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COMMENTARY



## The Use of NESTROFT for Screening Pregnant Women for Detection of β-Thalassemia Carriers

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The thalassemias and sickle cell disorders are widely distributed in India and cause a significant health burden [1, 2]. Community control programmes are the only way to reduce this burden. Screening for identification of carriers forms an integral component of a prevention programme [1, 2].

The often-debated questions are whom to screen and which would be the most appropriate technology to be used. The individuals targeted for screening in most studies include school or university students, newly-married couples, pregnant women, extended family members of a thalassemia major child, or members of communities where the prevalence of  $\beta$ -thalassemia carriers is higher than the average of 3 %-4 % in the Indian population. Pregnant women would be the most appropriate group as they are the ones at immediate risk but they often come late in the second trimester of pregnancy to the antenatal clinic for their first visit, especially in public hospitals, and it is often difficult to get their husbands for testing [3]. All these target groups have been screened in India by different centers [4–7]. Premarital screening is largely not acceptable due to fear of stigmatization.

The technologies used for screening and diagnosis, include the following:

Naked eye single tube red cell osmotic fragility test (NESTROFT); red cell indices; discriminant functions; flow cytometric osmotic fragility; and hemoglobin analysis by high-performance liquid chromatography (HPLC), capillary

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electrophoresis, or cellulose acetate electrophoresis for estimation of Hb  $A_2$ , Hb F or other hemoglobin variants.

A combination of these methods has been used by different investigators and each one has its advantages and limitations [8–13].

NESTROFT has been used as a primary screening test for population screening in many studies after Kattamis et al. first showed that a 0.36 % saline solution was sensitive and effectively detected more than 96 % of  $\beta$ -thalassemia carriers on screening [14].

The ideal approach would be to measure the red cell indices and quantitate Hb A<sub>2</sub>, Hb F, and any other hemoglobin variant by HPLC or capillary electrophoresis in every individual to ensure that all  $\beta$ -thalassemia heterozygotes as well as carriers of abnormal hemoglobins are identified. However, this is not a cost-effective strategy for massscreening programmes in a large country. Moreover, even with this ideal strategy, around 2 % of  $\beta$ -thalassemia heterozygotes who have normal red cell indices, and/or normal or borderline Hb A<sub>2</sub> levels will be missed.

NESTROFT has a high sensitivity but lower specificity as shown in several studies [8–13]. Using NESTROFT as a single first-line screening test has some limitations. Although it has a high negative predictive value, few  $\beta$ thalassemia carriers have been missed in different studies. In a quality control evaluation done during the large multicenter study of Indian Council of Medical Research (ICMR) under the Jai Vigyan programme, the main reasons for higher number of false negatives at some centers were the quality of water used, inaccurate dilution of the buffer, and frequent change of technicians. It was also shown that if NESTROFT was combined with measuring red cell indices in the first step itself using mean corpuscular volume (MCV) <80 fL and mean corpuscular hemoglobin (MHC) <27 pg as cut-off values, the  $\beta$ -thalassemia carriers

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missed on screening were only 1.8 % compared to using either of these approaches individually where the number of cases missed were far greater [7]. The value of combining NESTROFT and red cell indices as a first step has been shown in other smaller studies as well [10].

In a population where Hb S and Hb E are prevalent, NESTROFT alone is not sufficient as it would miss 25 %-40 % of carriers with these abnormal hemoglobins [7]. In countries like Thailand where Hb E is common, the dichlorophenol-indolphenol (DCIP) test has been included along with NESTROFT for preliminary screening of hemoglobinopathies and this was shown to be very effective and cost efficient [15]. The solubility test is widely used in India for screening for Hb S and could be used along with NESTROFT in population screening programmes where both  $\beta$ -thalassemia and Hb S are frequent. The flow cytometric osmotic fragility test has also been shown to be very sensitive in discriminating a group of carriers of hemoglobinopathies ( $\alpha$ ,  $\beta$ , and Hb S) as compared to NESTROFT which missed many Hb S carriers [13].

In this issue of the Journal, a study is presented where antenatal screening for β-thalassemia was done in 1000 antenatal women in North India using NESTROFT, complete blood counts (CBC), and HPLC in all the cases. The husband of every β-thalassemia carrier could also be screened and two couples at risk were identified. Both couples opted for prenatal diagnosis. One of the couples had an affected fetus and this pregnancy was terminated. They have shown that NESTROFT was more sensitive compared to MCV, MCH, and RBC count taken individually or when a combination of these three parameters was taken together. The specificity of NESTROFT was also shown to be the highest, closely followed by the RBC count. Surprisingly, a fairly large number of pregnant women who were heterozygous for \beta-thalassemia had MCV >80 fL (25.58 %) and MCH >27 pg (39.24 %). They conclude that although HPLC may be considered the gold standard for diagnosis of β-thalassemia, NESTROFT alone can be used for mass screening as it is sensitive, cost efficient, inexpensive, and simple to perform. Although HPLC was done on all the 1000 women, they have not mentioned whether they detected any abnormal hemoglobins as Hb D-Punjab and Hb Q-India are more common in the north Indian population.

The authors have also compared the mode of delivery, birth weight, and the gestational age at delivery of  $\beta$ -thalassemia trait and non $\beta$ -thalassemia trait women but did not find any significant differences, their findings being similar to earlier studies.

No population screening approach is 100 % accurate. Thus, the strategy for primary screening for  $\beta$ -thalassemias and other hemoglobinopathies will vary and depend on the

available infrastructure, equipment, and facilities as well as cost-benefit analysis for mass screening. Screening for  $\beta$ -thalassemia carriers can easily be integrated as a part of standard antenatal care in an institutional set up where both NESTROFT and CBC can easily be done. This will minimize the number of  $\beta$ -thalassemia carriers who would remain undetected.

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