ORIGINAL ARTICLE

Nestroft Validity for Screening of Extended Families of Thalassemia Patients

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ABSTRACT

Objective: To access effectiveness of NESTROFT as screening tool for detecting thalassaemia carriers in extended families of thalassaemia major /intermedia patients.

Patients and Methods: In a cross sectional study total no. of 25 families of index cases of Thalassaemia, attending Thalassaemia unit of Fatma Jinnah Medical college, Lahore, Pakistan, were visited and 561 samples were collected for CBC, red cell indices, Naked-Eye-Single-Tube-Red-Cell-Osmotic-Fragility-Test (NESTROFT), Hb Electrophoresis and DNA analysis (if required),without prior iron administration. Average extended family size was 23.Thisstudy was conducted for duration of 15 months.

Results: 515 NESTROFT tests were valid. 220 NESTROFT were positive and 295 NESTROFT were negative in detecting Thalassemia trait. Hb electrophoresis was performed as confirmatory test for Thalassemia trait. Sensitivity of NESTROFT was calculated as 55% and Specificity was 64%. Positive Predictive Value was 46% and Negative Predictive Value was 64%.

Conclusion: NESTROFT may not be a good screening test for mass screening of iron deficient high risk population.

Key words: NESTROFT, Validity, Screening, Thalassemia patients

INTRODUCTION

Thalassemia is one of the most common genetic disorders with 1.5% prevalence among general population, globally affecting 60,000 infants every year.[1] Thalassemia is more prevalent in areas of Mediterranean, Middle East, central Asia, Southern China and India.[2] Pakistan is located in East Mediterranean region of WHO and has carrier frequency for β-thalassaemia of 5.6 % in general population.[3]

Thalassaemia has striking impacts on economical constrained health systems in third world countries. An average Pakistani family with per capta income of $1368[4] cannot afford the expensive treatment cost of thalassemia. Approximately 40,000 cases of transfusion-dependent children with thalassaemia major presently registered and each year nearly 5250 are born in the country[5], almost 40% of health care budget will be spent on Thalassemia treatment with these high numbers.[6] Ideal option for prevention of single gene inherited disorder is by early detection and stopping the genetic transmission to next generation rather than expensive treatment. Worldwide screening and prevention programs adopt one or more of the following methods: extended and immediate family screening; newborn screening; premarital screening; pre-pregnancy screening; and chorionic villus sampling during the first trimester of pregnancy.[7]

Screening families of index cases is effective and low cost approach.[8] In Pakistan diagnostic test for thalassemia detection are expensive. DNA analysis cost Pk rupees 5000/, Hb Electrophoresis is Pk rupees 800/- and complete blood count with peripheral smear costs Pk rupees 200/. It is not possible to use these tests for general population because they are expensive, not available in rural setups and require technical staff. Rather inexpensive test is Naked-Eye-Single-Tube-Red-Cell-Osmotic-Fragility-Test (NESTROFT), used worldwide in differentiating between β-thalaessmia trait and iron deficiency anemia.[8-12] NESTROFT find outs osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) by direct vision without a spectrophotometer. Red cell osmotic fragility is reduced in BTT and positive test is indicator of thalassaemia.[13]

Though NESTROFT has been used in many countries for mass screening but bias exist between sensitivities and specificities for NESTROFT validity, especially with iron deficiency.[14] Pakistan has one of the highest prevalence in the world of iron deficiency anemia.[15] This study was designed with objective to evaluate the validity of NESTROFT in screening high risk groups, with very common iron deficient population, considering administration of iron to masses is not possible.

