An analysis of NESTROFT and Red cell indices in evaluating antenatal mothers for Beta Thalassemia trait

Safia R1, Jairajpuri ZS2, Khetrapal S3, Hassan MJ4, Gupta M5, Jetley S6.

Abstract

Background: The frequency of thalassemia trait is about 3% worldwide, while in developing countries like India, it is a major cause of burden on the health care system. Naked eye single tube red cell osmotic fragility test (NESTROFT) used in population screening for beta thalassemia trait. It is a simple, low cost, reliable and most suitable screening test for β BTT, with a sensitivity of 99.8% in regions with high prevalence rates. Material & Methods: The present study was conducted in the Department of Pathology and included a total of 174 antenatal cases, attending the Out Patient Department of Obstetrics and Gynecology of a tertiary health care center in New Delhi between June 2011 to January 2012. The aim of the study was to screen mothers antenatally for early detection of BTT and to test the validity of NESTROFT in detection of thalassemic carriers of this area. Results: 174 pregnant women attending antenatal clinics were screened for detecting hemoglobinopathy with the help of NESTROFT, red cell indices, haemoglobin electrophoresis and HPLC It was seen that only four cases were BTT while ten were of microcytic anaemia and two had normal red cell indices. Conclusion: antenatal diagnosis of BTT is an important screening programme to reduce the burden of birth of children suffering from thalassemia and other hemoglobinopathies.

Keywords: NESTROFT; Antenatal; Thalassemia

Introduction

Hemoglobinopathy is a common genetic problem worldwide with the commonest inherited being thalassemia. In developing countries like India, it is a major cause of burden on the health care system. The frequency of thalassemia trait is about 3% worldwide.1 In South-East Asia alone reside 50% of the thalassemic carriers of the world.2 The incidence in India however is variable ranging from 3%-18% in north and 1.3% in southern India.3 β Thalassemia Trait (BTT) is an asymptomatic condition resulting in microcytosis and mild naemia on the other hand β Thalassemia major is a much severe condition requiring lifelong blood transfusion. Thus, the birth of Thalassemia child places considerable strain, not only on the affected child and its family, but

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also on the community and nation at large. Genetic
counselling and prenatal diagnosis are effective
methods to reduce the incidence of genetic disorders
for which the population at risk needs to be identified.
Various haematological investigations are available
which are helpful in diagnosing BTT. However,
due to cost restrictions, increase time consumption
or cumbersome procedures requiring specialized
equipment, implementing them for screening is not
possible. Naked eye single tube red cell osmotic
fragility test(NESTROFT) used in population
screening for beta thalassemia trait, is a simple, low
cost, reliable and most suitable screening test for β
BTT, with a sensitivity of 99.8% in regions with
high prevalence rates. Thus, antenatal diagnosis
of BTT is an important screening programme to
reduce the burden of birth of children suffering
from thalassemia and other hemoglobinopathies.
Regarding this problem, most studies have been
conducted among urban population, and very few
have been reported in the semi-urban/slum and rural
population. The present study was conducted in such
area of a medical college, situated in New Delhi. The
aim of the study was to screen mothers antenatally for
early detection of BTT and to test the validity of
NESTROFT in detection of thalassemic carriers of
this area. The objective was finding the prevalence
of the BTT among the pregnant females attending the
ante-natal clinics, by using the NESTROF test
and to screen the pregnancies "at risk" of delivering
babies with Thalassaemia major and also making
them aware of its consequences among pregnant
women. The present study evaluates the efficacy of
NESTROFT in detection of beta thalassemia trait in
a semi-urban population of New Delhi.

**Material and methods:**
The present study was conducted in the Department of
Pathology and included a total of 174 antenatal
cases, attending the Out Patient Department of
Obstetrics and Gynecology of a tertiary health care
center in New Delhi between June 2011 to January
2012 and agreeing to give consent for the study.
Our hospital caters predominantly to a semi urban /slum population. These patients attended the hospital
for routine ante-natal check up in early pregnancy
(First Trimester). A detailed clinical history of all the
patients was taken.

