ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Naked Eye Single Tube Red Cell Osmotic Fragility Test Screening For Detection Of B-Thalassemia Trait – An Evaluation Against HPLC Method At Rajendra Institute Of Medical Sciences, Ranchi (A Tribal Zone).

Chandrahas Prasad[1], Shashi Bhushan Singh[2], Govind Kumar Gupta[3] Shanti Praksh[4], Tulsi Mahato[5] and Satish Chandra[6].

[1]Associate Professor, Department of Laboratory Medicine, [2] Lecturer Cum Statistician, Department of Preventive and Social Medicine, [3]Assistant Professor, Department of Orthopedics, [4]Associate Professor, Department of Anesthesiology [5]Professor, Department of Forensic Medicine ,[6]Professor, Department of pharmacology, Rajendra Institute of Medical Sciences (RIMS), Ranchi.

ABSTRACT

The objective of the manuscript is to evaluate the effectiveness of NACKED EYE SINGLE TUBE RED CELL OSMOTIC FRAGILITY TEST (NESTROFT) as screening tool for detection of B-THALASSEMIA TRAIT against the HPLC method. NESTROFT and HPLC METHOD were applied to blood sample of 84 patients of suspected 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.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Thalassemia is the commonest inherited haemogiobinopathy [1]. Prevalence 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.

Objectives

To evaluate the effectiveness of NACKED EYE SINGLE TUBE RED CELL OSMOTIC FRAGILITY TEST (NESTROFT) as screening tool for detection of B-THALASSEMIA TRAIT against the HPLC method.

MATERIAL 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 and Emergency to the Department of Laboratory Medicine (deals with investigation of genetic disease of referred cases) were selected for screening in year 2013. 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 were used and out of it HbA2 > 3.5% was treated as the gold standard for the diagnosis of the thalassaemia trait.

These tests were 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 a1 [4] and Kattamis et al [5].

ISSN: 0975-8585

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 $NaH_2Po_4\,2H_2O$
Add 1000 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.

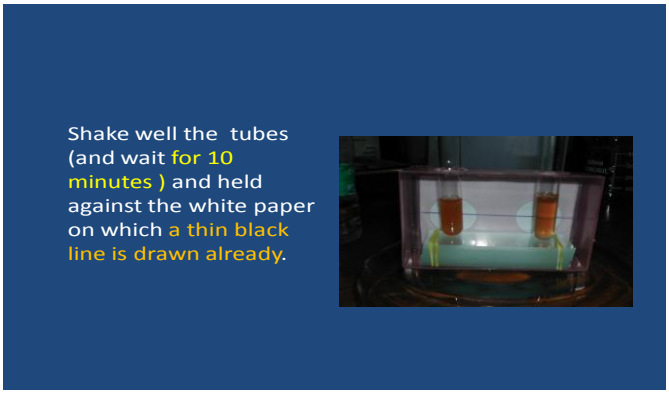
f) Shake well the tubes and left at room temp. For ½ an hour.

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

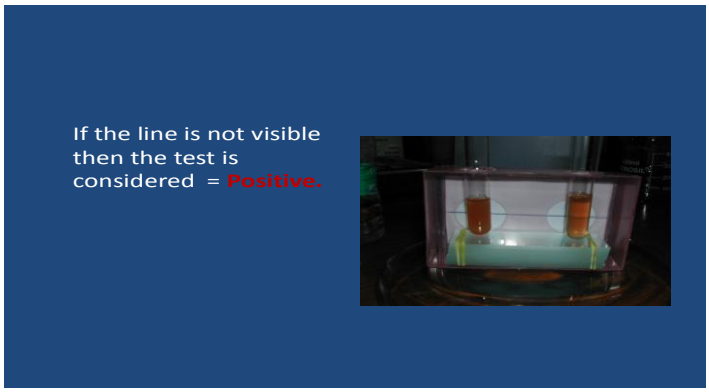
h) If the line is clearly visible through the content of tubes with Distilled Water tube (Control) and
Similarly visible through the content of tubes with buffered saline, then the test is considered "NEGATIVE".

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".



ISSN: 0975-8585



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

Table 1: HPLC and NESTROFT test result in cases of different types Hemoglobinopathies and 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

ISSN: 0975-8585

Table 2: 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 .

