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Role of NESTROFT as a Screening Tool for Beta-Thalassemia Trait among Pregnant Women Attending a Tertiary Care Hospital: A Cross-Sectional Study

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ABSTRACT

Background

Iron deficiency and thalassemia trait are the most common causes of microcytic hypochromic anemia. Serum ferritin and hemoglobin electrophoresis are required for the diagnosis of these conditions. These investigations are costly and not readily available in rural settings. Moreover, investigating a large population will overburden the health care facilities. Therefore, this study was conducted to determine the role of NESTROFT for identification of beta-thalassemia trait among pregnant women.

Material and Methods-

This cross-sectional study was conducted in the Department of Pathology of a tertiary care hospital for a duration of two years. All the pregnant women who had microcytic hypochromic anemia were evaluated with NESTROFT and hemoglobin electrophoresis. The quantitative data was represented as mean ± SD and qualitative data as frequency and percentage. The t-test was used for analyzing quantitative data and categorical data was analyzed using the chi-square test. The significant threshold of the p-value was set at <0.05.

Results

Total 312 pregnant women with microcytic hypochromic anemia were evaluated during the study period. The prevalence of beta-thalassemia trait was 3.85%, and NESTROFT exhibited sensitivity of 75%, specificity of 98%, positive predictive value of 60.3%, negative predictive value of 98.9%, and a Youden Index of 0.73.There was statistically significant difference in mean RBC count, mean MCV, mean MCH and mean RDW values among pregnant women with and without beta-thalassemia trait. Red cell distribution width Index had sensitivity= 66.8%, specificity= 96.6%, NPV=44.8%, PPV= 98.6%, and Youden Index=0.63.

Conclusion

NESTROFT is a simple and cost-effective screening tool for identification of beta-thalassemia trait among pregnant women.

Key Words: NESTROFT, Beta-thalassemia trait, Hemoglobin electrophoresis, pregnant women, RDW index

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INTRODUCTION

Iron deficiency and thalassemia trait are the most common causes of microcytic hypochromic anemia. Beta-thalassemia is the most common monogenic disorder characterized by defective β- globin chain production, and is highly prevalent in tropical and subtropical countries. India is estimated to have approximately 1-1.5 lakh children affected by thalassemia major, and 42 million carriers of beta-thalassemia, which is one of the highest in the world. [1] It is well established that some geographical areas and communities have high risk of thalassemia syndromes. Iron deficiency anemia is also a significant public health problem affecting nearly 52% pregnant women in India. [2] Distinguishing iron deficiency anemia from beta-thalassemia trait is challenging sometimes especially in pregnant women. Iron deficiency anemia is diagnosed by low serum ferritin levels, whereas beta-thalassemia trait is diagnosed by estimating Hb A2 levels by electrophoresis or high performance liquid chromatography. These investigations are expensive and tedious. Moreover, these tests are not widely available and affordable for many. Screening of large population for the cause of microcytic hypochromic anemia is time consuming as well not economical for resource poor country like India. Hence, simple, and inexpensive tests are the need of time especially for resource poor and high prevalence settings. Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) is a simple test which does not require skilled manpower and advanced lab machines. Various studies reported high sensitivity and negative predictive value for NESTROFT in children for beta-thalassemia trait screening. [3-4] It is an important mass screening tool to identify the beta-thalassemia trait without burdening health care resources.The exact prevalence of beta-thalassemia trait among pregnant women from Marathwada region of Maharashtra state is unknown. The literature on utility of NESTROFT as a screening tool for beta thalassemia trait among antenatal women from this region is limited. Therefore, this cross-sectional study was conducted to estimate the prevalence and determine the role of NESTROFT as a screening test for beta-thalassemia trait at a tertiary care centre.

Material and Methods

This cross-sectional study was carried out in the Department of Pathology of a tertiary care center for a period of two years from October 2018 to September 2020.All the admitted pregnant women who had microcytic anemia were included in this study. Women with known hemoglobinopathies or iron deficiency anemia, those received blood transfusion within previous three months, and those on retroviral therapy were excluded from the study. A written informed consent was obtained from the study participants for participation in the study and publication of data. 4 ml venous blood was collected in ethylenediamine tetra-acetic acid (EDTA) bulbs for complete blood count and was analyzed using Medonics Coulter M32 (Boule Diagnostics). The peripheral smear was examined for microcytic hypochromic red blood cells. The NESTROFT and Hemoglobin electrophoresis were performed on venous blood of those women who had microcytic hypochromic anemia. NESTROFT was performed using Biolab's Rapid Thalassemia kit, while hemoglobin electrophoresis was performed on Sebia Hydragel Hemoglobin [E] K 20. A cut off value of Hb A2 >3.5% was considered for diagnosing beta-thalassemia trait. Demographic characteristics, symptoms, other relevant history, physical findings, and all laboratory investigations were noted in a pre-designed proforma. After collecting the data, a master chart was prepared using MS-Excel. The data was analyzed using SPSS 22.0 (IBM corporation). The quantitative data was represented as their mean ± SD. Categorical and nominal data were expressed as frequency and percentage. The t-test was used for analyzing quantitative data and categorical data was analyzed using the chi-square test. The significant threshold of the p-value was set at <0.05.The ethical clearance was obtained from the Institutional Ethics Committee vide Letter no.363/2018.