Venous blood (4ml) was collected in ethylene
diamine tetra acetic acid (EDTA) vials from
pregnant mothers attending the antenatal clinics.
The anticoagulated blood was used for performing
haematological investigations including Complete
Blood Counts (CBC), NESTROFT’s, reticulocyte
count, haemoglobin electrophoresis and HPLC
wherever indicated. Reticulocyte count was done
with help of supravital stains (Brilliant Cresyl Blue)
and count was done manually under the microscope
by trained pathologists. Hemoglobin electrophoresis
and HPLC was done using Variant 2 (Biorad) and
D-10 (Biorad) respectively. NESTROFT was performed using 0.36% buffered saline solution. Two ml of buffer solution was taken
to which one drop of test blood sample was added, 2ml
distilled water was also taken in another test tube,
to which also the test sample was added, both tubes
were mixed well and left undisturbed for 30 min at
room temperature. After half hour both tubes were
shaken and then held against a white paper on which
a thin black line is drawn. Interpretation of the test
was done by experienced pathologist. The black line
should be clearly visible through the contents of the
control tube. If the line was clearly visible through
the contents of the tube labeled test, NESTROFT
was considered negative. If the line was not clearly
visible through the contents of the tube with 0.36%
buffered saline, the test was considered positive. If
the gestational age was less than 20 weeks, then the
couple was counselled to undergo Hb electrophoresis
to make a confirmatory diagnosis and subsequently,
to make a prenatal diagnosis. If the gestational age
was more than 20 weeks, the couple was counselled
to undergo electrophoresis for the confirmation
of the diagnosis and to make a prenatal diagnosis
for the subsequent pregnancies. The diagnosis of
iron deficiency anemia was made on basis of Hb
values<11g/dl, MCV<80fl, increased RDW, and
confirmed with serum ferritin<12µ/L AND serum
iron<60mg/dl.

The results were tabulated and relevant statistical
analysis was done. Variations of P<0.05 were
considered significant. Test of significance chi
square tests were used.

**Results:**
A total of 174 pregnant women attending
antenatal clinics were screened for detecting
hemoglobinopathy with the help of NESTROFT,
red cell indices, haemoglobin electrophoresis and
HPLC. In the present study, majority of the females
82(47.12%) were in the age group 21-25 years while
138 (79.31%) were in the age group of 20-30 years
(Figure 1).

All the 174 pregnant women were subjected to the
NESTROFT test as a primary screening test, 16 were
found to be positive and 158 negative. (Table 1)
Out of 174 subjects in the study, 78 (44.82%) had normal red cell indices while microcytosis (Mean Corpuscular Volume MCV<80fl) was seen in 96 (55.17%) of the women. Out of the women with microcytosis 4(2.29%) confirmed to be of BTT. 68 (73.91%) out of the 92 patients with microcytosis had decreased serum ferritin values while the rest 24 (26%) were not of IDA. (Table 1). Ferritin values vary according to pregnancy period due to hemodilution and this fact was considered in our study.

Table 1: Screening test results of NESTROFT

<table>
<thead>
<tr>
<th>Primary screening test NESTROFT</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16</td>
<td>9.19%</td>
</tr>
<tr>
<td>Negative</td>
<td>158</td>
<td>90.81%</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>100%</td>
</tr>
</tbody>
</table>

On further evaluating the NESTROFT positive results with HPLC for diagnosis of BTT, only 4 cases were true positive with HbA2>3.5% and 12 had HbA2 < 3.5% and comprised the false positive group. Hence, for detecting the BTT, the sensitivity and specificity NESTROFT were 100% and 92.9% respectively in our study. A cut off Hb A2 level of ≥ 3.5% was used to confirm the diagnosis of thalassemia trait and values between 3.2% and 3.5% were considered to be of borderline cases. Thus, the disease prevalence amongst the pregnant women who attended the ANC clinic at the HAHC hospital was 2.3% (with 95% CI of 0.63% to 5.78%).

