'NESTROFT'-AN EFFECTIVE SCREENING TEST FOR BETA THALASSEMIA TRAIT

Mamta Manglani, M.R. Lokeshwar, Vani V.G., Nishi Bhatia and Vijay Mhaskar

From the Division of Hematology-Oncology, Department of Pediatrics, L.T.M.M. College and L.T.M.G. Hospital, Sion, Mumbai 400 022.

Reprint requests: Dr. Mamta Manglani, 51, Sea Springs, B.J. Road, Band Stand, Bandra, Mumbai 400 050.

Manuscript received: May 7,1996; Initial review completed: May 24,1996; Revision accepted: March 14,1997

Objective: To evaluate the efficacy of NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) as a screening tool for detection of beta thalassemia trait. Design: Prospective study. Setting: Field camps in various parts of Gujarat and Maharashtra States. Methods: A total of 2525 subjects were screened. NESTROFT, complete hemogram including red cell indices and calculation of Mentzer's Fraction (MF) and discriminant functions (DF1-4) were done in all subjects. HbA_2 was performed in 830 initial subjects to compute sensitivity, specificity and predictive values for various parameters. Results: NESTROFT (sensitivity 94.4%), as a single screening parameter was superior to any of the other evaluated parameters individually, besides being cost effective. Mean corpuscular volume (MCV) <80 fl followed NESTROFT closely (sensitivity 93.7; p >0.05). MCV < 75fl had a significantly (p <0.001) lower sensitivity (87.3%) in comparison to both of these parameters. In contrast, MF, DF1, DF2, DF3 and DF4 did not meet the requirements of a good screening test with sensitivity values of 66.2%, 54.9%, 47.2%, 64.1% and 55.6%, respectively. NESTROFT in combination with MCV < 80 fl proved 100% sensitive. However, the combination was not cost effective. Conclusion: NESTROFT is a sensitive, cost effective, rapid and reliable screening test for detection of beta thalassemia trait in a population.

Key words: *Beta thalassemia trait, Naked eye single tube red cell osmotic fragility test, Sirreenina test.*

ALMOST 25 million people in India are carriers of the beta thalassemia gene with a mean prevalence of 3.3%(1) and 6000 to 8000 children are born every year with thalassemia major(2). Only 10 to 15% of these children receive optimal treatment(3); the cost of such treatment for one thalassemic child amounts to Rs. 90,000 to 1,00,000 annually at around 3 years of age, which increases as the child grows. The only cure available today is bone marrow transplantation, which is not affordable to almost all our patients.

The birth of a thalassemic child, thus, 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, emphasis must shift from treatment to prevention of such births in the future. Prospective prevention, which includes population education, mass screening, genetic counselling and prenatal diagnosis, is the only totally effective way to cope successfully with such a disease. Various screening parameters that are available include periph-

eral blood smear examination, red cell indices, osmotic fragility (quantitative), and free red cell porphyrins(4). All these tests including HbA2 estimation (confirmatory test for beta thalassemia trait) are 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 Fraglity Test) in comparison with various other screening parameters.

Subjects and Methods

Test Population and Sample Collection

The current study was undertaken in co-operation with two voluntary organizations. Camps for population screening were conducted in various parts of Gujarat and Maharashtra during the period January 1991 to May 1994. A total of 2525 subjects, below 45 years of age belonging to high risk communities, were included in this study. Out of these, 830 subjects (Representative Group) were screened for all parameters, namely, NESTROFT, mean corpuscular volume, (MCV), Mentzer's fraction (MF), discriminant functions (DF1-DF4; derived using various RBC indices) and HbA2. In the remaining 1695 (Study Group), HbA2 levels were estimated only when NESTROFT proved positive and/or MCV was < 80 fl. Awareness regarding thalassemia was created in the vicinity of camp sites by personnel belonging to the voluntary organizations. This was done variably by distributing pamphlets, having personal meetings, organizing meetings through schools and colleges, putting up posters at the camp sites and occasionally, by individual visits to the families in the given lcoality. Blood samples were collected by a team of two doctors, two laboratory technicians and a laboratory assistant. The laboratory personnel prepared the stock solution of NESTROFT in advance and packed a kit with the solution along with EDTA bulbs, syringes, disposable needles, scalp vein sets, spirit, cotton, gloves, distilled water and test tubes.

