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Role of Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) in Detecting Beta-Thalassemia Trait

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Summary:

Thalassemia is one of the commonest inherited diseases in Bangladesh. The birth of a thalassemic child places considerable strain, not only on the affected child and its family but also on the community and the nation at large. To reduce the burden of the society and to reduce the disease incidence by providing genetic counseling, detection of carrier is important.

The present study evaluates the role of 'Naked Eye Single Tube Red Cell Osmotic Fragility Test' (NESTROFT) in detecting ß-thalassemia trait.

The current study is a cross sectional study done during the period of September 2008 to August 2009. The study subjects were sibs, parents and relatives of thalassemia patients of age more than 1 year attending Pediatric department of Chittagong Medical College Hospital. Sample size was 50. Here subjects with lowered osmotic fragility test were detected and later on Hb- electrophoresis was done. All the data were recorded and analyzed by SPSS programme. The Sensitivity, Specificity and predictive value of positive and negative tests were computed and they were 92.6%, 80%, 92.6% and 80% respectively. False positive cases were found.

The present study found NESTROFT to be both sensitive and reasonably specific and of high negative predictive value. However, multicenter study with large sample is needed to recommend NESTROFT as a single screening test for detection of ß-thalassemia trait.

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Introduction:

Thalassemia is the most common inherited gene disorder in the world and varies in different population groups in the world.[1] Thalassemia is more prevalent in the Mediterranean basin, the Middle East, Southern eastern Asia, the South China, with reported carrier rates ranging from 2% to 25%. Recent data indicate that about 300,000-500,000 children are born each year with the severe homozygous states of these diseases (World Bank 2006, report of a joint WHO-March of Time meeting 2006).

Thalassemia is one of the commonest inherited disease in Bangladesh[2]. A conservative world report has estimated that 3 percent are carriers of beta-thalassemia and 4 percent are carriers of Hb-E in Bangladesh[3]. In Bangladesh, a study done on 735 school children the average prevalence of beta-thalassemia trait was 4.1% and Hb-E trait was 6.1% in Bengali school children. Among tribal school children in Chittagong, the average of beta-thalassemia trait was 4.2% and Hb-E trait was 41.7%[4]It is presumed that approximately six thousands thalassemic children are born each year in Bangladesh[1].

It's treatment is very cumbersome and costly. The only cure available today is bone marrow transplantation, which is risky and too costly for most of the patient. The cost of treatment for one thalassemic child in our country also approximately 70,000 to 90,000 taka per year[5]. Being a poor country, majority of thalassemia patients do not get adequate treatment.

The birth of a thalassemic child places considerable stain, not only on the affected child, but also on the family, community and the nation at large. With these limitations, emphasis must shift from treatment to prevention of such births in near future. This can reduce the disease incidence; hence reduce the burden of the society.

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The most effective approach is implementation of a carrier screening program offering genetic counseling, prenatal diagnosis and selective termination of affected fetus.[6]Various screening parameters that are available include peripheral blood smear examination, red cell indices,[7-9] osmotic fragility(quantitative), free red cell porphyrins[10], RBC count[11] and RDW[12]. All these test, including HbA_2 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 used for mass screening. NESTROFT is such a test used for detection of beta- thalassemia trait.

Various studies have been carried out in the world, especially in the developing countries similar to India, Myanmar, Iraq, Thailand[13-16] to see sensitivity and specificity of NESTROFT. This study has been undertaken to determine the validity of simple and inexpensive method, NESTROFT as a screening test for detection of heterozygous beta- thalassemia.

Methodology:

Study design: It is a descriptive cross sectional study.

Place of study: The study was carried out in pediatric department of Chittagong Medical College and Hospital.

Period of study: One year starting from September 2008.

Time of data collection: 15th September 2008 to 15th September 2009.

Study population: Sibs, parents and relatives of thalassemia patients attending pediatric department of Chittagong Medical College Hospital.

Sample size determination:Sample size was determined on the basis of the following formula. $N = Z^2pq$ Here, N=Sample size

e^2 Z=the value of standard variate at given confidence level
Usually at 1.96 which corresponds to 95% confidence limit.
P=Estimated prevalence which will be 4.1%
q=1-p that is 95.9%
e=Acceptable error.

Thus, N=37762

According to this formula, the calculated sample size is 37762.

