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Original article

Effectiveness of nestroft as a screening test for the detection of β-thalassemia trait

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ABSTRACT

The present study was carried out to evaluate the effectiveness of NESTROFT as a screening test for β-thalassemia trait. Naked Eye Single Tube Red cell Osmotic Fragility Test [NESTROFT] is an un-cumbersome and inexpensive test for the detection of β-thalassemia. NESTROFT was applied to 150 patients of β-thalassemia trait and 150 normal control samples. The test was successful in detecting 143 subjects with β thalassemia trait, and negative in all 150 control samples. Sensitivity of the test was 95.2% and specificity was 62.6%. Positive predictive value of NESTROFT was 60.5% and Negative predictive value of NESTROFT was 89.0%. NESTROFT is suitable for screening the suspected cases of β-thalassemia trait as it is easy to perform, can be used for field studies, inexpensive and does not require any sophisticated equipments. NESTORFT is found to be singly most cost effective and promising screening test to detect thalassemia heterozygotes.

1. Introduction

Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Findings in untreated or poorly transfused individuals with thalassemia major, as seen in some developing countries, are growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow (Galanello et al., 2010). Carriers for β-thalassemia are usually asymptomatic but may have mild hypochromic anemia (Haemoglobinopathies., 2007).

β thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia(Galanello et al.,2010). 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 (Bobhate et al., 2002).

Only 10 to 15% of these children receive optimal treatment (Choudhry et al., 1991). the cost of such treatment for one thalassemia child amounts to INR 90,000 to 1,00,000 annually at around 3 years of age, which increases as the child, grows (Manglani et al., 1997). The only cure available today is bone marrow transplantation, which is not affordable to almost all patients. Thus, the birth of a thalassemia child places considerable physical and economic strain, not only on the affected child and its family, but also on the community and the nation at large. With these limitations, the interest must be shifted from treatment to prevention of such births in the future. The prevention methods include population education, mass screening, genetic counseling and prenatal diagnosis. These are the only totally effective ways to end up successfully with increasing incidence of β thalassemia in India as well as in other countries(Srivastava et al.,1996). 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 (Shine and Lai, 1977).

All these tests are expensive and are not confirmatory tests for diagnosis of β thalassemia. The Hb A2 estimation is a confirmatory test for β-thalassemia trait is also expensive, time consuming and require sophisticated equipment. The need, therefore, is for a simple, low cost, rapid and reliable test which can be applied for mass screening. The present study evaluates the efficacy of one such test, NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test).

2. Subjects and methods

2.1. Subjects

The current case control study was undertaken from Western Maharashtra during the period 2009 to 2011. The study was approved by institutional ethical committee. In this study, a total of 150 subjects with β-thalassemia trait were included from general hospital, sangli, along with 150 normal control subjects. All the β-thalassemia trait subjects were confirmed by the Hb A2 level >3.5% by Hb electrophoresis were included in this group. The control subjects were having normal values of hematological parameters and Hb A2. Blood samples were collected in EDTA bulbs from individuals of either Sex from both groups. NESTROFT was performed using 0.36% buffered saline solution (Shine and Lai, 1977; Kattamis et al., 1981).

2.2. Method

2 ml of 0.36% buffered saline solution was taken in one tube (10 cm x 1 cm diameter) and 2 ml distilled water was taken in another tube. A drop 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. 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 = TP × 100/TN +FN

3. Results and discussion

NESTROFT test was carried out to find out the sensitivity and specificity of the test, due to abnormal osmotic fragility of red cell that could occur due to variety of reasons giving rise to altered shape and functioning of red cell. The red cells whose shape has been altered due to defective genes or whose functioning has been altered due to production of certain protein in less than normal amount show the positive test.

Blood samples of 150 beta thalassemia carrier and 150 normal healthy controls were selected for the study. The samples were subjected for NESTROFT as they were available. After analyzing the data it was found that out of 150 beta thalassemia carrier samples, 143 showed NESTROFT positive while 07 were NESTROFT negative. These 07 beta thalassemia carrier who showed NESTROFT negative was due increased in Hb concentration.

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 95.2%, though all previous studies by Bobhate S.K.(2002); Manglani M. (1997); Singh and Gupta, (2008). had shown sensitivity above 95%. The highest sensitivity was observed by Gorakshekar et al. (1990) which ranges between 98 to 100%. Similar results were obtained in the study of Srivastava et al., (1996), and Singh and Gupta (2008). and it was 98.4%. Raghavan et al. (1991) observed that sensitivity of NESTROF test to be 95.5% (Amini et al., 2011) noted in their study the sensitivity to be 100%.

Out of 150 healthy controls, 93 showed NESTROFT positive, where as 57 reflected NESTROFT negative, The 93 normal who showed NESTROFT positive this may be due to anemic 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 62.6%. 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 (Raghavan et al., 1991). the specificity was found to be 86.9 %. 82% specificity was noticed in the study of Amini SA et al. (2011) and Gorakshaker et al., (1990).

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). 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. 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.

Table 1

Table showing results of NESTOFRT test performed on known beta thalassemia carrier and control subjects.

	Control (n=150)	β thalassemia carrier (n=150)
Positive	93	143
Negative	57	07

Table 2

Table showing sensitivity and specificity of NESTROFT test.

NESTROFT	Sensitivity	Specificity	Positive predictive value	Negative predictive value
	95.2%	62.6%	60.5%	89.0%

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 60.5% and negative predictive was 89.0%. 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.

In conclusion, NESTROFT is a sensitive, cost effective, rapid and reliable screening test for detection of beta thalassemia trait in population. There are number of studies, which have been conducted to find out the incidence of beta thalassemia and the type of mutations prevalent.

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