NESTROFT

NESTROFT was performed using 0.36% buffered saline solution as advocated earlier(5,6). Two ml of the 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.

Complete Hemogram

Samples of blood taken in the EDTA bulbs were numbered, sealed and transported in ice flasks back to the central laboratory. Complete hemogram including hemoglobin, PCV, RBC count, MCV, MCH and MCHC were performed using Erma PC 604 cell counter within 12 to 24 hours after collection. MF and discriminant functions were calculated with the help of these parameters according to the following formulae with values suggestive of thalassemia trait considered as given below(7,8): (*i*) MF = MCV/RBC <13: *(ii)* DF1 = (0.23 x MCV) - (0.22 x RBC)-(0.93 x Hb)-3 <0; *(iii)* DF2=MCV - (RBC+5Hb+3.4) <0; *(iv)* DF3 = RBC x Hb/MCV >0.8; and *(v)* DF4=MCH/RBC < 3.8. Amongst the red

cell indices, MCV was evaluated using two different values, *i.e.,* < 75 fl and < 80 fl to decide the better cut off value for thalassemia trait.

Hemoglobin Electrophoresis

HbA2 levels were obtained using filter paper electrophoresis(9). A level of > 3.5% was considered as confirmatory of beta thalassemia trait as per our laboratory reference values.

Data Analysis

Data was fed into the computer and analyzed using a specially developed Foxbase software programme. The sensitivity, specificity and predictive values of positive and negative test were computed(10). 'Z' test was applied as a test of significance using the SPSS package to compare the efficacy of various parameters used for screening.

Results

Of the 2525 subjects included in this study, 1911 (75.7%) were above 12 years of age which was the targeted population. The male to female ratio was 1.48:1. One thousand and two subjects (39.7%) belonged to Lohana community, 426 (16.9%) were Gujaratis while there were 428 (17.0%) Punjabis, 560 (22.2%) Sindhis and 109 (4.3%) Maharashtrians.

Table I shows the analysis of results of NESTROFT, MCV, MF, DF1, DF2, DF3 and DF4 in the Representative Group. NESTROFT was positive in a total of 380 (45.7%) subjects out of 830. Out of these, in 134 cases (16.1%), it was true positive (HbA2 > 3.5%), whereas in the remaining 246 (29.6%), it was falsely positive. False negative test was seen in 8 (1.0%) subjects only. MCV was < 75 f 1 in 405 cases (48.7%), out of which thalassemia trait was confirmed in 124 (14.9%). However, MCV < 80 fl gave a higher yield in detection of thalassemia trait (133 out of 142). MF and various other discriminant functions showed a very high rate of false negative tests.

The sensitivity, specificity and predictive values of negative and positive tests of the screening parameters is depicted in *Table II.* NESTROFT was the most sensitive test with a sensitivity of 94.4% and a negative predictive value of 97.6%; MCV < 80 fl had a sensitivity of 93.7% and a negative predictive value of 95.8%; this compared closely with NESTROFT, while MCV < 5 fl had a lower sensitivity (87.8%, p <0.001) as well as a lower-negative predictive value (94.1%; p <0.001). MF and the discriminant functions did not meet the requirements of a good screening test, with a statistically low sensitivity and a negative predictive value as compared to NESTROFT (p <0.001). The sensitivity of MF was 66.2% and that of discriminant functions, DF1, DF2, DF3 and DF4 was 54.9%, 47.2%, 64.1% and 55.6%, respectively.

As shown in *Table III,* NESTROFT in combination with MCV < 80 fl detected 100% of thalassemia traits, thereby suggesting that in combination, the tests are supe-

TABLE I-*Comparison of Various Screening Parameters in the Representative Group (n=830)*

	Confirmed Thalassemia Trait (n=142)	
Screening Parameter	True Positive No. (%)	False Negative No. (%)
NESTROFT	134 (16.1)	8 (1.0)
MCV<80 fl	133 (16.0)	9 (1.18)
MCV<75 fl	124 (14.9)	18 (2.2)
MF	94 (11.3)	48 (5.8)
DF1	60 (7.2)	82 (9.8)
DF2	67 (8.1)	75 (9.0)
DF3	91 (10.9)	51 (6.1)
DF4	79 (9.5)	63 (7.5)