It became a big number for a single personto collect data and samples from the patients with in the decided time frame. Moreover, resource constrain is also an important factor. I therefore collected as many as possible, which was 50 in number.

Sampling technique:Sample was selected by non-probability convenience sampling technique.

Research instrument: case record form.

Inclusion criteria:

Any one >1 year of age among sibs, parents and relatives of thalassemia patients.

Exclusion criteria:

1. Subjects suffering from Hb-electrophoresis proved thalassemia or any other severe illness.
2. Subjects with moderate to severe anaemia.
3. Blood transfusion within last 90 days.

Results:

During the study period 50 subjects were screened. Among them 27 subjects were ²-thalassemia trait, 11 subjects were Hb-E trait, 10 were with normal hemoglobin and other 2 were Hb-E disease. Among the total 50 subjects, mild pallor was found in 9 subjects. Total NESTROFT positive cases were 30 and NESTROFT negative cases were 20 in number.

Among the total study subjects 28 (56%) were female and 22 (44%) were male. Female predominated in these series. Distribution of sex among the study subjects is shown in Table-I.

Table-I

Distribution of sex among the study subjects (n=50)

Sex	Frequency	Percentage (%)
Male	22	44.0
Female	28	56.0
Total	50	100.0

Age ranged between 2 and 52 years with a mean age of 30.12±10.02. Age distribution of the series is described in Table-II

Table-II

Distribution of age among the study subjects (n=50)

Age (years)	N	Mean	±SD	Median	Range
	50	30.12	10.02	30.50	2"52

Relation of study subjects with thalassemia patients was various. Among them no. of mother, father, brother, uncle, sister and aunt were respectively 22 (44%), 14 (28%), 4 (8%), 4 (8%), 3 (6%) and 3 (6%). Distribution of relation with thalassemia patients is shown in Bar Chart-01.

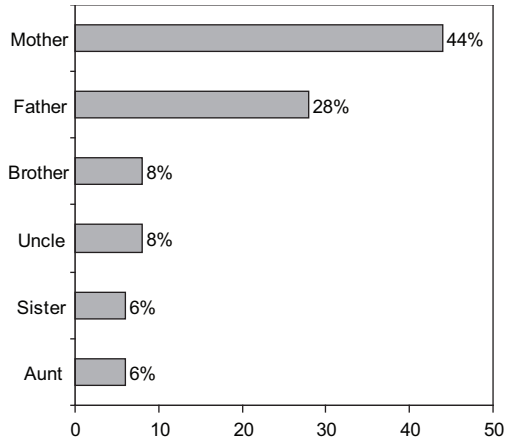

Fig.-1: *Distribution of relations of study subjects with affected persons (n=50)*

Among the 50 subjects carrier status of 5 persons (10%) were known from before. That is hemoglobin electrophoresis was done before they were included in the present study, which reflects awareness of the general population especially relatives of thalassemia patients regarding the carrier status of thalassemia. Carrier detection status of relatives of thalassemia patients is shown in Pie chart-01.

Distribution of pallor among the study subjects is described in Pie Chart -02. In the present, 9 subjects (18%) were mildly pale and 41 subjects (82%) were not pale.

Pie-chart-03 depicts that NESTROFT resultsamong the study subjects. It was positive in 60% of study subjects.

