

# Screening of $\beta$ -thalassemia trait among pregnant women with NESTROFT

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#### Abstract

The morbidity of  $\beta$ -thalassemia major has forced medical professionals to formulate screening tests to effectively screen β-thalassemia trait (BTT) of which naked eve single tube osmotic fragility test (NESTROFT) is the most cost effective test. Optimal time to screen for BTT appears to be during pregnancy, as at risk couples can be offered prenatal diagnosis. We screened 55 pregnant women attending antenatal clinic in a medical college hospital at Bangalore, with NESTROFT. Twelve (21.89%) were NESTROFT positive with mean hemoglobin of 11.2 mg % and packed cell volume of 32.6%. Catering predominantly to Vokkaliga community in whom literature reports increased prevalence of BTT, addition of a cost effective test like NESTROFT in the antenatal panel, appears promising.

### Introduction

At least 1.5% of the world's population are carriers of  $\beta$ -thalassemia trait (BTT) with 80-90 million patients and an estimated 60,000 new cases recognized each year.<sup>1</sup> South east Asian region countries alone account for 50% of the world's carriers,<sup>2</sup> carrier rates varying from 0-17% among different ethnic groups.<sup>3</sup>

Hence it is obvious that the prevention of thalassemia major can be reached by adopting screening programs to detect BTT individuals. The most efficient way of doing this has been found to be screening of pregnant women for possible BTT, so that prenatal diagnosis can be obtained by *at risk couples*.<sup>4</sup>

### **Materials and Methods**

This was an observational study of 2 months duration done in the Departments of Pathology and Obstetrics and Gynecology. We studied 55 pregnant women attending the antenatal clinic of Kempegowda Institute of Medical Sciences, Bangalore. The aim of this study was to screen pregnant women for potential BTT by naked eye single tube osmotic fragility test (NESTROFT) and evaluate the frequency of NESTROFT positivity. An informed consent to participate in the study was taken. After noting down age and period of gestation, all the women were questioned, to know about their awareness of thalassemia and responses were recorded. About 2 mL of venous blood was collected in K<sub>3</sub> ethylenediaminetetraacetic acid (1 mg/mL). Values of hemoglobin %, packed cell volume (PCV) and blood group were noted down from their antenatal laboratory investigation chart. NESTROFT was performed according to the standard procedure on all the 55 samples as follows: a stock solution of 10% buffered saline was prepared by dissolving 90 mg of sodium chloride, 13.65 mg of disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) and 2.43 mg of sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>) in 1 L of distilled water and pH adjusted to 7.4. This stock solution was refrigerated. Whenever NESTROFT was scheduled, a 1% solution was made from the stock solution [1 in 10 dilution with distilled water; 0.36% buffered saline was prepared by diluting 36 mL of 1% saline with 64 mL of distilled water to make 100 mL: 2 mL of 0.36% buffered saline was taken in one test tube (10×1 cm diameter) labeled test and 2 mL of distilled water in another tube labeled control]. A drop of test blood sample was added to each of the tubes and left undisturbed for half an hour at room temperature. After half an hour both tubes were shaken and then held against a white paper on which a thin black line is drawn. 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 con-

# Principle of naked eye single tube osmotic fragility test

A positive NESTROFT indicates that all red cells in the tested sample have not undergone lysis in 0.36% buffered saline. These unlysed red cells resulted in the hazy appearance of the contents of the tube and render the line on the paper indistinct. These red cells also sediment as a button at the bottom of the tube when it is left undisturbed for some time. Thus a positive NESTROFT indicates decreased red cell osmotic fragility and increased resistance to osmotic lysis.

## Results

sidered positive.

Among the 55 pregnant women 99% were

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unaware of thalassemia. Of the 55 screened for potential BTT, 12/55 were NESTROFT positive. Hence, frequency of NESTROFT positivity in pregnant women attending was 21.89%, with 50% of the positives belonging to 21-25 year age group. Most of the women in the study group *i.e.*, 39/55 were in the III trimester and hence 6 out of 12 *i.e.* 50% positives were in the III trimester. Most of the women with NESTROFT positivity had lower hemoglobin between 9-11 g % (P=0.05) with a mean hemoglobin of 11.4 g % and PCV of 32.6% (P=0.005).

# Conclusions

Unusually high percentage of NESTROFT positives was found in this study. This is comparable to a study by Colah *et al.*<sup>5</sup> with 14% NESTROFT positivity, where they screened 61,935 pregnant women. In a few studies from Karnataka, *Vokkaliga* community has shown relatively high percentage of BTT when compared to other communities.<sup>6,7</sup> In a rural based study by our institute, screening of BTT was



done in pregnant women by NESTROFT and the frequency of overall NESTROFT positivity was found to be 8.5%.<sup>7</sup> However when individual castes were considered 27.8% of NESTROFT positive pregnant women belonged to *Vokkaliga* community.<sup>7</sup> As our hospital caters predominantly to this community we attribute this high percentage of NESTROFT positivity to the same.

We could not confirm the findings by  $HbA_2$ estimation but review of literature shows specificity of 94.12%, sensitivity of 95.23%, positive predictive value of 41.02% and negative predictive value of 99.78%,<sup>8</sup> while some have documented a sensitivity and negative predictive value 0f 100%, specificity of 85.47% and positive predictive value of 66%.<sup>9</sup> NESTROFT also happens to be a very economical test with less than Rupees 2/test<sup>7</sup> and a high negative predictive value.<sup>8,9</sup>

As a pilot study in an urban hospital catering predominantly to *Vokkaliga* community this study demonstrates high frequency of NESTROFT positivity in pregnant women in this community elucidating the need for routine screening of all pregnant women with NESTROFT combined with other red cell indices and HbA<sub>2</sub> estimation.

So we propose the addition of NESTROFT for screening of BTT in pregnant women among the antenatal tests especially in high risk communities like the *Vokkaligas* especially in resource restricted countries like India.

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