PATIENTS AND METHODS

This cross-sectional study was conducted at Thalassaemia of Sir Ganga Ram Hospital, Lahore. Study subjects were identified from families the index case of thalassemia, registered at Sir Ganga Ram Hospital Lahore. Informed consent was taken for investigations for research purpose .Index cases were encouraged to bring maximum family members on the test day. All family members were given family number and a family tree was made. Samples were taken for CBC, red cell indices, NESTROFT, Hb Electrophoresis and DNA analysis (if required).Iron supplements were not given to study subjects considering the fact that Pakistani population has high prevalence of iron deficiency and sensitivity and specificity of test is effected by iron status. Diagnostic efficiency of test is only valuable if it is not affected by iron deficiency. Tests were performed by well trained technicians under supervision of experienced medical staff. Cross checking of every 10^{th} sample was done to assure quality of testing. Total number of 25 families visited at their homes and blood samples were collected. Average Extended family size was 23.Total561 tests were sent with 247 males and 314 females. Samples lost were 45 because of inappropriate batch of buffered saline solution. Statistical analysis was performed on 515 samples Male 229 and female 286.

Blood samples were collected under sterile conditions and complete blood count was performed on all patients. Hb electrophoresis was used as gold standard to diagnose Thalassemia trait. Alkaline cellulose acetate and acidic citrate agar electrophoreses are the most widely utilized methods for haemoglobin analysis.[16] NESTROFT was validated for its sensitivity and specificity in study population without prior administration of iron. For NESTROFT ,stock solution of 10% buffered saline (pH 7.4) was prepared by taking NaCl 90g, Na_2HPO_4 13.655 g and NaH_2PO_4, $2H_20$ 2.4 g. dissolved in one liter of water. One of buffered saline was prepared by 1:10 dilution with distilled water of stock solution. Further 0.36% buffered saline was prepared by diluting 36 ml of 1 % buffered saline with 64 ml distilled water to make 100 ml. To perform test ,2mL of 0.36% buffered saline was taken in one tube (10x1 cm diameter) and 2 ml distilled water was taken in another. A drop of blood was added to each of the tubes, which were left undisturbed for half an hour at room temperature.

All families were given family number with reference to the index case representing family. Demographic & personal data and CBC results were collected and entered in registers by designated staff. Data was stored in Microsoft Excel and analyzed in SPSS version 20 by two persons in order to reduce errors.

RESULTS

The study results of 515 subjects revealed that NESTROFT found 101 true positive BTT cases. Also 214 true negatives were identified. Overall results are shown in table 1. Flow chart (1) for NESTROFT results along with Hb electrophoresis clearly shows low sensitivity and specificity of NESTROFT. Sensitivity was 55.5% and specificity was 64.3% of NESTROFT. The positive predictive value (PPV) was 46% and negative predictive value (NPV) was 72.5%. Such low PPV shows that NESTROFT is not much useful as mass screening test. Complete blood count showed significant values for differentiating, between thalassaemia trait and no trait. Haemogram values are reported in table 2. Mean with standard deviation were calculated for Hb in grams, RBC $*10^{12}$ l, MCV in fl, MCH in pg and MCHC in gl were measured. Males and females of BTT had microcytosis, hypochromia and low HB. Mild anaemia was also present in non- BTT subjects.

Table 1: Comparison of thalassemia vs NESTROFT

NESTROFT	Thalassaemia Trait +ve	Thalassaemia Trait -ve	Total
+ve	101 (TP)	119 (FP)	220
-ve	81 (FN)	214 (TN)	295
Total	182	333	515

Sensitivity = 101/(101+ 81)x100 = 55.5%
Specificity = 214/(214+119)x100 = 64.3%
Positive Predictive value=101/{101+119)x100=46%

Negative Predictive value=214/(214+81)x100=72.5%
Accuracy = 101+214/(101+214+119+81)x100=41.7%

