESTROFT and HPLC METHOD were applied to blood sample of 84 patients of suspected of cases of B-Thalassemia and other haemoglobinopathies. Out of 84, Beta Thal Trait 13 cases (15.4%) , Delta Beta Thal Trait 9(10.7%), Thal Major 5(5.9%), HPFH 7(8.3), Sickle Homo 12 (14.2 %), Sickle Trait 10(11.9%) Sickle Thal Trait 7 (8.3%) & IDA 21(25%) cases were detected by HPLC. The NESTROFT test was successful in detecting 12/13 subjects with B-Thalassemia trait. Sensitivity of the test was 92.31 % and specificity was 63.38 %. The test was positive in detecting other haemoglobinopathies like sickle cell disease also. The test proved to be simple, cheap easy to perform and adaptable for mass screening coming close to an ideal screening test for B-Thalassemia trait.

KEYWORDS

B-Thalassemia trait, Naked eye single tube red cell osmotic fragility test (NESTROFT), HPLC.

Introduction

Thalassemia is the commonest inherited haemogiobinopathy [1]. Preval ence of Thal assemia trait varies from 1.0-14.9% in various regions of India'. In our country, it is estimated that there would be about 25 million carrier and about 8000 children would be born each year inheriting a major haemoglobin disorder. The incidence of these genetic disorder can be reduced markedly by genetic counselling and prenatal diagnosis[3]. Determination of Red cell indices, Haemoglobin Chromatogram and HbA_2 level can be used for identification of B-Thalassemia heterozygotes[4]. However, these techniques are time consuming and expensive for population screening. The most effective and feasible approach for a vast country like ours is preventive genetics and major efforts need to be directed for applying simple and unexpensive screening test. NESTROFT is suitable test for screening the suspected cases of B- Thalassemia trait as it is easy to perform, inexpensive and does not require any sophisticated equipments.

MATERIALS AND METHODS

Since the concentration of 0.36% buffered saline was more efficient in detecting heterozygous beta-thalassaemia patients than the four other saline strengths (i.e. 0.35%, 0.37%, 0.38% and 0.39%). [22]

In this study buffered saline (i.e. 0.36%) has been used by various workers to study its effect on the osmotic fragility of red cells and working out the reliability of this concentration in detecting the beta-thalassaemia trait.

Total of 84 **patients referred from the OPD, Indoor & Emergency to the Department of Laboratory Medicine** (deals with investigation of genetic disease of referred cases) were selected for screening. The criteria for selection of cases were: i) Those suspected for Thalassemia & Hemolytic disorders. ii) In High risk community. iii) Parents and siblings of known Thalassemic major patients.

NESTROFT, complete haemogram and HbA2 estimation. HbA2 > 3.5% was treated as the gold standard for the diagnosis of the thalassaemia trait.

The test was also studied in patients of sickle cell disease and other forms of Hemoglobinopathies.. NESTROFT was carried out in these patients as advocated by Mehta et a l[4] and Ka ttamis et al'.

Nestroft test

Principle :

Limit of hypotonicity which red cells can withstand. There is a decreased in osmotic fragility in red cells in Beta Thalassemia.

Preparation & Procedures:

10 % stock solution of buffered saline

a)

90 g		Nacl
13.65 g		Na_2Po_4
2.4 g		$NaH_2Po_42H_2O$
Add	10000 ml	Distilled water

b) 1% buffered saline is prepared by 1: 10 dilution with Distilled water

c) Five buffered saline solutions with

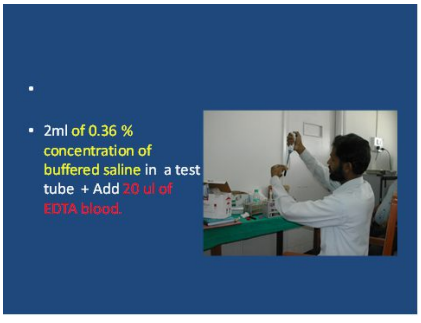
Concentration	1% buffered saline	Distilled water
0.35 %	35 ml	65 ml
0.36 %	**36 ml**	**64 ml**
0.37 %	37 ml	63 ml
0.38 %	38 ml	62 ml
0.39 %	39 ml	61 ml

d) 2 ml of each five concentration of buffered saline in five different test tube
add 2 ml of Distilled water in the 6th test tube.

e) add 20 ul of EDTA blood in each test tube .

f) Shake well the tubes and left at room temp. for ½ an hour.

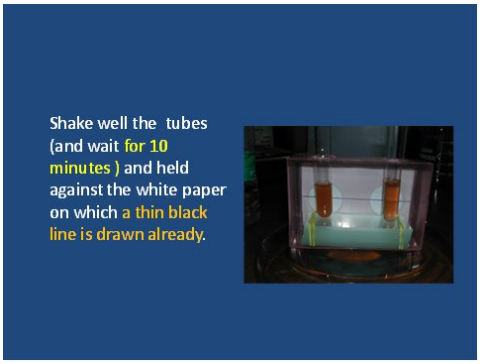
g) Shake well all tubes and held against the white paper on which a thin black line is drawn already.

