EVALUATION OF NAKED EYE SINGLE TUBE RED CELL OSMOTIC FRAGILITY TEST IN DETECTING β-THALASSEMIA TRAIT

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ABSTRACT

The Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) was applied to 4 groups of subjects: (i) Normal; (ii) Proven β-thalassemia trait carriers; (iii) Iron deficiency anemia; and (iv) other hemoglobinopathies, to evaluate its effectiveness as a screening test for β-thalassemia minor. The test was successful in detecting 105/110 subjects with β-thalassemia trait. The sensitivity of the test was 95.5% and specificity was 87%. The predictive value of the positive test was 70.5% and that of the negative test was 98.3%. NESTROFT was also positive in 9/17 subjects with HbS trait, in 3/3 subjects with HbD trait and in 1/1 subjects with HbE trait. The test proved to be simple, cheap, easy to perform and adaptable for field surveys, coming close to an ideal screening test for β-thalassemia minor.

Key words: *β-thalassemia minor, Red cell osmotic fragility test.*

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The birth of a thalassemic child places considerable health and economic strain not only on the affected child and its family but also on the community. Since it is a severe and incurable disease, emphasis must shift from treatment of the affected child to prevention of such births in future. Identifying carriers of the thalassemic gene plays an important part in preventing this. Screening for thalassemia minor is costly, laborious and needs technical expertise. The present study was carried out to evaluate the effectiveness of NESTROFT(1) also known as one tube osmotic fragility screening(2) as a screening test for thalassemia minor.

Material and Methods

All patients attending the Hematology OPD at our hospital and all relatives of thalassemic children diagnosed at our Thalassemia Outdoor Transfusion Centre were routinely tested for the following: Hemoglobin, red cell count, red cell indices on an *ERMA* 400 counter, HbA2 estimation by paper electrophoresis and elution, serum iron, total iron binding capacity, transferrin saturation and the naked eye single tube red cell osmotic fragility test (NESTROFT).

Nestroft was carried out as advocated by Mehta *et al.*(1) and Kattamis *et al.*(2) as follows:

A. *Preparation of Reagent:* Stock solution of 10% buffered saline (pH 7.4) was prepared by taking NaCl 90 g, Na_2HPO_4 13.65 g, and Na_2HPO_4. $2H_2O$ 2.4 g and dissolving them in distilled water. The final volume was then adjusted to one litre.

For convenience, a 1% solution is made from the above by 1 in 10 dilution with distilled water. 0.36 buffered saline is prepared by diluting 36 ml of 1% saline with

64 ml distilled water, to make 100 ml.

B. *Test:* Two millilitre of buffered 0.36% saline was taken in one tube (10 cm × 1 cm diameter) and 2 ml distilled water was taken in another tube. A drop of blood is added to both tubes and they were left undisturbed for half an hour at room temperature. After half an hour both tubes were shaken and then 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 is similarly visible through the contents of the tube with the buffered saline, the test is considered negative. If the line is not clearly visible, the test is considered positive.

The subjects were divided into 4 groups:

(*i*) *Group I:* 150 subjects who were normal with regard to abnormal hemoglobins, iron status and had normal hematological values.

(*ii*) *Group II:* This group comprised of 110 patients with β-thalassemia trait, proved by complete hematological investigations.

(*iii*) *Group III:* This group consisted of 189 patients with iron deficiency anemia (transferrin saturation <16%) but otherwise normal hematologically.

(*iv*) *Group IV:* 21 patients with other abnormal hemoglobin variants (17 HbS traits, 3 HbD traits, 1 HbE trait).

The number of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) were determined. The sensitivity, specificity and predictive values were calculated as follows:

(*i*) Sensitivity = $(TP \times 100)/(TP + FN)$

(*ii*) Specificity = $(TN \times 100)/(TN + FP)$

(*iii*) Predictive value of a positive test = $(TP \times 100)/(TP + FP)$

(*iv*) Predictive value of a negative test = $(TN \times 100)/(TN + FN)$

Results

A total of 470 subjects were screened. The results of NESTROFT in the 3 groups–normals, β-thalassemia traits, and iron deficiency anemia (IDA) are shown in *Table I*. NESTROFT was positive in 149 cases; of these 105 had β-thalassemia trait (true positive, TP) and 44 did not have β-thalassemia trait (false positive, FP). The test was negative in 300 cases; of these 5 had β-thalassemia trait (false negative, FN) and 295 did not have the trait (true negative, TN). Sensitivity of NESTROFT was 95.5% and specificity was 87.0%. Predictive value of a positive test was 70.5% and the predictive value of a negative test was 98.3%. NESTROFT was also positive (*Table II*) in 9/17 of subjects with HbS trait, in 1/1 subject with HbE trait and in 3/3 subjects with HbD trait.

TABLE I – *Results of NESTROFT*

NESTROFT	Group I (n=150) Normals	Group II (n=189) IDA	Group III (n=110) β-Thalassemia trait
+ve	13 (8.6)	31 (16.4)	105 (95.5)
–ve	137 (91.4)	158 (83.6)	5 (4.5)

Figures in parentheses represent percentages.

TABLE II – *NESTROFT in Abnormal Hb Variants*

NESTROFT	HbS (n=17)	HbE (n=1)	HbD (n=3)
+ve	9	1	3
–ve	8	0	0

Discussion

The purpose of this study was to evaluate the effectiveness of NESTROFT as a screening test for β-thalassemia trait. In our experience NESTROFT was both sensitive (95.5%) and specific (87.0%) for the identification of β-thalassemia trait. Mehta *et al.*(1) also found similar results of 95% sensitivity and 82% specificity. Kattamis *et al.*(2) found the test effective in detecting almost 100% of subjects with β-thalassemia trait, while it gave a false positive in nearly 10% of normal subjects.

Though NESTROFT was positive in 16.4% of IDA patients as well as in 55.0% of patients with sickle cell trait and in 100% of patients with HbD and HbE trait, their detection is of benefit as each of these conditions has its own health implications. Kattamis *et al.*(2) also found the test useful in picking up these conditions. When used as a population screening test, this will prove to be a beneficial fallout of this test.

The cost of performing a single NESTROFT comes to less than Re. 1.00. It is easy to perform, as much technical expertise is not required. Besides, no initial capital outlay in the establishment of automated cell counters is required. The stock solution once made, keeps well in a stoppered bottle, and thus can be used in field surveys. A single individual can perform 40 to 50 tests in an hour.

The confirmatory tests needed for diagnosing β-thalassemia trait are costly, laborious and time consuming. By excluding normal subjects and thus restricting further investigations for the precise diagnosis to the small proportion of positive subjects, NESTROFT reduces the time, cost and labor. Thus, NESTROFT seems to be valuable as a single screening test in areas with limited laboratory facilities and economic resources as well as for mass screening.

A practical approach would be to perform NESTROFT on an accessible unmarried cohort of people: adolescents at school leaving or before starting college, or young adults starting a job. Positive cases could then be examined for raised HbA2. When one considers the repeated yearly expenses(3) of bringing up a child with thalassemia, preventing thalassemic births by diagnosing and counselling β-thalassemia carriers, becomes the more feasible and attractive alternative.

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