Original Article

Assessing NESTROFT as a preliminary screening tool for thalassemia in the Malayali tribes of Dharmapuri district, Tamil Nadu, India

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Abstract

Background: Mutations affecting transcription, translation, or beta-globin stability, among other stages of beta-globin production, cause beta-thalassemia. **Methods:** Beta-thalassemia results in a high red blood cell count with a low mean corpuscular volume (60–70fl) and mean corpuscular hemoglobin (19–23pg), and Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) is recommended for mass screening of populations. Among tribals in Dharmapuri district, this cross-sectional analysis evaluated the efficacy of RBC and NESTROFT against HPLC, regarded as the gold standard, in a study involving 484 subjects. **Results:** Findings indicated that out of the 484 samples, 73 tested positive for the beta-thalassemia trait through HPLC. The NESTROFT test demonstrated 87% sensitivity, 98.5% specificity, a positive predictive value of 99.3%, and a negative predictive value of 73.3%. In the multivariate analysis, NESTROFT and hemoglobin showed high significance with *P* values of 0.003 and 0.000, respectively. **Conclusion:** NESTROFT's high sensitivity is particularly noteworthy due to the absence of research among the Malayali tribes in the Dharmapuri district. Adoption of this preliminary test shows promise in detecting the disease at the local level, providing important information for early detection, especially considering the sizeable tribal population.

Keywords: Beta-thalassemia, HPLC, NESTROFT, RBC indices, Tamil Nadu, tribes

Introduction

Hemoglobinopathies are a group of hemoglobin synthesis abnormalities that are inherited from one generation to the next.[1] India has the biggest concentration of individuals belonging to tribal communities anywhere in the globe.[2] The tribal people in India are exposed to considerable health concerns due to the presence of β-thalassemia and hemoglobinopathy.[3] Developing screening methods that are both efficient and effective will continue to be of utmost importance in the prevention of beta-thalassemia trait for the foreseeable future. The results of the Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) might be improved with the regular use of RBC indices obtained from automated cell counters.[4] Patients still face challenges and difficulties while attempting to get treatment for thalassemia major. Because prevention is of such critical importance, the search for an effective screening tool has been a goal for a very long time. There is a test known as NESTROFT[5] that is considered to be a potentially useful diagnostic tool. With a sensitivity of up to 99.8% for detecting thalassemia carriers, it is believed to be simple, economical, fast, and objective, particularly in regions where the illness is quite widespread.[6]

There are no studies on NESTROFT in Tamil Nadu, except a thesis, which showed the parameters of mean corpuscular

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Received: 31-01-2024 **Revised:** 05-03-2024
Accepted: 18-04-2024 **Published:** 14-06-2024

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_157_24

How to cite this article: Pichamuthu BG, Arunachalam KD, Kosalram K. Assessing NESTROFT as a preliminary screening tool for thalassemia in the Malayali tribes of Dharmapuri district, Tamil Nadu, India. J Family Med Prim Care 2024;13:2767-71.

volume (MCV) and mean corpuscular hemoglobin (MCH) do not exhibit statistical significance in distinguishing between beta-thalassemia trait and iron deficiency anemia. Although NESTROFT surpasses RBC indices in accuracy, it is associated with higher rates of false positives.[7] High-performance liquid chromatography (HPLC) analysis is considered the gold standard for diagnosing thalassemia trait, but because of its high cost and the need for advanced equipment, it is not used in large-scale screening programs.[8] In most cases, people of the indigenous population who belong to tribal communities live in secluded, unspoiled landscapes, which are far from metropolitan culture. It is acknowledged that they were the ones who first inhabited the country.[9] Tamil Nadu, a state located in the southern region of India, is recognized as having one of the most diverse tribal population landscapes[10] as it is home to 36 distinct types of tribal communities. Thalassemia, which is more prevalent among Indian tribal tribes, is one of the monogenic diseases that are experienced by the greatest number of people in the Indian subcontinent.[11] The implications of thalassemia for tribe members who are affected by it and their families are severe, and they have a significant impact on their emotional, psychological, and economic well-being.[12]

There are 36 Scheduled Tribes (STs) in Tamil Nadu according to the state government, and they are distributed among 38 districts. According to the census completed in 2011, there were a total of 794,697 STs in the state. Of them, there were 401,068 males and 393,629 females. 660,280 persons who were classified as ST were living in rural regions, while 134,417 were living in urban areas, which accounted for 1.1% of the entire population of Tamil Nadu.[13]

The utility, cost-effectiveness, and reliability of the NESTROFT test have been established by numerous investigations.[14] Although there is a significant incidence of hemoglobinopathies in Tamil Nadu, there is a lack of information about the general prevalence of these ailments in the indigenous tribes of the state. The primary objective of this research is to assess the effectiveness of NESTFORT in the field, particularly in comparison to HPLC, from the perspective of a large number of tribal populations living in the Dharmapuri area of Tamil Nadu.