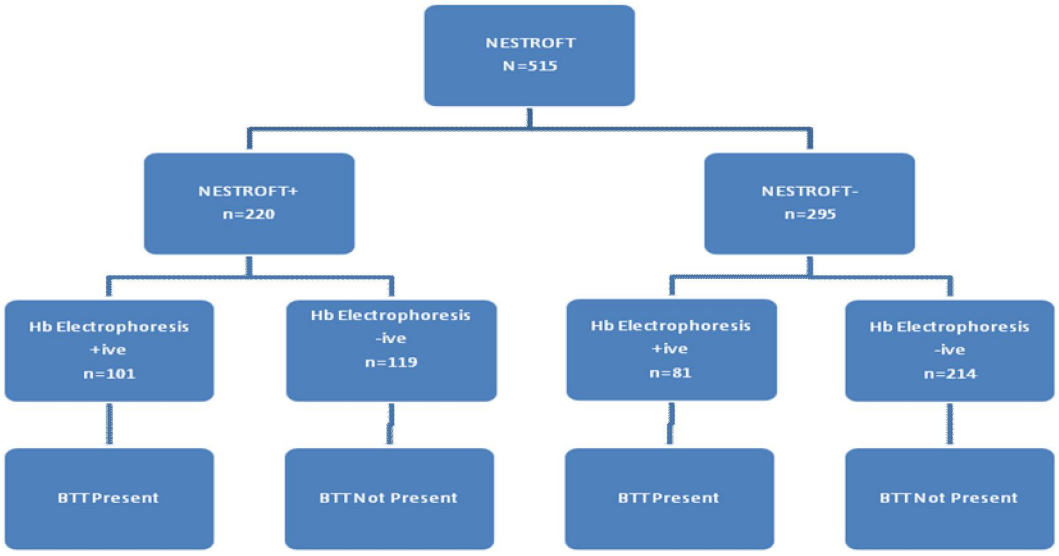
DISCUSSION

Pakistan has an estimated 5% prevalence of β-thalassaemia in general population, though variation is seen in different regions. In many rural and urban health set ups basic laboratory facilities are missing and being a low income country, health system cannot take the burden of expensive thalassaemia screening tests. Screening of family members of Thalassemia patients with a low cost test is suggested and used by many scientists. NESTROFT is considered as low cost test and yielded a high sensitivity and specificity in many studies but our study results are considerably different from past studies. We found a sensitivity and specificity of 55.5% and 64.3% respectively in our study population without prior administration of iron. Technically it is not possible to administer iron in resource constrained setups. Lack of compliance and

Table: 2 Haemogram of study subjects

Lab Parameter	BTT +ve on electrophoresis (182)	BTT-ve on electrophoresis (333)	P value
Hb g/dl (±SD)	(Male =82, Female=100)	(Male=147, Female=186)	
Male	12.7±7.1	12.04±3.3	0.314
Female	10.93±0.78	11.61±2.40	0.006
RBCx10^{12}/L(±SD)			
Male	6.05±0.71	4.7±0.98	0.000
Female	5.6±0.45	4.4±0.74	0.000
MCV fL (±SD)			
Male	65.4±5.02	79.30±10.7	0.000
Female	65.16±4.4	80.88±9.91	0.000
MCH pg (±SD)			
Male	19.8±2.07	25.94±8.02	0.000
Female	19.1±2.08	26.0±4.41	0.000
MCHC g/L(±SD)			
Male	30.22±1.5	31.71±3.15	0.000
Female	29.72±1.28	32.0±2.77	0.000

Fig. 1: Validity of NESTROFT in detection of thalassemia



unawareness in our population are also obstacles in giving iron supplements regularly before testing for NESTROFT. Study from Pakistan previously reported sensitivity and specificity of 93% and 88%.[9] From different parts of world different sensitivity and specificity was reported with 95% &86%.[14] 97.7% and 83.3%[10], 97.9% and 76.8%[12], 98.4% and 66.6%.[17]

Positive predictive value documented 94% by Chow[14] and 46.5% by Ritu[12] which is close to our study findings PPV 46%. Negative predictive value of our study was 72.5% which vary from previous studies of Chow[14] and Ritu[12] with NPV of 88% and 99.4% respectively. These findings are suggestive of the fact that NESTROFT is not sufficient alone to label anyone with life threatening disorder like Thalassemia or even worse to skip the diagnosis. Proper gold standard testing is mandatory in extended families of index cases.

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