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Original Article

Validity of Naked Eye Single Tube Red Cell Osmotic Fragility Test for the Diagnosis of β-Thalassemia –A Case Control Study

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Abstract

This study was conducted at the department of paediatrics, Rajshahi Medical College Hospital over a period of 2 years to see the sensitivity, specificity, positive predictive value and negative predictive value of Naked Eye Single Tube Red cell Osmotic Fragility (NESTROF) test for the diagnosis of β-thalassemia. Fifty eight children with β- thalassemia were taken as case and 58 children were taken as control. Among the control 29 children were normal (without anaemia) and 29 children had anaemia other than β-thalassemia. Hemoglobin electrophoresis was done to all children to enroll them as case or control. Then NESTROF test was performed in all cases and controls. The data was analyzed by SPSS V.12. This study showed that sensitivity, specificity, positive and negative predictive value of NESTROF test was 96.55%, 72.41%, 77.77% and 95.45% respectively.

Key Words: Anaemia, Thalassemia, β-thalassemia, Naked Eye Single Tube Red Cell Osmotic Fragility (NESTROF) test.

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Introduction

Thalassemia is one of the common congenital disorders in the worldwide including Bangladesh. Three percent of the world's population carries genes for beta thalassemia.[1] No population group is completely free from thalassemia.[2] There are two types of thalassemia: (1) Alpha thalassemia and (2) Beta thalassemia. Alpha thalassemia is rarely seen in our country while beta thalassemia is common.[3] the world population of carriers of beta thalassemia trait is reported to be more than 100 million worldwide. About 100000 children with thalassemia major are born each year. Bangladesh also lies in the thalassemia belt.[4] Parents who carry abnormal gene pass it to their children. Beta thalassemia carriers are normal except that some of them are mildly anemic. The carrier status of β-thalassemia trait in our population is 4.1%. In Bangladesh the expected births of β-thalassemia major infants annually are about 1040 and Hb E beta thalassemia are about 6443. Expected total beta thalassemia major patients in our country is 52,015.[5] The birth of a thalassemic 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, emphasis must shift from treatment to prevention of such births in the future. Prospective prevention, which includes population-education, mass screening, genetic counseling and prenatal diagnosis, is the only totally effective way to cope successfully with such a disease. Various screening parameters that are available include peripheral blood smear examination, red cell indices, osmotic

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fragility (quantitative), and free red cell porphyrins. As against this, a single test of NESTROFT costs very little. 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, therefore, reinforces NESTROFT singly, as the most cost effective and promising screening test to detect thalassemic patients.

Material and Methods

This case control study was carried out in the department of pediatrics, RMCH from January, 2009 to December, 2010. A total of 116 children were included in this study. Among them 58 were taken as cases and another 58 were taken as control. Cases were defined as children of 1 to 12 year old suffering from beta thalassemia where diagnosis was confirmed by Hb electrophoresis. Among the controls 29 were anaemic children other than beta thalassemia and E beta thalassemia and 29 normal children. Patients were selected from all indoor units and outpatient department of pediatrics of RMCH. The siblings of β-thalassemia children were also included. After introduction with the subject and the attendants, an informed consent was taken from them. Demographic details including age and gender and a brief, relevant clinical history was recorded. The history included a complete family history of pallor requiring blood transfusions or a confirmed diagnosis of thalassemia. Confounding variables (age, gender, family history of thalassemia) were controlled through matching. Next naked eye single tube red cell osmotic fragility test was done on all the cases and controls. Other hematological parameters were also assessed.

Nestrof Test

It was carried out by the procedure as advocated by Gomber et al.[6] A stock solution of buffered saline was prepared by taking Nacl 90gm, Na2PO4 13.655 g and NaH2 PO42HO2 2.4 g, and dissolving in 11 of distilled water. From this 1 per cent buffered saline was prepared by 1:10 dilution with distilled water. The working solution of 0.36 per cent buffered saline was prepared by diluting 36 ml of buffered saline with 64 ml of distilled water to make 100 ml. Two ml of buffered saline 0.36 per cent was taken in one tube (10 x 1 cm diameter) and 2 ml of distilled water was taken in another tube. EDTA blood (20/µl) was added to both these tubes. Tubes were shaken well and then were left undisturbed for half an hour at room temperature. After half an hour contents of both these tubes were shaken and the tubes were held against a white paper on which a thin black line was already drawn. The line was clearly visible through the contents of the tube containing distilled water (control). If the line was similarly visible through the contents of the tube with buffered saline then test was considered as negative where as if line was not visible then the test was considered positive. The principle of NESTROFT is based on the limit of hypo tonicity which the red cells can withstand. There is a pronounced decrease in osmotic fragility of red cells in β-thalassaemia. Sensitivity, specificity, positive and negative predictive value of NESTROF test was calculated by using statistical formula. Data analysis was done by SPSS program 12 V.