Materials and Methods

We conducted a cross-sectional study among Malayali tribals in Dharmapuri district, Tamil Nadu, involving a total of 484 subjects. This research is part of an ICMR-funded initiative focusing on tribal communities, with the principal investigator (PI) and research scholar expressing interest in assessing the efficacy of NESTROFT as a field-level test for thalassemia. The Institutional Review Board and Ethics Committee (IRB Reg. No. IR800008555) granted ethical approval. All study participants provided informed consent diligently. We developed a comprehensive questionnaire to gather data, encompassing demographic variables and diagnostic values obtained from the complete blood count (CBC), NESTROFT, and the confirmatory test, HPLC.

A vacuum-enabled tube filled with K2 EDTA anticoagulant was used to draw a 3 ml intravenous blood sample. At our laboratory in the study area, we performed NESTROFT and CBCs on all samples within 3 hours of collection. We performed the CBC using an automated hematology analyzer (SYSMEX POCH). We tested all the blood samples for NESTROFT using freshly made 0.36% saline and obtained readings using a transparent test tube. We pipetted 20 microliters of whole blood (2 µl) into a glass tube and shook it with 4.0 milliliters of freshly made 0.36% buffered saline solution. The tube remained undisturbed at room temperature for approximately 20 minutes. After another shake, we determined the outcome by observing three distinct black lines that were visible from a standard distance behind the tube. When lines were visible, the results were recorded as negative for the beta-thalassemia trait, when they were not visible, and when they were partially apparent as "unsure of the beta-thalassemia trait." The HPLC test was done as a confirmatory test using Bio-Rad at SRM Hospital and Research Centre, and this HPLC is used as a gold standard.

As part of doctoral studies, the principal author, a public health specialist with a strong interest in hemoglobinopathies, conducted this study. The doctoral study intends to investigate the incidence of sickle cell anaemia and thalassemia among the Malayali Tribes in Dharmapuri district, Tamil Nadu, and initial detection strategies that are both affordable and effective. Focusing on this specific disease and tribal population makes sense because a) there are not many recent studies on hemoglobinopathies in Tamil Nadu and b) we want to understand how different preliminary tests, like NESTROFT, solubility, and RBC indices, are used in field settings among the tribal communities. Further investigation is necessary to explore the particular mutations and molecular traits that make up this susceptible group.

Data analysis

SPSS software was used for the data analysis to calculate *P* values. We used the Pearson Chi-square test to investigate the relationship between two nonparametric variables. In addition, we calculated and compared the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic value of each formula with the outcomes of NESTROFT and CBC. We conducted binary logistic regression and multiple logistic regression analyses to investigate relationships between factors.

Results

A total of 23 individuals were found to carry the sickle cell trait, while 73 individuals were identified as carriers of the beta-thalassemia trait through HPLC analysis among the 484 respondents. Despite fresh reagent preparation before the study, six samples were not detected as positive by the NESTROFT test. However, 67 cases with an A2 greater than 4 were correctly

classified as positive. Conversely, ten blood samples tested positive in the NESTROFT test but were negative in the HPLC estimation, although the NESTROFT test accurately diagnosed 401 blood samples as negative for thalassemia. Table 1 presents the plotted information.

The NESTROFT test demonstrated solid diagnostic performance in detecting beta-thalassemia and sickle cell traits among 484 respondents. With an 87% sensitivity and 98.5% specificity, the test exhibited a favorable balance between identifying true positives and avoiding false positives. A high PPV of 99.38% underscores the reliability of positive results, while a 73.3% NPV indicates moderate confidence in negative results.

Though NPV is relatively lower, these metrics collectively emphasize NESTROFT's effectiveness. Integrating it with other methods like HPLC could enhance overall screening accuracy, indicated in Table 1.

For the analysis of RBC indicators, Hb, RBC, MCV, MCH, and NESTROFT were considered. Among the total 484 samples, 280 had anemic Hb less than 12. In RBC, those values were higher than (4.5–5.6 × 1012/L), and 238 samples were higher. In MCV (80–95 fl), 252 samples were lower. In MCH (27–34 pg), 362 samples had lower MCH. There was a nonsignificant negative correlation between HbA2 and Hb (r = -0.163, $P = 0.079$) [Table 2]. Twenty-six study participants had serum ferritin levels less than 10 ng/dl, indicating iron deficiency. Among these, 20 subjects had normal HbA2 levels.