Table 3: Distribution of cases according to diagnosis on NESTROFT screening

<table>
<thead>
<tr>
<th></th>
<th>NESTROFT +</th>
<th>NESTROFT -</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTT</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>MHA/IDA</td>
<td>10</td>
<td>82</td>
<td>92</td>
</tr>
<tr>
<td>NORMAL</td>
<td>2</td>
<td>76</td>
<td>78</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td>158</td>
<td>174</td>
</tr>
</tbody>
</table>

On evaluation of diagnosis NESTROFT positive and negative cases, it was seen that only four cases were BTT while ten were of microcytic anaemia and two had normal red cell indices. Of the 158 NESTROFT negative cases 51.9% were in the MHA category and 48.1% in the normal red cell indices category, no false negative case was seen in the present study. The red cell parameters were analyzed for their mean values ± standard deviation depicted in Table 4. The P value to see statistical significance of the mean values was also further calculated, where P value <0.05 was taken to be statistically significant. All red cell parameters including Haemoglobin(Hb), Mean corpuscular volume, Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Red Cell Distribution Width (RDW) were found to be statistically significant Pvalue<0.05 while Red blood cell (RBC) count and Haematocrit values (HCT) were not statistically significant(P value >0.05). Reticulocyte counts were within normal range of adult.
the health-care professionals, pregnant women and their families. Majority of the studies were based on population including all ages and both sexes while our study was based on antenatal mothers. The majority of the screened mothers (47.1%) were in the age group 21-25 years with the mean age of 24.72 years and SD ±3.76. This was in concordance to 60% women in this age group in a study by Parikh et al. However other authors have also reported a similar age group and a female preponderance in their studies.3,8 The distribution of β thalassemia gene in the Indian subcontinent is not uniform possibly due to ethnic diversity, it exists in different regions with varying frequency, ranging from <1-17% with an average of 3%.9 Disease prevalence in the present study was 2.3% in pregnant women. A prevalence of 3.4% of β thalassemia carrier state, among antenatal women has been reported by Chakraborti et al.10 and 3.1% by Sujatha et al in previous studies.

Effective screening will continue to be the backbone of preventive strategies against BTT especially in countries where prevalence is high and resources limited. Consequently, there is considerable emphasis on strategies to optimize the cost–benefit ratio of mass screening.11 Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) can be a very useful screening tool for BTT because of low cost and less technically advanced and easy result availability. It is based on the principle of decreased red cell osmotic fragility and increased resistance to osmotic lysis that occur due to altered shape and functioning of red cell.12,8 The sensitivity and specificity of NESTROFT in detection of BTT in the present study were 100% and 92.9% respectively, in concordance with other authors, 95.2% sensitivity and 94.1% specificity10 and 85.4% and 100%3. Although considered to be an effective screening test, authors have argued that the success of NESTROFT in large-scale screening programmes is lessened because of the associated false-negative error rate.11 Since the costs of treating β-thalassaemia major cases is high that could result from the β-thalassaemia carriers missed by NESTROFT, it has been suggested that information from the haematological parameters output by automated cell counters should be combined with the results of NESTROFT to improve the yield from screening strategies for BTT.11 In evaluating a potential screening programme, false-negative and false-positive error rates should be considered in addition to cost. The false negative results could not be accurately assessed due to limitations, the false