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Results

A total of 312 pregnant women with microcytic hypochromic anemia were enrolled in the study. Among study participants, 41.7% were between 18–24-year age, 36.5% were between 25-30 year age, 16.4% were 31-35 year, and 5.4% were > 35 years of age. There were 69.6% Hindu women, 29.8% Muslims, and 0.6% Christians in the study. All the women diagnosed with beta-thalassemia trait were in the second and third trimester. The demographic and other characteristics of study participants are shown in Table 1.

Table 1: Demographic details and other variables among study participants (n=312)

Variables	Frequency (n)	Proportion (%)
Age (year)		
18-24	130	41.7
25-30	114	36.5
30-35	51	16.4
>35	17	5.4
Religion		
Christian	2	0.6
Hindu	217	69.6
Muslim	93	29.8
Parity		
Primi-para	162	51.9
Multipara	144	46.2
Grand-multipara	6	1.9
Trimester		
First	39	12.5
Second	74	23.7
Third	199	63.8

Out of the 312 women, 15 women (4.8%) had positive NESTROFT, and 297 women (95.2%) had negative NESTROFT. Total 12 pregnant women (3.85%) had beta-thalassemia trait (Hb A2>3.5%) by hemoglobin electrophoresis. Therefore, 6 women were false positive, and 3 women were false negative by NESTROFT as shown in Table 2. The overall prevalence of beta-thalassemia trait in pregnant women was reported as 3.85%. This study reported a sensitivity of 75%, specificity of 98%, positive predictive value of 60.3%, and negative predictive value of 98.9% for NESTROFT for diagnosis of beta-thalassemia trait.

Table 2: Comparison of NESTROFT and Hb electrophoresis among study participants

NESTROFT	Hb Electrophoresis		Total
	Hb A2>3.5%	**Hb A2 <3.5%**	
Positive	9 (TP)	6 (FP)	15
Negative	3 (FN)	294 (TN)	297
P value <0.00001			

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Table 3 illustrates the mean values of various red cell indices and hemoglobin fractions among pregnant women with and without beta-thalassemia trait. The mean hemoglobin value (8.65 ± 1.14 vs 8.41 ± 1.93, p=0.5), mean red cell count (4.70 ± 0.62 vs 3.45 ± 0.83, p=<0.001), and mean Hb A2 levels (4.94 ± 0.43 vs 2.59 ± 0.51, p=<0.001) were higher in pregnant women with beta-thalassemia trait. However, no statistical significance was seen for hemoglobin levels, and red cell distribution width among women with and without beta-thalassemia trait. Similarly, mean MCV value (60.54 ± 5.78 vs 68.64 ± 8.19, p=<0.001), mean MCH value (18.65 ± 3.17 vs 68.64 ± 8.19, p=<0.001), and mean RDW values (16.38 ± 2.69 vs 19.10 ± 4.64, p=<0.001) were significantly lower in women with beta-thalassemia trait. A statistically significant correlation was seen in mean red cell count (p<0.002), mean corpuscular volume (p<0.007), mean corpuscular hemoglobin (p<0.009), Mentzer Index (p<0.001), and red cell distribution width Index (p<0.00001) among women with and without beta-thalassemia trait.

Table 3: Various red cell parameters among antenatal women with and without beta-thalassemia trait

Parameters	Diagnosis		p-value (T-test)
	BTT (n=12)	**No BTT (n=300)**	
Hb (gm/dl)	8.65 ± 1.148	8.41 ± 1.932	0.66
RBC (million/L)	4.70 ± 0.620	3.45 ± 0.834	**< 0.001**
HCT (%)	26.15 ± 3.420	25.93 ± 5.743	0.833
MCV (fl)	60.54 ± 5.789	68.64 ± 8.192	**< 0.001**
MCH (pg)	18.65 ± 3.176	25.06 ± 5.863	**< 0.001**
MCHC (%)	33.15 ± 2.035	32.46 ± 2.861	0.278
RDW (%)	16.38 ± 2.698	19.10 ± 4.647	**0.005**
HBA (%)	93.52 ± 1.671	97.08 ± 0.605	**< 0.001**
HBF (%)	1.54 ± 1.618	0.32 ± 0.433	0.024
HBA2 (%)	4.94 ± 0.432	2.59 ± 0.513	**< 0.001**

The diagnostic accuracy of various red cell parameters is shown in Table 4. Among various red cell parameters, MCH value <27 has shown 100% sensitivity, and negative predictive value. Similarly, Mentzer Index had high specificity of 98.3%, and negative predictive value of 97.9%. The red cell distribution width index also had high specificity (96.6%), and negative predictive value (98.6%).

