NESTROFT AS A SCREENING TOOL TO IDENTIFY BETA THALASSAEMIA TRAIT IN PAEDIATRIC CASES
T. A. Santhi¹, M. Menaga²

¹Assistant Professor, Department of Pathology, Government Tirunelveli Medical College, Tirunelveli, Tamil Nadu.
²Assistant Professor, Department of Community Medicine, Government Tirunelveli Medical College, Tirunelveli, Tamil Nadu.

ABSTRACT

BACKGROUND
Thalassemia is the most common genetic disorder worldwide. Indian Academy of Paediatrics has reported a frequency of thalassemia trait of 3-18% in Northern India and 1-3% or even less in the Southern part of India. Thus, the objectives are 1) To detect cases of β-thalassemia trait in paediatric patients presenting with anaemia using NESTROFT as a screening tool. 2) To compare the various red cell indices and discriminant functions derived from them in identifying thalassemia trait. 3) To correlate the RBC indices with the peripheral smear findings.

MATERIALS AND METHODS
100 patients in the age group of 0-12 years, presented with features and symptoms of anaemia to the inpatient and outpatient ward, Department of Paediatrics, in a medical college hospital. Their blood samples were subjected to basic complete haemogram, NESTROFT (Naked eye single tube red cell osmotic fragility test) and haemoglobin variant studies by electrophoresis. The results were analysed statistically.

RESULTS
The sensitivity, specificity, positive predictive value and negative predictive value of NESTROFT and various discriminant functions Mentzer index, Shine & Lal index and Srivastava’s formula were statistically showed statistically significant difference and were ideal for screening tools to identify the β thalassemia trait cases. The association of NESTROFT with other indices evaluated with chi-square test (χ²) was found to be statistically significant. The mean MCV, mean MCH, mean RDW in β–thalassemia trait was lower than non-β-thalassemia trait which is statistically significant (p<0.001).

CONCLUSION
NESTROFT is a suitable test for screening for beta-thalassaemia and the common haemoglobinopathies seen in India. It is easy to perform, simple, inexpensive.

KEYWORDS
Beta Thalassemia Trait, NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test), HPLC.

HOW TO CITE THIS ARTICLE: Santhi TA, Menaga M. Nestroft as a screening tool to identify beta thalassaemia trait in paediatric cases. J. Evid. Based Med. Healthc. 2017; 4(17), 989-993. DOI: 10.18410/jebmh/2017/194

BACKGROUND
Thalassemia is the most common genetic disorder worldwide. It occurs with a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East, Indian sub-continent, Burma, South East Asia, Melanesia and Islands of Pacific.¹ Approximately 3% of the world population carry β-thalassemia gene. In India frequencies between 3.5 and 14.9% have been reported.² Indian Academy of Paediatrics has reported a frequency of thalassemia trait of 3–18% in Northern India and 1-3% or even less in the Southern part of India.³ In India, there are around 20 million carrier cases of β-thalassemia and around 8000-10,000 children are born every year with β-thalassemia major.⁴ The birth of a thalassemia child places considerable health and economic strain not only on the affected child and his/her family but also on the community.

Thalassemia is a group of disorders, each resulting from an inherited abnormality of globin production a condition collectively known as haemoglobinopathies.⁵ Thalassemia are classified into α and β by decreased or absent synthesis of α and β globin chain respectively. β-thalassemia is the most common form of inherited haemoglobinopathy.⁶

Since, thalassemia is a severe and incurable disease, emphasis has to be shifted from the treatment of an affected child to the prevention of such births in future. Identifying carriers for β-thalassemia patients, thus plays an important role in preventing this, β-thalassemia trait presents with microcytic hypochromic blood picture similar to the picture of iron deficiency anaemia and anaemia of chronic disease.⁷ These diagnostic methods are costly and hence constitute a significant burden on public health economy particularly in developing countries like India. This made the way for developing a simple and economic screening test for the detection of carries of β-thalassemia.
AIMS AND OBJECTIVES
This study was aimed-
1. To detect cases of β- thalassemia trait in paediatric patients presenting with anaemia using NESTROFT as a screening tool.
2. To compare the various red cell indices and discriminant functions derived from them in identifying thalassemia trait.
3. To correlate the RBC indices with the peripheral smear findings.