Results

The age range of the study population varied from 1 year to 12 years with a mean age of 4.6 ±2.9 Years (Table: 1). There was slight male preponderance with a male and female ratio of 37: 21 (Figure 1). Majority patients were β-thalassemia trait (51.72%) followed by β-thalassemia major (32.76%) and only 15.52% were β-thalassemia intermedia (Figure 2). The Hematological parameters in different types of β-thalassemia are showen in Table 2. The mean level of hemoglobin in β-thalassemia trait (BTT) was 10.84±1.45 g/dl, in β-thalassemia intermedia (BTI) was 8.55±1.36 g/dl and in β-thalassemia major (BTM) was 4.98±0.89g/dl. Total count of RBC in BTT was 5.88±0.65 M/µl, BTI was 4.82±0.46 M/ µl, and BTM was 3.7± 0.38 M/µl. Mean value of MCV was 65.68±6.46%, 64.31±4.62% and 59.34±4.6 fl in BTT, BTI and BTM respectively. Mean value of MCH was 20.93±2.42 pg, 19.47±1.74 pg and 16.36±1.57 pg in BTT, BTI and BTM respectively. The reticulocyte percent was mean±SD in BTT was 1.85±0.58% followed by BTI and BTM was 5.03±1.23% and 7.47±1.78% respectively. Table 3, 4 and 5 shows the result of hemoglobin electrophoresis in

different types of thalassemia. Among 58 cases NESTROFT was positive in 56 (96.55 %) cases and negative in 2 (6.67%) cases. It was 100% positive in both beta thalassemia major and intermedia group but 93.33% positive in trait (Table 6). NESTROFT gave positive results in 27.6% controls and negative results in 72.4% controls. It was positive in 100% (1/1) sickle cell trait, 60% in HbE disease followed by, HbE trait 55.55% and Iron deficiency anaemia 28.57%. It was also positive in 7% normal children (Table 7).

Table 1: frequency distribution of patients by age

Age in groups	**Frequency**	
	N	%
1-2 years	15	25.9
2-5 years	26	44.8
5-8 years	10	17.2
>8 years	7	12.1
Total	58	100

Figure 1: Frequency of distribution of patients by sex

Figure 2: frequency distribution of cases by clinical types of beta thalassemia

Table 2: Hematological parameter in different types of beta thalassemia

Parameter	Diagnosis			P value
	Minor	Intermediate	Major	
Hb gm/dl	10.84±1.45	8.55±1.36	4.98±0.89	0.000
Total count of RBC	5.85±0.65	4.82±0.46	3.7±0.38	0.000
MCV (fl)	65.68±6.46	64.31±4.62	59.34±4.6	0.001
MCH (pg)	20.93±2.42	19.47±1.79	16.36±1.57	0.000
Reticulocyte count (%)	1.85±0.58	5.03±1.23	7.47±1.78	0.000

Table 3: Result of Hb electrophoresis in BTT

Types of Hb	**Mean (%)**	**SD**	**Minimum (%)**	**Maximum**
HbA	93.91	1.47	90.80	96.20
HbF	1.43	1.14	0.00	4.20
HbA_2	4.72	1.11	3.60	8.60

Table 4: Result of Hb electrophoresis in Beta thalassemia intermedia

Types of Hb	**Mean (%)**	**SD**	**Minimum (%)**	**Maximum**
HbA	72.37	13.00	46.80	87.30
HbF	26.21	12.46	9.30	49.70
HbA_2	2.78	0.55	1.90	3.50

Table 5: Result of Hb electrophoresis in Beta thalassemia major

Types of Hb	**Mean (%)**	**SD**	**Minimum (%)**	**Maximum**
HbA	3.48	1.75	0.70	7.70
HbF	93.63	1.87	89.30	96.70
HbA_2	2.76	0.71	1.10	3.80