TABLE **II**—*Comparison of Efficacy of Various Screening Tests*

Parameter	Sensitivity (%)	Specificity (%)	Negative predictive value (%)	Positive predictive value (%)
NESTROFT	94.4	64.2	97.6	35.3
MCV<80fl	93.7	40.6	95.8	32.5
MCV<75fl	87.8*	59.2	94.1	30.6
MF	66.2*	82.8	89.5	44.3
DF1	54.9*	91.3	84.5	50.0
DF2	47.2*	86.6	85.1	42.1
DF3	64.1*	82.8	89.0	41.7
DF4	55.6	79.7	85.0	36.1

* Statistically significant difference as compared to NESTROFT

TABLE III- *Efficacy of Various Parameters lndividually and in Combination with NESTROFT*

	Individual sensitivity (%)	sensitivity in combination with NESTROFT (%)
NESTROFT	94.4	-
MCV < 80 fl	93.7	100.0
MCV<75fl	87.3	98.2
MF	66.2	94.0
QP2	54 .9	93.8
DF2	47.2	93.1
DF3	64.1	91.9
DF4	55.6	91.9

rior to either of them individually or in combination with any other tests.

Analysis of various tests performed in the study group *(Table IV)* shows detection of 9.8% thalassemia carriers, when only NESTROFT positive samples were subjected to HbA2 estimation. However, when MCV < 80 fl was combined with NESTROFT, the detection increased to 10.1%. The gene prevalence in various communities was found to be as follows: Lohanas-14.6%, Punjabis -9.3%, Sindhis-*7-7%*> Gujarahs-3.4% and Maharashtnans-1-7%.

Amongst the parameters evaluated in the present study, NESTROFT was found to be the most sensitive (94.4%). Similar results using NESTROFT have been observed by previous workers. The sensitivity of this test usually ranged between 95% to 100%(5,ll). A high sensitivity has also been documented with 0.4% buffered saline instead of 0.36%(12). In a study on 133 Myanmar patients with thalassemia trait, NESTROFT proved superior to various RBC indices and derived discriminant functions(13).

In the present study we also evaluated the significance of 2 different values of MCV with regard to identification of thalassemia trait. We found that MCV < 80 fl (sensitivity-93.7%) compared closely with NESTROFT (sensitivity-94.4%), whereas MCV < 75 fl had a significantly lower sensitivity (87.3%, p <0.001) as compared to NESTROFT. In an earlier Indian study on red cell indices in thalassemia trait, MCV < 80 fl was observed in 94.2% cases, which

TABLE IV - *Analysis of Study Group (n=1695)*

Parameter	True + Ve No. (%)	False + Ve No. (%)	Total + Ve No. (%)
NESTROFT	166 (9.8)	261 (15.8)	427 (25.2)
MCV<80fl	162 (9.8)	380 (22.4)	542 (32.0)
MCV<75fl	154 (9.1)	276 (16.3)	430 (25.2)
MF	81 (4.8)	129 (7.6)	210 (12.4)
DF1	60 (3.5)	115 (6.8)	175 (10.3)
DF2	69 (4.1)	193 (11.4)	262 (15.5)
DF3	81 (4.8)	277 (16.3)	358 (21.1)
DF4	58 (3.4)	76 (4.5)	134 (7.9)
NESTROFT + MCV < 80	171 (10.1)	405 (23.9)	576 (34.0)

compares well to that seen in our series(14). In contrast, another report found MCV < 75 fl to be 100% sensitive and superior to NESTROFT in detecting subjects with beta-thalassemia trait(11). The specificity of MCV < 80 fl (40.55%) was significantly lower ($p < 0.001$) than NESTROFT (64.2%) in the present series as reported earlier(11). MF < 13 was a less sastisfactory parameter to detect thalassemia trait, with a sensitivity of only 66.2%. In contrast, MF values of < 14 (taken as significant for thalassemia trait) were seen in 82.6% of heterozygotes in another Indian study(14). The discriminant functions-DF1, DF2, DF3 and DF4 were even less efficacious in identifying thalassemic heterozygotes as has also been shown by other workers(8,13,14).