MATERIALS AND METHODS
100 patients in the age group of 0-12 years, presented with features and symptoms of anaemia to the inpatient and outpatient ward, Department of Paediatrics, Tirunelveli Medical College Hospital, Tirunelveli during the period of August 2008 to March 2009 were included. All these patients were subjected to basic hematologic investigations and those who presented with Hb less than 11.5 gms % and MCV of less than 80 fl, were included in the study. Peripheral smear examination were done to exclude any haematological malignancy and Beta thalassemia major.

Selected Patients were subjected to the following Investigations
1. Complete blood haemogram— Total RBC count, MCV, MCH, MCHC and RDW were calculated in all selected patients using automated cell counter Sysmax – K21, 22 (Transasia Biomedicals).
2. Peripheral smear analysis.
3. NESTROFT was carried out in these cases with fresh working solution. Discriminant functions like Mentzer’s Index, England and Fraser index, Green and King Index, Srivastava’s formula, Shine and Lal Index, RDW index were carried out with the data obtained from the cell counter.

>13 Iron Deficiency Anaemia
<13 Beta Thalassemia trait.

Positive value – Iron deficiency anaemia.
Negative value – Beta thalassemia trait.

Srivastava’s Formula
MCV 2xRDW /Hbx100

RESULTS

<table>
<thead>
<tr>
<th>NESTROFT</th>
<th>Total Subjects</th>
<th>β –Thalassemia Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=100)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>97</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Results of NESTROFT Especially in β –thalassemia Trait N=100

Table 1 shows total of 100 paediatric patients were screened, NESTROFT was positive in 3.9 cases of β-thalassemia trait. No false positive. It was negative in 1.9 cases and 96 cases were non-β- thalassemia trait. Sensitivity, specificity, positive predictive value and negative predictive value was 64.2%, 94.4%, 97.6% and 35.3% respectively.

<table>
<thead>
<tr>
<th>NESTROFT</th>
<th>Positive</th>
<th>Negative</th>
<th>Chi-square Test (x²)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Mentzer’s Index</td>
<td>BTT</td>
<td>14</td>
<td>93.3</td>
<td>1</td>
</tr>
<tr>
<td>Non-BTT</td>
<td>40</td>
<td>47.1</td>
<td>45</td>
<td>42.9</td>
</tr>
<tr>
<td>Srivastava’s Formula</td>
<td>BTT</td>
<td>33</td>
<td>89.1</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2. Comparison of Various Discriminant functions with NESTROFT

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Discriminant Function</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mentzer's Index</td>
<td>52.9%</td>
<td>93.3%</td>
<td>25.9%</td>
<td>97.8%</td>
</tr>
<tr>
<td>2.</td>
<td>England Fraser index</td>
<td>90.2%</td>
<td>84.7%</td>
<td>92.6%</td>
<td>80.4%</td>
</tr>
<tr>
<td>3.</td>
<td>Green and King index</td>
<td>85.75%</td>
<td>92.2%</td>
<td>87.0%</td>
<td>91.3%</td>
</tr>
<tr>
<td>4.</td>
<td>Srivastava's formula</td>
<td>66.7%</td>
<td>89.2%</td>
<td>61.0%</td>
<td>91.3%</td>
</tr>
<tr>
<td>5.</td>
<td>Shine and Lal index</td>
<td>94.1%</td>
<td>63.8%</td>
<td>98.2%</td>
<td>34.9%</td>
</tr>
<tr>
<td>6.</td>
<td>RDW index</td>
<td>87.8%</td>
<td>94.1%</td>
<td>88.9%</td>
<td>93.5%</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the Various Discriminant Function N=100

Table 3 shows various discriminant functions Mentzer index, Shine & Lal index and Srivastava's formula were statistically significant and were ideal for screening tools to identify the β thalassemia trait cases.

