International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Study of Effectiveness of NESTROFT and Solubility Test as a Screening Test for the Detection of Haemoglobin Disorder at Nanded Region of Maharashtra

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Received: 12/08//2014 *Revised: 25/08/2014* *Accepted: 26/08/2014*

ABSTRACT

We screened 1000 cases of thalassemia trait and sickle cell carriers already diagnosed and confirmed by High Performance Liquid Chromatography (HPLC) by using NESTROFT and Solubility test. NESTROFT was successful in detecting 922 subjects with β thalassemia trait, and negative in all 1000 control samples. Sensitivity of the NESTOFT was 92.2% and specificity was 62.6%. Positive predictive value of NESTROFT was 60.5% and Negative predictive value of it was 89.0%. Solubility test was positive in 990 samples while it was negative in all controls. The sensitivity of solubility test was found to be 94.8% and specificity of study was 87.8 %. Positive predictive value of this study is 97.73% and negative predictive value of this study is 94.40 %

NESTROFT and Solubility are suitable for screening the suspected cases of β-thalassemia trait and sickle cell carrier respectively as they are easy to perform, can be used for field studies, inexpensive and does not require any sophisticated equipment. NESTORFT and Solubility are found to be most cost effective and promising screening test to detect thalassemia trait and sickle cell anaemia respectively.

Keywords: NESTROFT, Sickle cell carrier, Solubility test, Thalassemia trait.

INTRODUCTION

Inherited disorders hemoglobinopathy and thalassemia are the commonest genetic disorders in the world. In many countries they constitute major public health problem. [1]

In India almost 25 million people are carriers for β thalassemia and 8000 children are born every year with thalassemia major. Prevalence of thalassemia trait varies form 1.0-14.9% in various regions of India, having the average incidence of beta thalassemia trait in India of 3.3% with 1-2 per 1,000 couple being at risk of having an affected offspring each year. [2] (Bobhate et al., 2002).

Sickle cell disorder is the second most common haemoglobin disorder next to thalassemia in India. [3]

Combating with these inherited disorder prevention is best remedy. *Sc*reening will continue to be the backbone of preventive strategies against β-thalassemia trait, especially in countries where the prevalence is high. [4-7]

There are various screening parameters available for the diagnosis of β-thalassemia trait, include peripheral blood smear (PBS) examination, red cell indices, osmotic fragility (quantitative), and free red cell porphyrins. [8] (Shine and Lai, 1977).

All these tests are expensive and are not confirmatory tests for diagnosis of β thalassemia. The HbA2 estimation is a confirmatory test for β-thalassemia trait is also expensive, time consuming and require sophisticated equipment.

Various screening tests are available for diagnosing sickle cell disorder. Out of which sickling tests is simple and cheap to perform but false positive and false negative results are not uncommon [9] The HbS estimation on HPLC is a confirmatory test for sickle cell disorder is also expensive.

The present study evaluates the efficacy of such low cost rapid tests NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) and Solubility for mass screening of Haemoglobin disorders.

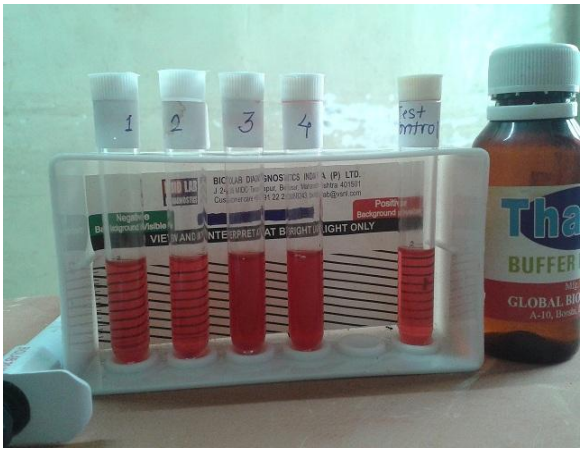
METHODOLOGY

The current case control study was undertaken at Dr. Shankarrao Chavan Govt Medical College Nanded during the period Jan.2011to Dec. 2013. In this study, a total of 1000 subjects with β-thalassemia trait and1000 subjects with sickle cell carrier were included along with 1000 normal control subjects for both NESTROFT and solubility tests. All the β-thalassemia trait subjects with Hb A2 level 4 -7 % and Sickle cell carrier with HbS range 31-40 % detected by HPLC were included in this group. The control subjects were having normal HPLC report. Blood samples were collected in EDTA bulbs from individuals of either Sex from both groups. NESTROFT was performed using 0.36% buffered saline solution [8,10] (Shine and Lai, 1977; Kattamis et al., 1981).