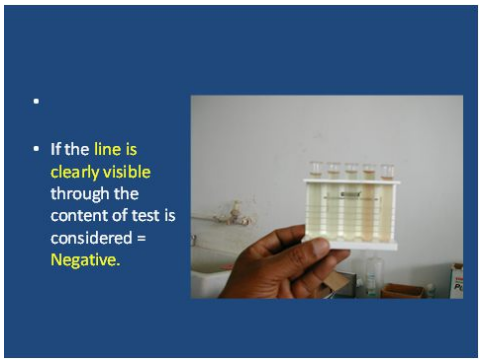


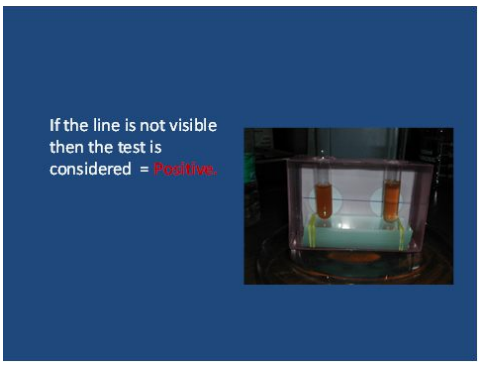
h) If the line is clearly visible through the content of tubes with Distilled Water tube(Control) and similarly visible through the content of tubes with buffered saline, then the test is considered "NEGATIVE".

I) If the line is not visible then the test is considered "POSITIVE".

j) In the case of a blurred line test is considered " DOUBTFUL".







Interpretation of doubtful case is taken positive/ negative.

k) In case of normal sample if the test is negative – called TRUE NEGATIVE(TN)
If POSITIVE – called - FALSE POSTIVE (FP)

l) In the case of carriers if the test is POSITIVE it is called TRUE POSITIVE (TP)
If NEGATIVE is called FALSE NEGATIVE (FN)

All the subjects were also screened by mainly HPLC . A few cases were screened by Haemato analyzers when ever required in the laboratory.

The subjects were divided into following categories after taking into consideration HPLC (a gold standard procedure) , Haematological Nestroft test parameters.

OBSERVATIONS

Total cases screened = 84

HPLC & Nestroft test result in cases of different types Hemoglobinopathies & others

	1	2	3	4	5	6	7	8	Total
Findings of cases	Beta Thal Trait	Delta Beta Thal Trait	Thal Major	HPFH	Sickle Homo	Sickle cell Trait	Sickle Thal Trait	IDA	
by using HPLC	13	9	5	7	12	10	7	21	84
by using Nestroft	12	3	0	0	8	2	1	0	26

Nestroft test result in the following diseases

Disease	Positive	Doubtful	Negative
Beta Thal Trait	12	1	0
Delta Beta Thal Trait	3	4	2
Thal Major	0	1	4
HPFH	0	1	6
Sickle Homo	8	1	3
Sickle cell Trait	2	5	3
Sickle Thal Trait	1	1	5
IDA	0	13	8
Total	26	27	31

Note : Doubtful results were assumed as negative

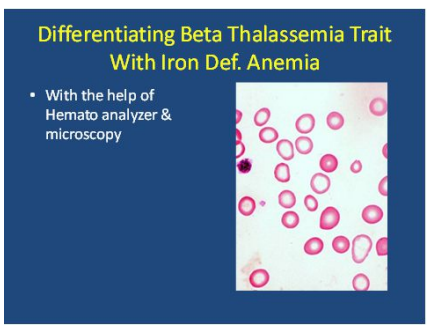
Among the 84 patients with different types of Hemoglobinopathies and Iron deficiency Anemia on HPLC, NESTROFT was positive for 26, 'doubtful' for 27 and negative for 31.

Of the 21 patients with other haemoglobin disorders (IDA), None were positive on NESTROFT and 13 were 'doubtful' and negative for 8.

To differentiate Beta Thalassemia from Iron Deficiency Anemia followings points were considered :

When there is red cell picture of microcytic hypochromic anemia
Discriminant functions in distinguishing Beta Thalassemia Trait and Iron Deficiency Anemia

Points	BTT	IDA
Morphology	Microcytic hypochromic may be with inclusion bodies	Microcytic hypochromic may be with ring cells
RDWSD	<46 fl (N 38 to 58)	>46
RDW CV	< 16 % (N 11 to 19)	>16
Hb	Minimal decrease(around 5 million)	May be markedly decrease(<5 million)
Nestroft test	Positive	Negative
Hb A2	>3.5 % up to (5 – 9 %)	
HbF	>2% up to (5 to 15%)	
Hepatomegaly	May be present	May not be present
Spleenomegaly	May be present	May not be present



Of the 13 patients with Beta Thalassemia Trait on HPLC, 12 were positive on NESTROFT and 01 was 'doubtful'.