Table 4: Mean Values of Various Red Cell Parameters

<table>
<thead>
<tr>
<th>Red cell Parameter</th>
<th>BTT (Mean±SD)</th>
<th>NBTT (Mean±SD)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Hb(gm/dl)</td>
<td>8.87±1.6</td>
<td>10.64±1.61</td>
<td>0.020</td>
</tr>
<tr>
<td>RBC count (X106)</td>
<td>4.33±0.70</td>
<td>3.95±0.51</td>
<td>0.301</td>
</tr>
<tr>
<td>HCT(%)</td>
<td>30.27±4.37</td>
<td>33.23±4.24</td>
<td>0.093</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>68.67±4.60</td>
<td>78.98±8.39</td>
<td>0.024</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>23.12±0.54</td>
<td>27.03±3.18</td>
<td>0.041</td>
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<td>MCHC(%)</td>
<td>29.1±1.68</td>
<td>31.92±1.90</td>
<td>0.048</td>
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<tr>
<td>RDW(%)</td>
<td>26±4.12</td>
<td>39.76±15.5</td>
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Discussion:
The identification of β thalassemia trait is important especially in developing countries where resources are limited and management expensive. Not only does the major form of the disease compromise quality of life it is an extra financial burden on the family. Also, as the red cell morphology in beta thalassemia trait is microcytic hypochromic; these patients are often misdiagnosed, as those suffering from iron deficiency anaemia and given unnecessary iron medication.5 Hence, the aim of the present study was to screen the pregnant women attending the antenatal clinic of our hospital by NESTROFT test to assess the burden of β thalassemia trait of antenatal mothers of this areas well as offer counselling and prenatal diagnosis.

Beta thalassemia is the commonest inherited hemoglobinopathy, population screening has identified the prevalence of β-thalassemia carrier status to be as high as 17% in certain communities in India.6 The classical heterozygous form of BTT is usually asymptomatic. The family history of thalassemia is important however a significant number of patients do not have previously affected family members. The prevalence of BTT is known to vary from 1.0%-14.9% in various regions of India.8 In the present study the disease prevalence was 2.3% of the screened 174 cases. The lesser number of women ready for screening in the present study as compared to other studies,2,6 could be attributed to lesser awareness about hemoglobinopathies amongst

In the age group 21-25 years with the mean age of 24.72 years and SD ±3.76. This was in concordance to 60% women in this age group in a study by Parikh et al. However other authors have also reported a similar age group and a female preponderance in their studies.3,8 The distribution of β thalassemia gene in the Indian subcontinent is not uniform possibly due to ethnic diversity, it exists in different regions with varying frequency, ranging from <1-17% with an average of 3%.9 Disease prevalence in the present study was 2.3% in pregnant women. A prevalence of 3.4% of β thalassemia carrier state, among antenatal women has been reported by Chakraborti et al.10 and 3.1% by Sujatha et al in previous studies.

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positive results however were 6.8% in present study which was lower than 15.32% reported by Pipiani et al. The lower values in our study could be attributed to small sample size. Previous workers have also reported false positive rates ranging from 16.4% to 18.5%. The positive and negative predictive values (PP, NPV) reflect the presence or absence of disease when a test is positive or negative. Although, we could not reliably calculate these values due to limitation of the sample size reflecting the true prevalence of the disease, the role of these values is highlighted in many reports.

As has been suggested, haematological parameters by automated cell counters could be combined with the results of NESTROFT to improve the yield from screening test for BTT. However, authors suggest that red cell parameters have a limited use and can be influenced by several non genetic causes such as nutritional status and heritability only explains only ~50% of the variability in these parameters. A comparison of the mean values of different parameters along with their P values was analyzed in the present and a significant statistical association was drawn between BTT and Non BTT all red cell parameters except red cell count and and haematocrit values. However in a study by Patel et al a significant association was seen in all red cell parameters. Among the various parameters, the role of RDW is important but has limited specificity. Values of MCV and MCH were significantly lower in BTT cases in our study in concordance with other studies although it is recommended that MCH is used to screen thalassemia as this parameter is more stable than MCV. Nutritional anaemia commonly coexists both at a geographic and individual level, and can severely confound the red cell parameters. An increase in MCV in BTT patients has been attributed to possible concomitant Vitamin B12 or folic acid deficiency or could be due to significant intramedullary lysis of the red cells leading to a spillage of immature erythroblasts into peripheral circulation. According to Sujatha et al, RBC > 5 x
References:


12. Sarda H, Nivedita S R, Shivilingaiah N. Screening of β thalassemia trait among pregnant women with NESTROFT. Thalassemia Reports 2015; 5:4430