NESTROF Test (Fig.1):

We used readymade 0.36% buffered saline solution (Thal-S reagent) of Global Biosciences Company.

Take 2 ml of 0.36% buffered saline solution (Thal-S reagent) was taken in one tube (10 cm x 1 cm diameter) and 2 ml distilled water was taken in another tube. A 20 µ of blood was added to each tube and they were left undisturbed for 1/2 an hour at room temperature. Both the tubes were then shaken and held against a white paper on which a thin black line was drawn. The line was clearly visible through the contents of the tube containing distilled water. If the line was similarly visible through the contents of the tube with the buffered saline, the test was considered negative. If the line was not clearly visible, the test was considered positive. A positive test indicates lowered red cell osmotic fragility, suggestive of thalassemia trait.



(Figure 1): 1,2-NESTROFT Negative,3,4-NESTROFT positive .

Solubility Test (Fig.2):

We used Dithionate Qualitative Solubility Test Kit for this study. Test is a rapid solubility test employing properties of reduced HbS. Some other haemoglobins known to give the same positive reaction as haemoglobin S are haemoglobin C (Harlem), C (Georgetown), and Memphis/S.

a) Reagents:

R1: Dipotassium phosphate 2.3 mol/L,Detergent-0.4 g/LpH 7.1(+/-0.1) Contain stabilizers and preservatives.
R2: Sodium dithionate 57mmol/L
R3: White saponin 1%

b) Working Reagent Preparation:

Add 1 vial of R2 and R3 in bottle of R1. Rinse R2 and R3 with R1 for 3 times and make sure that either content of R2 and R3 are transferred to R1.Mix gently for 15 mins.

c) Specimen Collection and Preparation:

Use anti coagulated whole blood. If blood is used immediately, sufficient sample may be obtained by a finger or heel puncture. Never use a clotted specimen.

d) Procedure Outline:

Add 20 µl of blood sample to 2.0 ml working reagent in 12x75 mm test tube.Wait for 10 minutes at room temperature. Observe for turbidity by holding the test tube one inch in front of lined card.

e) Results:

Positive tests - lines on card cannot be seen through the test solution.

Negative test - lines on card can be seen through the test solution

(Figure 2): Sample 1,2-Solubility negative . Sample 3,4-Solubility positive.

The Sensitivity, specificity, positive and negative predictive values were calculated by using following formulae.

Sensitivity = TP × 100/TP + FN
Specificity = TN × 100/TN + FP
Predictive value of a positive test = TP × 100/TP +FP
Predictive value of a negative test = TN × 100/TN +FN

RESULTS

NESTROFT:

Blood samples of 1000 beta thalassemia carrier and 1000 normal healthy controls were selected for the study. The samples were subjected for NESTROFT as they were available. After analysing the data it was found that out of 1000 beta thalassemia carrier samples, 954 showed NESTROFT positive while 46 were NESTROFT negative(Table:2). These 46 beta thalassemia carrier who showed NESTROFT negative was due increased in Hb concentration.

Sensitivity of the NESTOFT was 92.2% and specificity was 62.6%. Positive predictive value of NESTROFT was 60.5% and Negative predictive value of it was 89.0 %(Table: 2).

Table no: 1

NESTROFT	β-Thalassemia Trait N=1000	Control N=1000
Positive	954	179
Negative	46	821

Table: 2

TESTS	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Nestrof test	95.4%	82.1%	84.20%	94.69%

Solubility Test:

Blood samples of 1000 sickle cell carrier and 1000 normal healthy controls were selected for the study. The samples were subjected for solubility test as they were available. After analysing the data it was found that out of 1000 sickle trait samples, 948 showed positive while 52 were solubility negative(Table:3).

The sensitivity was found to be 94.8% and specificity of study was 87.8 %.

Positive predictive value of this test is 97.73% and negative predictive value of this test is 94.40 %

Table: 3

SOLUBILITY	Sickle trait N=1000	Control N=1000
Positive	948	122
Negative	52	878

Table: 4

TESTS	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Solubility test	94.8%	87.8%	97.73%	94.40%

DISCUSSION

NESTROF test:

At constant hypotonic Nacl solution hypochromic red blood cells are able to uphold certain amount of water and remain intact whereas the normal erythrocyte cannot and explode. Due to unlysed RBCS NESTROFT become positive in Thalassemia trait.