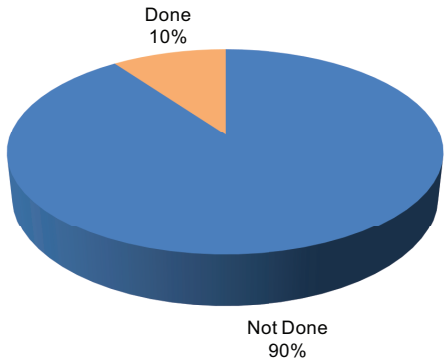

Fig.-2: *Distribution of carrier detection status of study subjects.*

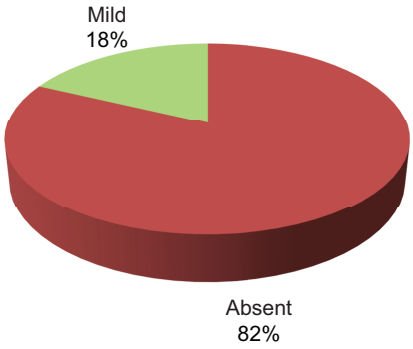

Fig.-3: *Distribution of pallor among the study subjects.*

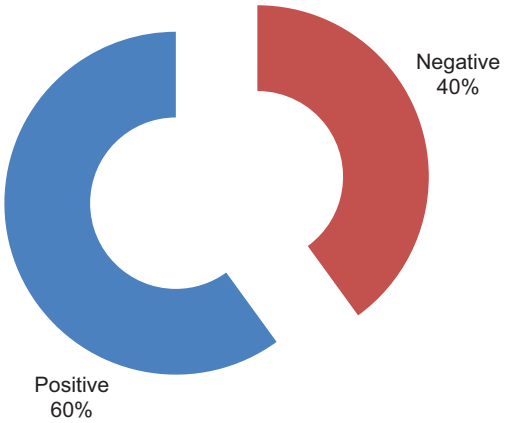

Fig.-4: *Distribution of NESTROFT results among the study subjects.*

Bar Chart-02 describe the 27 (54%), 2(4%), 11(22%) of study subjects were detected respectively as ^{2}-thalassemia trait, Hb-E disease, Hb-E trait by Hb-electrophoresis, while 20% were normal.

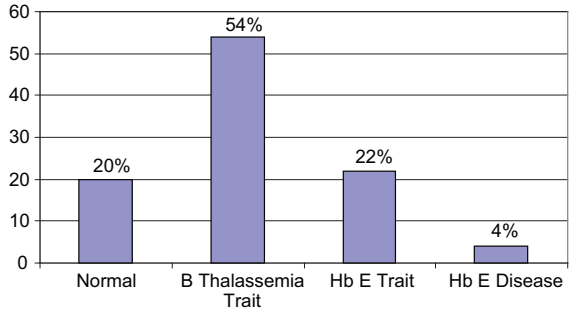

Fig.-5: *Distribution of Hb electrophoresis results (n=50)*

Bar Chart-03 shows distribution of hemoglobin electrophoresis results ignoring Hb-E disease and Hb-E trait. Among total 37 cases, 27 (73%) cases were detected as ²- thalassemia trait and 10(27%) cases were normal on hemoglobin electrophoresis.

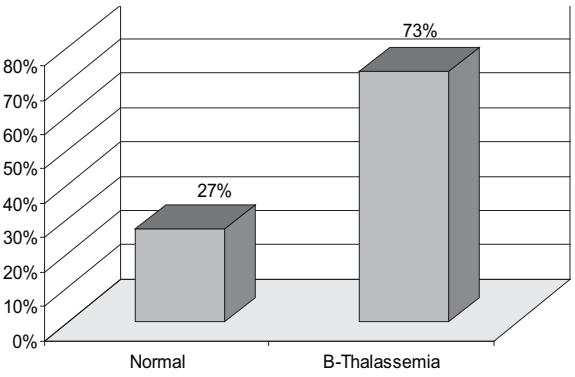

Fig.-6: *Distribution of Hb electrophoresis results (n=37)*

Table-03 describes the distribution of normal and abnormal hemoglobin on Hb- electrophoresis among the total 50 subjects. Here, 80% patients were detected to have abnormal hemoglobin on Hb-Electrophoresis.

Table-III

Distribution of normal and abnormal hemoglobin on Hb- electrophoresis (n=50)

Hb-Electrophoresis Result	Frequency	Percentage (%)
Normal	10	20.0
Abnormal	40	80.0
Total	50	100.0

Table-04 shows distribution of pallor by NESTROFT results with X² significance among the total 50 subjects. About 89% of the patients with mild pallor were detected NESTROFT positive, while among the subjects with normal hemoglobin about 54% were NESTFORT positive.Pearson's Chi-square test was done to find out the association between pallor and NESTROFT results. However, just significant was found (P=0.05).Fisher's Exact test was also just significant P-0.05.

Table-05 describes that 20% of subjects with normal hemoglobin and 70% of subjects with abnormal hemoglobin pattern were NESTROFT positive. Pearson's Chi-square test was done to find out the association between abnormal hemoglobin pattern and NESTROFT result. Highly significant association was found (P<.01). Fisher's Exact test was also significant P=0.006 (P=<.01).

Table-06 depicts the distribution of Hb-electrophoresis results by NESTROFT with X² significance (n=50). It shows that among the ²-thalassaemia traits, 92.6% were NESTROFT positive, while among the normal patients, only 20% were NESTROFT positive. Pearson's Chi-square test done to find out the association between NESTROFT and Hb-Electrophoresis results. It was found to be statistically highly significant (P<0.001).