Table 6: Result of NESTROFT in cases (n=58)

NESTROFT	**Positive**		**Negative**	
	N	%	N	%
BTT (n=30	28	93.33	02	6.67
BTI (n=9)	09	100	00	00
BTM (19)	19	100	00	00
Total	56	96.55	02	3.45

BTT= Beta Thalassemia Trait, BTI= Beta Thalassemia Intermedia, BTM= Beta Thalassemia Major

Table 7: Result of NESTROFT in controls (29 anemic other than beta thalassemia and 29 normal child), n=58

Type of controls	Number of controls	Frequency			
		Positive		Negative	
		N	%	N	%
Hb E disease	10	06	60.00	04	40.00
Hb E trait	09	05	55.55	04	42.86
Sickle cell trait	01	01	100	00	00
Iron deficiency anemia	07	02	28.57	05	71.43
Kala-azar	01	00	00	01	100
Chronic liver disease	01	00	00	01	100
Normal subject	29	02	7.0	27	93
Total	58	16	27.6	42	72.4

Calculation of validity of NESTROFT in the diagnosis of Beta thalassemia:

Sensitivity : = x100 s $= \frac{56}{56+2} x100$ $\frac{56}{58} x100$ = = 96.55%		thalassemia case n=58 a (true positive) = 56 c (false negative) = 02 b (false positive) = 16(control) d (true negative) = 42 (control)

Specificity = $\frac{42}{16+42} x100$ = $\frac{42}{58} x100$ = 72.41%

Predictive value of a positive test = $\frac{56}{56+16} x100$ = $\frac{56}{72} x100$ = 77.77%

Predictive value of a negative test = $\frac{42}{2+42} x100$ = $\frac{42}{44} x100$ = 95.45%

Accuracy = $\left[\frac{56+42}{56+42+16+2} x100 \right] = \frac{98}{116} x100$ =84.48%

Discussion

Naked Eye Single Tube Red cell Osmotic Fragility (NESTROF) test is described as an important screening test for β-thalassemia. It is a simple and easily reproducible test with a high degree of sensitivity and specificity. However, there is paucity of literature on NESTROF test. No work is reported from Bangladesh where the prevalence of β-thalassemia is high. Though all the previous studies have shown a sensitivity that is above 90%, the specificity has varied from 70% to100%. The sensitivity of NESTROF test in the present study is 96.55%. This result is quite comparable to many studies.[6,7,8,9,10,11] The specificity of NESTROF test in the present study is 72.41%. This result is almost similar to 71.7% as reported by Suri et al [10] but lower than several other studies like Gomber et al, Mehta et al, Bobhate et al and Javeria et al.[6,7,8,11] In this study we found the negative predictive value of NESTROF test is 95.45%. The result is comparable with many of the previous studies.[6,10,11,12,13] The important point brought to the notice is that the presence of negative test almost rules out the possibility of β-thalassemia. The application of this test for screening the cases before further investigations would reduce the work load on specialized laboratories. The positive predictive value of the test has significance in a particular population with

high prevalence of the disease. The positive predictive value of NESTROF test in present study is 77.77% which is higher than the study by Suri et al, Gorakshaker et al and Mehta et al.[10,12,13] but lower than Gomber et al and Javeria et al. [6,11]

We have found false positive result in 16 childs which comprises 27.6% of control group. Among them 02 had severe iron deficiency anaemia, 06 had haemoglobin E disease, 05 had haemoglobin E trait, 01 had sickle cell trait and 02 were normal child. The false positive rate of this study is quite comparable with 24% as reported by Mehta et al.[7] Severe iron deficiency anaemia can be the cause of false positive NESTROF test due to decreased osmotic fragility. Thus the false positive results may affect the specificity of the test in a population with a high incidence of iron-deficiency anaemia.[14] Therefore, subjects positive with NESTROF test need to undergo further investigations to confirm the diagnosis. The test also needs careful standardisation.

Conclusion

Naked eye single tube red cell osmotic fragility test is a reliable, cost-effective, rapid and easily interpreted test for diagnosis of β-thalassemia. It is a highly sensitive and specific test for all type of β-thalassemia including trait. So it will be suitable for large scale use in a developing country like Bangladesh which has got limited financial and technical resources.

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