In the present study, from the results it was found that, the NESTROFT to be very sensitive, though not highly specific. The sensitivity was found to be 95.4 % which matches study by Bobhate S.K.(2002); Manglani M. (1997); Singh and Gupta, (2008) had shown sensitivity above 95% [2,11,12] The highest sensitivity was observed by Gorakshekar et al. (1990) which ranges between 98 to 100%. [13] Similar results were obtained in the study of Srivastava et al., (1996), and Singh and Gupta (2008)and it was 98.4%. [14,12] Raghavan et al. (1991) observed that sensitivity of NESTROF test to be 95.5%. [15] Amini et al., 2011 noted in their study the sensitivity to be 100%. [16]

Out of 1000 healthy controls, 179 showed NESTROFT positive, whereas 821 reflected NESTROFT negative, The 179 normal who showed NESTROFT positive this may be due to anaemic disorders. Table 2 shows the sensitivity, specificity, positive predictive value and negative predictive values for NESTROFT in the present study. Table 1 lists the distribution of NESTROFT observations among the β-thalassemia trait and control samples.

In our study the specificity was 82.1%. The specificity reported in previous studies was ranges between 82-91%. The highest specificity was observed in the study of Mehta et al and it was 91%. In the study of Raghavan the specificity was found to be 86.9 %. [15] 82% specificity was noticed in the study of Amini SA et al. (2011) and Gorakshaker et al., (1990). [16, 13]

In a study from North Indian Punjabi population, the test showed a sensitivity of 100%, specificity of 85.47%, a positive predictive value of 66% and a negative predictive value of 100% (Piplani et al., 2013). [17] In a study by Indranil et al. (2012) NESTROFT showed an overall sensitivity and specificity of 95% and 95.8% respectively in detection of heterozygous and double heterozygous states of beta-thalassemia. [18] The comparison of the sensitivity and specificity of NESTROFT using 0.32%, 0.34%, and 0.36% buffered saline; Chow J et al (Jason et al.,2005) recommend the use of 0.36% saline, which gave definitely positive results in 81 of 85 patients of β thalassemia trait. [19]

The estimated predictive value for positive and negative NESTROFT will vary depending on the prevalence. The positive predictive value in our study was found to be 84.20% and negative predictive was 94.69 %. NESTROFT still showed a very high negative predictive value. Our data therefore confirm that negative NESTROFT is very useful in excluding beta thalassemia.

NESTROFT as a single screening parameter is superior to any other simple tests like MCV and is more cost effective. It has also been noted that to increase the effectiveness of screening, a combination of test has been used by the laboratory such as NESTROFT followed by MCV thereby

achieving sensitivity up to 100%. Hence, though ideal a combination of these two tests considerably increases the cost of screening, thus defeating the feasibility of utilizing them in areas with limited laboratory facilities and economic resources.

From the above studies it can be concluded that NESTROFT test is easy to perform and can be used for field studies, which does not requires sophisticated equipment or technical expertise and can be done from capillary blood obtained by finger prick. This, therefore, reinforces NESTROFT singly, as the most cost effective and promising screening test to detect thalassemia heterozygotes.

Solubility Test

Solubility tests differentiate between sickling and non-sickling haemoglobin. Red cells are haemolysed in presence of high phosphate buffer and HbS if present is reduced by dithionite. Reduced HbS form insoluble polymers, which refract light and solution become turbid (positive).

Vasaikar et al found that, the Solubility test to be very sensitive. The sensitivity was found to be 100% and specificity of study was 91.66%. Positive predictive value of this study is 80% and negative predictive value of this study is 100 %. [20] Sure et al (2000) screened 3,246 samples from tribal populations of the Dhule and Gadchiroli districts of Maharashtra by solubility test, Hb electrophoresis and automated HPLC showed that the overall sensitivity of solubility test is 93.8% and specificity is 100%. [21] Comparing to Surve et al study we got good sensitivity but less specificity was found.

A sickle solubility test should always be performed when the presence of a significant proportion of haemoglobin S is suspected. It will be positive, except in the early neonatal period when the percentage may be below the detection limit. It follows that a negative sickle solubility test in a neonate with variant haemoglobin consistent with haemoglobin S does not exclude a diagnosis of sickle cell trait. [22]

CONCLUSION

In conclusion, NESTROFT and SOLUBILITY are sensitive, cost effective, rapid and reliable screening tests for detection of beta thalassemia trait and sickle cell carrier in populations. Both tests have overall good sensitivity and are effective tests for screening thalassemia trait and sickle cell trait.

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How to cite this article: Shewale SP, Meshram DP, Sameer MA et. al. Study of effectiveness of NESTROFT and solubility test as a screening test for the detection of haemoglobin disorder at Nanded region of Maharashtra. Int J Health Sci Res. 2014;4(9):49-54.

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