To increase the effectiveness of screening, a combination of tests have been used by a number of laboratories(12). In the present study too, incidentally it was found That NESTROFT in combination with the next best parameter (MCV < 80 fl), did not miss identification of even a single case of thalassemia trait, thereby achieving a sensitivity of 100%. This met the requirement of an ideal screening test. However, MCV determination by manual RBC count is often unreliable, thus necessitating an automated particle cell counter. Hence, though ideal, a combination of these 2 tests would considerably increase the cost of screening, thus defeating t he feasibility of utilizing them in areas with limited laboratory facilities and economic resources. As against this, a single test of NESTROFT costs as little as Rs 1.50. Additionally, it is easy to perform, can be used for field studies, does not require sophisticated equipment or technical expertise and can be done from capillary blood obtained by finger prick. This, there fore, reinforces NESTROFT singly, as the most cost effective and promising screening test to detect thalassemic heterozygotes.

In conclusion, NESTROFT is a sensitive, cost effective, rapid and reliable screening test for detection of beta thalassemia trait in a population,

Acknowledgement

The authors wish to thank Dr. (Mrs) S.S. Deshmukh, Dean, L.T.M.G. Hospital, Sion, Mumbai for permitting us to carry out this work and publishing this article. We also wish to acknowledge the help rendered for organising camps by Ghelani Charitable

Trust, Mumbai & Mrs. Jasmine Majethia, Liaison officer, LTMG Hospital, Sion, Mumbai

REFERENCES

1. Verma IC, Choudhry VP, Jain PK. Prevention of thalassemia: A necessity in India. Indian J Pediatr 1992; 59: 649-654.
2. Varawalla NY, Old JM, Sarkar R, Venkatesan R, Weatherall DJ. The spectrum of p-thalassemia mutations on the Indian subcontinent: The basis for prenatal diagnosis. Br J Hematol 1991; 78:242-247.
3. Choudhry VP, Desai N, Pati HP, Nanu A. Current management of homozygous beta thalassemia. Indian Pediatr 1991: 28: 1221-1229.
4. Shine I, Lai S. A strategy to detect p-thalassemia minor. Lancet 1977; 1: 692-694.
5. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting p-thalassemia trait. J Med Genet 1981; 18: 266-270.
6. Mehta BC, Gandhi S, Mehta G, Kamath P, Singhal S, Mehta JB. A simple rapid screening test for p-thalassemia trait. Indian J Hemat 1990; 8: 5-9.
7. Lukens JN. Iron metabolism and iron deficiency. *In:* Blood Diseases of Infancy and Childhood, 6th edn. Eds Miller DR, Bachner RL. St. Louis, C.V. Mosby Company 1989; pp 170-198.
8. Collaborative Study on Thalassemia. An ICMR Task Force Study. New Delhi, Indian Council of Medical Research, 1993; pp 14-15.
9. Black MB, Miller H, Wen J. Quantitative determination of hemoglobin A2 by filter paper electrophoresis. Am J Clin Path 1966; 46:483-485.
10. Raghavan K, Lokeshwar MR, Birewar N, Nigam V, Manglam MV, Raju NB. Evaluahon of naked eye single tube red cell osmotic fragility test in detecting p-thalassemia trait. Indian Pediatr 1991; 28: 469-472.
11. Mehta BC, Gandhi S, Mehta JB, Kamath P. Naked eye single tube red cell osmotic fragility test for B-thalassemia: Population survey. Indian J Hemat 1988; 6:187-190.
12. Cao A, Galanello R, Furbetta M, Muronni PP, Garbato L, Rosatelli C, *et al.* Thalassemia types and their incidence in Sardinia. J Med Genet 1978; 15:443-447.
13. Han KE, Han AM, Win K, Myint TT. Thalassemia in the Outpatient Department of the Yangon Children's hospital in Myanmar: Basic hematological values of thalassemia traits. South East Asian J Trop Med Pub Health 1992; 23: 264-268.
14. Madan N, Trehan P, Taneja N, Ghai OP, Sood SK. Red cell indices in beta-thalassemia trait and iron deficiency. Indian J Med Res 1982; 75: 719-725.