The binary logistic regression analyses revealed various relationships between blood parameters and the result. The association between hemoglobin and odds was statistically significant (P value = 0.003), with an adjusted odds ratio of 0.293, suggesting a reduction in odds of approximately 70.7% for every unit increase. With odds ratios of 1.421 and 1.212, respectively, MCV and MCH displayed nonsignificant correlations. With a 2.492 odds ratio, MCV (RBC) showed a significant positive relationship (P value = 0.046). Surprisingly, MCV NESTROFT showed a strong 52.9 odds ratio and a highly significant association (P value < 0.001). These results highlight both significant and nonsignificant associations in the complex relationships between blood parameters and the examined outcome, explained in Table 3.

Positive: Turbidity impairs the visibility of a dark, bold line drawn on white paper. Negative: A clear red solution through which the dark line is easily seen. The identification is shown in Figure 1.

Table 1: Description of NESTROFT results

HPLC Results	No. of respondents	NESTROFT Positive	NESTROFT Negative
Positive	73	67	6
Negative	411	10	401

Table 2: Evaluation of 'Nestroft' as a screening test for the beta-thalassemia trait

Statistical parameters	(%) Percentage of affected individuals	95%CI
Sensitivity (%)	87.01%	77.41%-93.59%
Specificity (%)	98.53%	96.82%-99.46%
PPV (%)	99.38%	98.63%-99.72%
NPV (%)	73.73%	61.15%-83.34%

Discussion

Hemoglobinopathies, encompassing thalassemia and sickle cell disease, present a significant global health burden, affecting millions of individuals worldwide. Estimates suggest that approximately 7% of the global population carries genetic variants for these disorders, with over 300,000 babies born annually with severe forms. Regions such as South Asia, the Middle East, and parts of Africa bear a disproportionate burden due to higher prevalence rates and limited access to healthcare services, exacerbating the impact on affected individuals and their families. The socioeconomic consequences extend beyond health, with families facing financial strain from the costs of ongoing medical care, including transfusions and supportive therapies, alongside diminished productivity and educational opportunities for affected individuals. Efforts to mitigate this burden involve improving access to screening, genetic thalassemia, and affordable treatments through collaborative initiatives between governments, healthcare organizations, and international agencies. By raising awareness, enhancing access to care, and investing in research and innovation, there is potential to alleviate the global impact of hemoglobinopathies and enhance the well-being of affected individuals worldwide.

The main motive of this study is to find out the effectiveness of using NESTROFT and another preliminary test in Tamil Nadu, Dharmapuri District. There are very limited studies done, especially in Tamil Nadu and tribal areas. Due to the large tribal population in Tamil Nadu, this study will be effective in encouraging further research on thalassemia among the tribals.

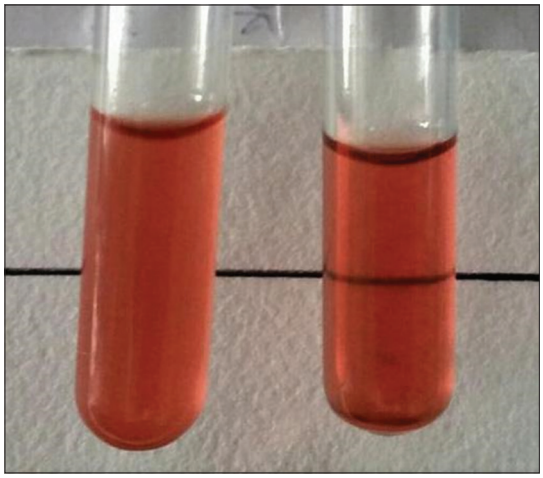

Figure 1: NESTROFT test

Table 3: Multivariate analysis for all preliminary tests and HPLC

Abnormal values	Positive 73	Normal 411	Crude odds ratio, *P* (95% CI)	Adjusted odds ratio, *P* (95% CI)
Hemoglobin ≤12 g/dl (*n*=280)	47 (64)	184 (44.7)	0.342,0.000 (0.204-0.574)	0.293,0.003, (1.296-0.665)
MCV <74fl (*n*=252)	65 (89)	123 (30)	5.855,0.0001 (3.061-11.2)	1.421,0.524 (0.482-4.184)
MCH <74fl (*n*=362F V)	62 (85)	120 (30)	9.5, 0.0001 (2.9-30)	1.212,0.718 (.279-5.271)
RBC (4.5-5.6) (*n*=238)	7 (10)	90 (21.8)	4.962,0.000 (2.7-9)	2.492,0.046 (1.016-6.110)
NESTROFT positive (*n*=67)	67 (92)	6 (24)	72.488, 0.0001 (32-159)	52.9,0.000 (22.126-123)