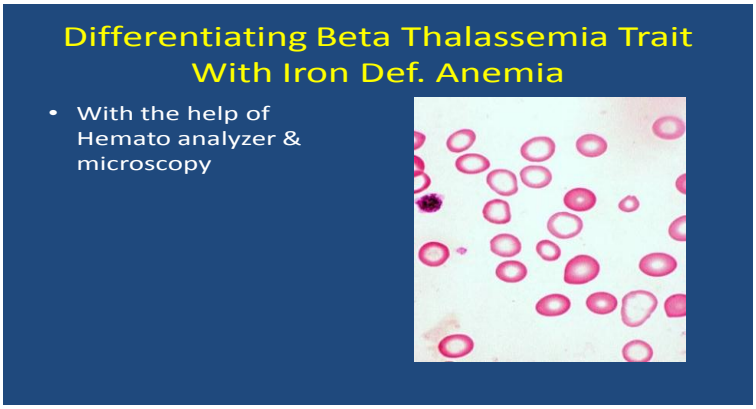
Of the 21 patients with other haemoglobin disorders (IDA), none were positive on NESTROFT and 13 were 'doubtful' and negative for 8.

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

ISSN: 0975-8585



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

Table 3: Comparison between HPLC and 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%
4) 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

Results of NESTROFT especially 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 a negative predictive value 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 none was also positive in 25.0 % of subjects with iron-deficiency anemia.

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 [5], Mehta et al [13], 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 K [6], NESTROFT was positive in 29.46% and negative in 70.6% cases of sickle cell disease. Similar study by Thomas et a1 [9] reported that NESTROFT was positive 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.

Colah [7] 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 an 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.

ACKNOWLEDGEMENT

Author acknowledges the support of faculty of various departments and the Director of RIMS, Ranchi. Author is giving thanks to Mr. Jawed Akhtar, senior lab tech. for rendering technical help and lab work support during the course of this study, which is gratefully acknowledged.

REFERENCES

[1] Sukumar PK. Abnormal HaemogIobins in India. In Trends in haematoi ed. Sen NN and Basu AK, Chatterjee Memorial Volume, 1975; 225-253.
[2] Chauhan DM, Chauhan V. Indian J Haematol Blood Transf 1992; 10: 1-20.
[3] Thomas B, Shrivastava A, Jayasselan L, Dennison D and Chandy M. Indian J Med 1996; 104: 194-197.
[4] Mehta BC, Gandhi S, Mehta JB, Kamnath P. Indian J Hematol 1988; 6: 187-90.
[5] Kattamis C, Effrenov C, Pootrakul S. J Med Gent 1981; 18(4): 266-70.
[6] Raghavan K, Lokeshwar MR, Birewar N, Nigam V. Manglani MV, Raju NB. Indian Pediatr 1991; 28: 469- 72.

[7] Gorakshaker AC, Colah R, Nadkarni A, Desai S. Nati Med J Indian 1990; 3: 171-3. Colah R. JAPI 1994; 42(10): 810- 814.
[8] Balgir RS. Nat Med J India 1999; 12:234-8.
[9] ICMR task force study. Indian Council of Medical Research: Collaborative study on thalassaemia. New Delhi: Indian Council of Medical Research, 1993.
[10] Balgir RS. Curr Sci 2000; 79:1536-7.
[11] Choudhry VP, Desai N, Pati HP, et al. Indian Pediatr 1991; 28:1221-9.
[12] Kattamis C. Screening of haemoglobinopathies (thalassaemia and other abnormal haemoglobins). In: Bichel H, Guthrie R, Hammersen G, eds. Neonatal Screening for Inborn Errors of Metabolism. New York: Springer Verlag, 1980: 133-47.
[13] Mehta BC, Gandhi S, Mehta JB, et al. Indian J Haematol 1988; 6:187-90.
[14] Gorakshaker AC, Colah R, Nadkarni A, et al. Nat Med J India 1990; 3:171-3.
[15] Raghavan K, Lokeshwar MR, Birewar N, et al. Indian Pediatr 1991; 28:469-72.
[16] Kattamis C, Efremove G, Portrakul S. J Med Genet 1981; 18:266-70.
[17] Thomas S, Srivastava A, Jeyaseelan L, et al. Ind J Med Res 1996; 104:194-7.
[18] Maheshwari M, Arora S, Kabra M, et al. Indian Pediatr 1999; 36:1119-25.
[19] Sirichotiyakul S, Tantipalakorn C, Sanguansermsri T, et al. Int J Gynaecol Obstet 2004; 86:347-50.
[20] Manglani M, Lokeshwar MR, Vani VG, et al. Indian Pediatr 1997; 34:702-7.
[21] Gomber S, Sanjeev and Madan N. J Trop Pediatr 1997; 43:363-6.
[22] Singh S P, Gupta S C Singh S P, Gupta S C Singapore Med J 2008; 49 (10) : 823