Table-07 describes the distribution of Hb-electrophoresis results by NESTROFT with X² significance (n=37)

Among 27 cases of beta-thalassemia trait, NESTROFT was found positive in 25 (92.6%) subjects and 2 (7.4%) cases were found NESTROFT negative; among 11cases of Hb-E trait the test was found positive in 1 (9.1%) and was negative in 10 (90.9%) subjects ; among 2 cases of Hb-E diseases all the subjects i.e. 100% were found NESTROFT positive; among 10 normal subjects 2(20%) were found positive and 8 (80%) were found negative. Pearson's Chi-square test was done to find out the association between NESTROFT and ²-thalassemia carrier. It was found to be statistically highly significant (P<.001). Fisher's Exact test was also highly significant P=0.000 (P=<.01).

Table-IV

Distribution of pallor by NESTROFT results with X^2 significance (n =50)

Pallor	Nestroft Result				Total	
	Positive		Negative			
	n	%	n	%	n	%
Mild	08	88.9	01	11.1	09	100.0
Absent	22	53.7	19	46.3	41	100.0
Total	30	60.0	20	40.0	50	100.0

X^2=3.817. df=1. P=0.051. Just Significant

Table-V

Distribution of normal and abnormal Hb-electrophoresis results by NESTROFT (n=50)

Hb-Electrophoresis Result	Nestroft Result				Total	
	Positive		Negative			
	n	%	n	%	n	%
Normal	02	20.0	08	80.0	10	100.0
Abnormal	28	70.0	12	30.0	40	100.0
Total	30	60.0	20	40.0	50	100.0

X^2=8.333. df=1. P=0.004. Highly Significant

Table-VI

Distribution of Hb-electrophoresis results by NESTROFT with X^2 significance (n=50)

Hb-Electrophoresis Result	Nestroft Result				Total	
	Positive		Negative			
	n	%	n	%	n	%
Normal	02	20.0	08	80.0	10	100.0
B-thalassaemia trait	25	92.6	02	7.4	27	100.0
Hb E trait	01	9.1	10	90.9	11	100.0
Hb E disease	02	100.0	00	0.0	02	100.0
Total	30	60.0	20	40.0	50	100.0

X^2=31.829. df=3. P=0.000. Highly Significant.

Table-VII

Distribution of Hb-electrophoresis results by NESTROFT with X^2 significance (n=37)

Hb-Electrophoresis Result	Nestroft Result				Total	
	Positive		Negative			
	n	%	n	%	n	%
Normal	02	20.0	08	80.0	10	100.0
B-thalassaemia trait	25	92.6	02	7.4	27	100.0
Total	27	73.0	10	27.0	37	100.0

X^2=19.498. df=1. P=0.000. Highly Significant.

Table-VIII

Usefulness of NESTROFT in heterozygous beta- thalassaemia: comparison of data with previous studies.

	Singh[19] et al (2008)	kattamis[14] et al (1981)	mehta[17] et al (1988)	Gorakshaker[18] et al (1990)	Raghavan[21] et al (1991)	Thomas[22] et al (1996)	Manglani[10] et al (1997)	Maheshwari[6] et al (1999)	SiIrichotiyakul[20] et al (2004)
Sensitivity (%)	97.7	98.4	95.0	98-100	95.5	98.4	94.4	91.0	97.6
Specificity (%)	83.3	91.0	82.1	82-84	87.0	66.6	64.2	95.0	72.9
Positive Predictive Value (%)	95.5	91.3	73.1	50.0	70.5	81.5	97.6	55.0	33.6
Negative Predictive Value (%)	90.9	98.3	97.0	99-100	98.3	96.5	35.3	99.0	99.5

Sensitivity, specificity, positive and negative predictive values of the test were calculated as validity statistics by the following formulae:

Sensitivity: 100 x TP ÷ (TP + FN)
Specificity: 100 x TN ÷ (TN + FP)
Positive predictive value: 100 x TP ÷ (TP + FP)
Negative predictive value: 100 x TN ÷ (TN + FN)

Here, TP = True positive i.e. have disease and have positive test.
FP = False positive i.e. no disease but have positive test.
TN = True negative i.e. no disease but have negative test.
FN = False negative i.e. have disease but have negative test.