Comparison between HPLC & Nestroft test in case of Beta Thal Trait

Nestroft	HPLC		
	Positive	Negative	Total
Positive	12	26	38
Negative	1	45	46
Total	13	71	84

The number of True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) were determined.

Sensitivity, specificity and predictive values were calculated as follows:
1) Sensitivity = TP x 100/TP+FN = 93.3 %
2) Specificity = TN x 100/TN+FP = 63.38 %
3) Predictive value of a positive test TP x 100/ TP+FP 4) = 31.58%
Predictive value of a Negative test = TP x 100/ TN+FN= 97.83%

Positive NESTROFT blood samples are subsequently confirmed for B Thalassemia Trait by HPLC = 13

Table-1: Results of Nestroft specially in B-Thalassemia trait

NESTROFT	Total Subject n= 84	B-Thalassemia trait n = 13
Positive	38	12
Negative	46	1

Findings

A total 84 patients were screened, NESTROFT was positive in 12(13) cases of B-Thalassemia trait (True Positive, TP). There were no False positive. It was negative in 1(13) cases. (False negative, FN) and 71 cases did not have ii-Thalassemia trait (True Negative, TN). Sensitivity of NESTROFT was 93.3 % and specificity was 63.38 %. Positive predictive value was 31.58% and negative predictive values was 97.83 %.

NESTROFT was also positive in 2/10 cases of sickle cell trait and 8/12 cases of sickle Homo , 1/7 cases of Sickle Thal Trait and However, none of the Thal Major subjects 0/5 cases showed positive NESTROFT test.

DISCUSSION

The purpose of this study was to evaluate the effectiveness of NESTROFT as a screening test for Beta Thalassemia trait.

Although screening of the thalassaemia trait using 0.36% buffered saline was successful in detecting 97.7% of subjects with this trait, but non was also positive in 25.0 % of subjects with iron-deficiency anaemia.

In our study NESTROFT was both sensitive (93.3) and specific (63.38%) for identification of B-Thalassemia trait.

The result is comparable with the studies of Kattamis et al,(9) Mehta et al,(6) Gorakshakar et al, [14] Raghavan et al, [15] Thomas et al, [17] Maheshwari et al [18] and Sirichotiyakul et al,[19] who reported values of 98.3%, 97.0%, 99%–100%, 98.3%, 96.5%, 99.0% and 99.5%, respectively.

The specificity in the present study was 63.38 %, which is comparable to results obtained by Mehta et al,[13] Gorakshakar et al [14] and Raghavan et al.[15] The negative predictive value of the test in carriers during the present study was 97.83 %.

Kattamis[5], Raghawan[6], Gorashker[7], Thomas[3] and Mehta[4] reported the sensitivity and specificity of NESTROFT in the range of 95 to 98.4% and 66.6 to 91% respectively.

None of the The Thalassemia Major in our study showed positive NESTROFT test. Positive predictive value of NESTROFT in our study was 31.58% and Negative predictive value was 97.83%.

Kattamis[5] in his study reported Positive Predictive Value of 91.3% and Negative Predictive Value of 98.3% for NESTROFT.Though NESTROFT was positive in '40% cases of sickle cell trait, 23.63% cases of sickle cell disease and 100% cases of Thalassemia major, their detection is of major benefit as each of these conditions has its own health implications.

In the study by Raghavan K6, NESTROFT was positive in 29.46% and negative in 70.6% cases of sickle cell disease. Similar study by Thomas et a19 reported that NESTROFT was postive in 56.26% and negative in 43.75% cases of sickle cell disease.

Kattamis[5] et al also found the test useful in picking up patients of sickle cell disease. When used as a population screening, this will prove to be beneficial aspect of the test.

Colah8 reported that NESTROFT does not miss 'out any Beta - Thalassemia heterozygous and helps to pick up cases of sickle cell disease also.

NESTROFT with 0.36% buffered saline still showed a very high negative predictive value. The present data therefore confirms that a negative NESTROFT is very useful in ruling out beta-thalassaemia.

NESTROFT has thus emerged as an inexpensive, most sensitive and specific test of population screening for the beta-thalassaemia trait, and is considered suitable for large scale use in developing countries like India, which has limited financial and technical resources.

Conclusions

We conclude that, NESTROFT is a suitable test for screening for beta-thalassaemia and the common haemoglobinopathies seen in India. It is easy to perform, simple, inexpensive and does not require sophisticated equipment. Subjects who are NESTROFT 'positive' or 'doubtful' deserve further investigation.

The NESTROFT test is very cheap, cost effective and easy to perform. The stock solution once made can be kept well in a stoppered bottle. At one time one can perform 40-50 tests in a hour. Thus, NESTROFT seems to be valuable as a screening test in our country with low socio-economic status.

A practical approach would be to perform NESTROFT in high risk community for detection of heterozygotes of B-Thalassernia and positive cases would then be examined for raised HPLC . When one considers the repeated yearly expenses of bringing up a child with Beta -Thalassemia, preventing Thalassemia births by diagnosis and counseling (Beta - Thalassemia trait cases becomes the more feasible and practical approach.

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