Table 4: Diagnostic accuracy of red cell parameters for identifying beta-thalassemia trait

Red cell parameter	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden Index
RBC count	> 5 million/L	66.6	2.0	2.6	60.3	-0.32
MCV	< 60 fl	41.6	85.3	10.1	97.4	0.26
MCH	<27 pg	100	36.3	5.8	100	0.36

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RDW	<14%	16.7	91.7	7.5	96.4	0.08
Mentzer Index	<13	50	98.3	54.9	97.9	0.48
RDWI	< 220	66.8	96.6	44.8	98.6	0.63
NESTROFT	Positive	75	98	60.3	98.9	0.73

DISCUSSION

In the present study, the prevalence of the beta-thalassemia trait among pregnant women admitted in a tertiary care centre was found to be 3.85%. This is in accordance with the finding of Satpute S et al study which reported the prevalence of 3.1%. [5] The most pregnant women in our study were between 18-24 years of age (41.7%). Similarly, Gosavi M et al study had 53.4% women in the 21–25-year age group 32.1% in the 26-30 years age group. [6] In this study, 69.6% women were Hindu, 29.8% were Muslim and 0.6% were Christian. This is in accordance with the previous studies by Gosavi M et al, Kulkarni P et al which also had majority of Hindu women followed by Muslim and Christian women. [6,7] In the present study, 63.8% pregnant women were in the third trimester, 23.7% women in the second trimester, and only 12.5% women in the first trimester. A study by Amna A et al had 57% women in the third trimester, 19% women in the second trimester, and 24% women in the first trimester.[8] In another study by Bhukhandwala D S et al, 52.6% women were in the second trimester, 37.1% women in the first trimester while 10.3% women were in the third trimester.[9] In this study, NESTROFT showed sensitivity of 75%, specificity of 98%, positive predictive and negative predictive values of 60.3%, and 98.9%. The Youden Index was 0.73. A study by Mendiratta S L et al on antenatal women for beta-thalassemia trait, found that NESTROFT had sensitivity= 78.48%, specificity= 94.14%, PPV=53.45%, and NPV= 98.08%. [10] Gosavi M et al study also reported sensitivity of 84%, specificity of 96.2%, PPV of 69.5% and NPV of 98.3%. [6] In the present study, the mean red blood cell count was higher among women with beta-thalassemia trait compared to women without beta-thalassemia trait. Similarly, the mean MCV, mean MCH, and mean RDW values were lower among women with beta-thalassemia trait. This is in accordance with the results of previous studies. No statistically significant difference was observed in mean hemoglobin values among women with and without beta-thalassemia trait which is in accordance with the results of Gosavi M et al study.[6] However, few previous studies have shown statistically significant difference in mean hemoglobin levels among women with and without beta-thalassemia trait.In the present study, red cell distribution width index had a sensitivity of 66.8%, specificity of 96.6%, PPV of 44.8%, and NPV of 98.6%. Bhushan R et al study also reported similar results where RDWI had sensitivity of 63.4%, specificity of 99.6%, PPV of 90.9%, and NPV of 98.1%. [11] Similarly, this study also showed high sensitivity and NPV of 100% for MCH value, while specificity of 36.3% and PPV of 5.8% only. The previous studies by Mendiratta S L et al and Baliyan M also reported similar sensitivity of 60-77% and higher NPV of 96% for MCH. [10,12] In the present study, low sensitivity of 16.7%, high specificity of 98.3%, PPV of 54.9%, and NPV of 97.9% was observed for Mentzer Index which is in accordance with the findings of Bhushan R study which reported similarly high specificity and NPV, while low sensitivity for Mentzer index. [11]

CONCLUSION

The prevalence of beta-thalassemia trait among pregnant women from Marathwada region is found to be 3.85%. The study also establishes that

NESTROFT is a reliable, simple cost-effective screening tool for identification of beta-thalassemia trait in pregnant women. Other red cell parameters including Mentzer Index and Red Cell distribution width Index along with NESTROFT can be reliably included in the initial investigation protocol during antenatal visits to identify beta-thalassemia trait among pregnant women having microcytic hypochromic anemia. It will help in providing prenatal genetic testing and counseling to expectant mothers and their families prior to delivery.

Authors Contribution- Amrita Singh Chauhan: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. **Anil R Joshi:** Conceptualization, Writing- Reviewing and Editing, Supervision. **Smita Hilalpure**: Investigation, Data Curation

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