Blood analysis using HPLC is considered the gold standard for diagnosing the thalassemia trait; however, the high cost of sophisticated and expensive equipment prevents the use of this method in large screening programs. For field research and mass screening, NESTROFT has proven to be an effective, efficient, quick, and reasonably priced tool.[15]

The present study conducted a comprehensive analysis of blood parameters and diagnostic test outcomes in 484 respondents, focusing on NESTROFT's performance in detecting beta-thalassemia. Through HPLC analysis, the researchers identified 23 cases of sickle cell trait and 73 cases of beta-thalassemia trait among the respondents. Notably, NESTROFT exhibited robust diagnostic performance, correctly classifying 401 cases as negative for thalassemia and accurately identifying 67 cases with an A2 greater than 4.

The diagnostic accuracy metrics for NESTROFT were commendable, with an 87% sensitivity and 98.5% specificity, demonstrating a balanced ability to identify true positives and avoid false positives. The high PPV of 99.38% underscores the reliability of positive test results, while the 73.3% NPV indicates a moderate level of confidence in negative results. While the NPV is relatively lower, the overall diagnostic performance of NESTROFT is noteworthy. A study done in tertiary care hospitals showed 100% sensitivity, 100% specificity, 100% PPV, and 100% NPV.[15] The results have the highest values because the study is hospital-based. A study done in the year 2021 showed similar results: a sensitivity of 84.2%, a specificity of 96.2%, a PPV of 69.5%, and an NPV of 98.3%.[9] A study done among pregnant women showed the high efficiency of NESTROFT, with a sensitivity of 98.3%, a specificity of 96.7%, a PPV of 96.7%, and an NPV of 98.3%.[16]

In the analysis of RBC indices, the study identified 280 cases of anemia (Hb less than 12), 238 cases with elevated RBC values (4.5–5.6 × 10^12/L), 252 cases with lower MCV values (80–95 fl), and 362 cases with lower MCH values (27–34 pg). Furthermore, the study identified a nonsignificant negative correlation between HbA2 and Hb (r = -0.163), indicating a potential relationship between these two parameters.

The binary logistic regression analyses revealed diverse associations between blood parameters and the outcome. While the association between hemoglobin and odds was statistically significant (*P* value = 0.003), indicating a reduction in odds with increasing hemoglobin levels, MCV and MCH did not exhibit significant correlations. However, MCV (RBC) showed a significant positive relationship (*P* value = 0.046), and MCV (NESTROFT) demonstrated a strong association with a 52.9 odds ratio (*P* value < 0.001).

Numerous tribal communities in India were found to have different types of hemoglobinopathies; sickle cell anemia and beta-thalassemia were the most prevalent diseases. Consanguineous marriages in the tribal community primarily cause these illnesses, which are hereditary or genetic. They also tend to run in families.[17]

These findings collectively underscore the complexity of relationships between blood parameters and diagnostic outcomes and suggest enhancing overall screening accuracy by integrating NESTROFT with other methods, such as HPLC. Additionally, the study highlights the importance of considering multiple blood parameters for a comprehensive understanding of the hematological profile and thalassemia traits among the study population. The observed relationships and diagnostic performance metrics contribute valuable insights to a broader understanding of these hematological conditions.

The findings of this study hold significant relevance for primary care physicians, especially those serving in regions with a substantial tribal population like Tamil Nadu's Dharmapuri District. By demonstrating the effectiveness of NESTROFT as a preliminary screening tool for thalassemia among tribal communities, this research provides valuable insights that can inform the practice of primary care physicians in their efforts to improve healthcare delivery and disease management in such populations. Primary care physicians play a crucial role in the early detection and management of hemoglobinopathies, and integrating cost-effective screening methods like NESTROFT into routine practice can enhance their ability to identify at-risk individuals promptly. Moreover, the study's emphasis on the importance of considering multiple blood parameters for a comprehensive understanding of thalassemia traits underscores the need for primary care physicians to adopt a holistic approach to patient evaluation and diagnosis. By incorporating the findings of this study into their clinical practice, primary care physicians can contribute to reducing the burden of hemoglobinopathies and improving the overall health outcomes of tribal communities in Tamil Nadu and similar regions worldwide.

Conclusion

The study's objective is to evaluate the efficacy of employing NESTROFT and additional preliminary tests, particularly in

Tamil Nadu's tribal communities, with a particular emphasis on Dharmapuri District. Importantly, very little research has been done in this particular context, especially concerning the effects of thalassemia on the health of Tamil Nadu's tribal population. This study has the potential to provide important new insights because of the significant tribal population in the area. Its results could provide an important starting point for future studies on thalassemia in the particular setting of Tamil Nadu tribal communities, which would help us comprehend the health issues these communities face.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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