Mean of the RDW values of the thalassemia trait cases in the study was 15.23. Optimum cut off value of RDW to differentiate between BTT and IDA was found to be 15.23. The mean RDW of β–thalassemia trait was significantly lower than non β–thalassemia trait. Since the difference of mean 2 is statistically significant p <0.01. Mean RBC count is higher in β-thalassemia trait as compared to non β-thalassemia trait cases.

Table 4. Association between NESTROFT with other Indices N=100

Table 4 shows the association of NESTROFT with other indices evaluated with chi-square test (x^2) was found to be statistically significant.

Table 5. Comparison of Red Cell Indices N=100

Table 5 shows by employing the independent t-test, the mean MCV mean MCH were lower than non β–thalassemia trait cases which is statistically significant. (p value <0.001).

Mean RBC count is higher in β-thalassemia trait as compared to non β-thalassemia trait cases.
The mean red blood cell count values of our study group was almost similar to that of the observation of Madhan N et al loccit (1994) with 5.6x10^6 cells/cumm. The mean MCV of our study group was 62.8fl. This was similar to the observation Madhan N et al in 1994. (or) slightly lower than the observation made by Mohamed et al in 1999. The mean RDW of our study was 14.3%. This was slightly lower than the values of Madhan N et al loccit (1994) with 16.6%. 

Comparing the sensitivity, specificity, positive and negative predictive value of various discriminant functions were found. Mentzer's index and Srivastava's formula were the most useful screening test than the others. This goes in hand with the observation of Manglani et al (1994) study, the sensitivity and specificity positive predictive value and negative predictive value of Mentzer's Index were respectively 66.2%, 82.8%, 89.5% and 44, 3%. In our present study sensitivity specificity, positive predictive value and negative predictive value were respectively 73.3%, 59.9%, 25.9% and 97.8% which compared to the other studies and found that sensitivity is higher than other studies.

Manglani et al loccit (1994) found that sensitivity specificity, positive predictive value and negative predictive value of Srivastava's formula respectively, 55.6%, 79.7%, 85.0% and 36.1%. In our study showed sensitivity, specificity, positive predictive value and negative predictive value of 89.2%, 66.7%, 61.1% and 91.3% which shows comparatively higher sensitivity value.

**DISCUSSION**

This study was done to evaluate the usefulness of NESTROFT along with various other RBC indices and discriminant function in detecting cases of beta thalassemia trait and to differentiate them from non-beta thalassemia trait cases.

Regarding the usefulness of the NESTROFT as a screening test for the detection of beta thalassemia trait our observation was almost similar to that of Manglani et al (1997) who have screened 830 cases from general population from various region for our country. The NESTROFT positive cases were confirmed with Hb electrophoresis. Similar observations was also found by Mehta et al, Ragavan et al, Thomas S. Srivastava et al.

The sensitivity, specificity, positive predictive value and negative predictive value 64.2%, 94.4%, 97.6% and 35.3% respectively. Our study also showed similar observation and 3 cases were positive for beta thalassemia trait by Hb electrophoresis. Our positive predictive value was lower than that of the other similar studies due to the small size of the study group included. We have also tried to correlate the RBC indices and other discriminant function with other similar studies.

The mean haemoglobin value of our study group was 10.68 m/dl which was lower than that of similar observation made by Madhan N et al (1999) with a haemoglobin of 11.6 gm/dl. Das Gupta et al (1994) with a mean haemoglobin of 11.2 gm/dl. and Mohamed et al (1999) with 11.3 gm/dl.

**CONCLUSION**

The NESTROFT positively correlated with other discriminant function and various red cell indices. In this Mentzer's Index, Shine and Lal index, Srivastava's formula were ideal for screening. Analysis of various red cell indices shows MCV value is comparatively lower in beta thalassaemia trait. So we conclude various red cell indices, discriminant function and NESTROFT will be useful to detect cases of beta-thalassemia trait in the general population.

**REFERENCES**