In this study, sensitivity of NESTROFT was 92.6% and specificity was 80%, predictive value of a positive test was 92.6% and predictive value of negative test was 80%.

Discussion:

Any programme for the prevention of Cooley's anaemia requires, as a preliminary step, the reliable identification of young people with thalassemia. Screening for beta- thalassemia trait is extremely difficult. This is mainly because of the heterogeneity of beta- thalassemia variants[17]. In spite of these difficulties, many attempts have been made to establish a screening test capable of detecting all beta-thalassemia variants. Various screening tests for carrier screening have been developed, viz. Determination of red cell indices, HbA, HbA² and HbF level estimation; however, all these techniques are time consuming and expensive for population screening.

In the present study, mean age was 30.12±10.02 years and female subjects predominated, they constitute 56%. Carrier status of 10% of study subjects was known from before. That is hemoglobin-electrophoresis was done before the persons were included in the present study, which reflects awareness of general population especially relatives of thalassemia regarding carrier of thalassemia.

In this study, 18% of study subjects were found mildly pale. Among them 2 were found normal on haemoglobin electrophoresis. They were not further investigated for detection of iron deficiency anaemia, as we know that iron deficient subjects may be NESTROFT positive. Among the rest of the pale subjects, there were 3 beta-thalassemia trait, 2 Hb-E trait and Hb-E disease.

From the result of this study it was shown that NESTROFT was successful in detecting 92.6% of subjects of beta- thalassemia trait. It was also shown that 100% of Hb-E disease was detected.

Table –08 shows the usefulness of NESTROFT in heterozygous beta- thalassaemia along with comparison of data with previous studies.

The present study found the NESTROFT to be more sensitive though not as specific, which correlates with other studies. Table-08 compares the sensitivity, specificity, positive and negative predictive values of the NESTROFT in the present study with those of other similar studies. All the previous reports have shown a sensitivity that is above 91%, the specificity has varied from 64.2%-95%. The sensitivity reported in other studies (Table-08). The specificity in the present study was 80%, which is comparable to obtained by Mehta et al.[17]Gorakshakar et al[18] and

Singh et al[19]. The negative predictive value of the test in carriers during the present study was 80%. The result is low but comparable with the study of Singh et al.[19]. Manglaniet al[10] reported much lower value 35.5%. Kattamis et al,[14]Mehta et al,[17]Gorakshakar et al[18] and

Sirichotiyakul et al,[20] who reported negative predictive values of 98.3%.97.0%,99-100%, 98.3%, 96.5%, 99.0% and 99.5% respectively.

In the present study, the test led to both high sensitivity and specificity; this is a desirable factor for judging the effectiveness of a test. Calculation of the negative predictive value in the test helps to rule out the possibility of beta-thalassemia trait in the general population. The application of this test for screening the cases before further investigations would reduce financial implications faced in performing other costly tests on the general population. The positive predictive value of the test has significance in a particular population with high prevalence of the disease. The positive predictive value was high (92.6%) and comparable to the studies conducted by Kattamis et al[14] and Singh et al,[19] who reported values of 91.3% and 95.5% respectively; higher then 73.1% reported by Mehta et al,[17]50% reported by Gorakshaker et al,[18] 705.% reported by Raghavan et al,[21] 81.5% reported by Thomas et al,[22] 55% reported by Maheshwari et al,[6] and 33.6% reported by Sirichotiyakul et al [20]and lower than 97.6% reported by Manglani et al.[10]

Although detection of the thalassemia trait using NESTROFT was successful in 92.6% of subjects with this trait. It also gave a false positive test in 20% of the normal individuals, which is quite comparable with 18.5% reported by Gomber et al.[23] Although this test is easy to perform, fast, cheap and does not require sophisticated equipment, there are certain limitations of this test. As observed during the study, it gives false positive results in the case of patients with pallor. This would affect the specificity of the test in a population with high incidence of nutritional anaemia. Therefore, subjects positive with NESTROFT need to undergo further investigations to confirm the diagnosis. The test also needs careful standardization.

In this study NESTROFT showed high sensitivity and specificity, also high negative predictive value. NESTROFT has thus emerged as an inexpensive, most sensitive and specific test of population screening for the beta-thalassemia trait, and is considered suitable for large scale use in developing countries like Bangladesh which has limited financial and technical resources.

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