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Utility of haematological parameters and NESTROFT as screening tools for thalassemia trait- A study in a tertiary care hospital

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ABSTRACT

Background: Thalassemia being one of the commonest causes of anaemia, screening remains is the backbone for diagnosing traits. Considering expensive confirmatory diagnostic tests, aim of this study was to study utility of haematological parameters and NESTROFT as screening tools for thalassemia trait.
Materials and Methods: It is a one and half year prospective study from June 2017 to December 2018, carried out in outdoor patients investigated in central laboratory of a tertiary care centre. A total of 5000 cases were randomly selected based on low haemoglobin values as per WHO Criteria. All the haematological parameters of the complete hemogram performed, were thoroughly analysed. Mentzer Index (MI) and Ehasani Index (EI) were calculated. NESTROFT was performed in all study samples and results were compared with haematological parameters. Results were compiled and statistically analysed.
Results: Out of total 5000 cases with low haemoglobin, mean haemoglobin was 10.12+1.29. Age ranged between 1-82 years with mean age 36.22+16.50 years with female preponderance. NESTROFT was positive in 152 cases (3.04%) and haematological parameters such as RBC, Hemoglobin, MCV were found to be significant ($p<0.05$) in those cases. The sensitivity, specificity, PPV and NPV of MI and EI with NESTROFT was found to be 94.74%, 89.77%, 22.50%, 99.82% and 94.74%, 89.44%, 21.95%, 99.82% respectively.
Conclusion : This study concludes that both haematological parameters and NESTROFT have to be used in conjunction as the primary screening tool for thalassemia traits in general population. Thus making it an ideal contender to be adopted in rural settings of developing countries, for early diagnosis and decreasing the morbidity associated with thalassemia.

1. Introduction

In India about 25 million people are identified as carrier and 6000 – 8000 children are born every year with thalassemia major[1]. For early diagnosis and management of thalassemia syndromes, screening remains the backbone for diagnosing thalassemia trait and other haemoglobinopathies to identify the prevalence in developing country. [1]

For screening purposes, a test which is inexpensive, requires a small amount of blood, does not require sophisticated equipment and can be applied on a large scale of population is usually preferred. These requirements are met by a modified osmotic fragility test "NESTROFT" (Naked Eye Single Tube Red Cell Osmotic Fragility Test) a test first described by Kattamis et al. It is simple to perform, cost effective and has high sensitivity and specificity. [2]

Combination of NESTROFT and application of various haematological formulas to various parameters helps to predict thalassemia trait, thus having an added value as

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screening tools.

The aim of this study was to apply NESTROFT to a suspicious group of thalassemia trait identified using various haematological parameters. Thereby correlating NESTROFT with these parameters and socio-demographic profile of patients to identify prevalence of thalassemia trait in outdoor patients of a tertiary care centre.

2. Materials and Methods

This study was prospective, observational and random selection study which was carried out in Pathology department at a tertiary care health centre, over a period of one and half years (June 2017- December 2018). A total of 5000 patients coming for treatment in various specialities with low haemoglobin formed the study group.

2.1. Inclusion criteria

All patients with low haemoglobin (as per WHO guidelines) irrespective of age, gender, caste, religion attending central pathology service laboratory (OPD). Exclusion criteria- Antenatal clinic (ANC) patients as samples of ANC patients are not processed in central pathology service laboratory of our hospital.

EDTA samples were collected and various hematological parameters such as RBC count, total haemoglobin concentration, haematocrit, RDW, MCV, MCH and MCHC using fully automated Cell counter (Model PCE-210 of ERMA INC Company) were estimated. Anaemic patients were identified based on cut off haemoglobin concentration to particular age and sex as per WHO guidelines.[3,4]

These patients were counselled, and Informed consent was taken for carrying out NESTROFT with recollection of EDTA sample. Mathematical formulas to predict type of anaemia was applied on significant haematological parameters. Hospital Ethics committee approval was attained prior to the study.

Different RBC indices and mathematical formulas used in the study wereas follows[4]

MDHL index: Mean Density of Hb/liter of blood;

MCHD index: Mean Cell Hb Density.

Socio - demographic profile and questionnaire was filled up. NESTROFT was done as per procedure and results of NESTROFT were signed off to the patients.

2.2. Principle of nestroft

Normally red cells put in saline solution begins to lyse at a saline concentration of 0.4-0.5% and lysis is complete at 0.32%. However, in thalassemia trait due to alteration in osmotic resistance of affected RBC's (Value & surface area ratio changes) lysis which begins at a saline concentration between 0.4-0.35% may not be completed even at 0.1% solution. NESTROFT is at a saline concentration of 0.36%.[5]

2.3. Procedure of test

2ml of 0.36% buffered saline was taken in sample test tube (T) and 2ml distilled water was taken in control test tube (C). 20 μl of blood was added to both tubes and are left undisturbed for half an hour at room temperature. Both tubes were shaken and held against a white paper with a black line. The line is clearly visible through the control tube(C). If the line was visible in (T), test is negative. If the line was not clearly visible in (T), test is positive[5] as shown in Figure 1.

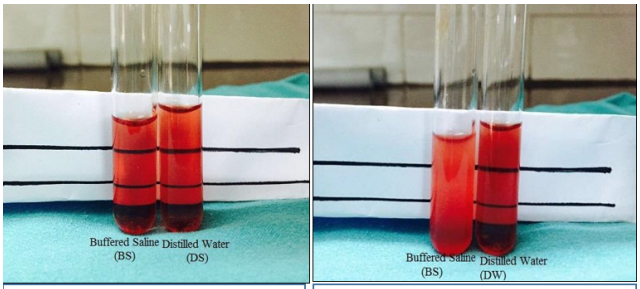

Fig. 1:

All NESTROFT positive patients were counselled and referred to haematology OPD to undergo Hb electrophoresis by High Performance Liquid Chromatography (HPLC) (D10-BIORAD) method. Hb A2 level >3.5% was taken as the gold standard to diagnose beta-thalassemia trait.

The data was analysed using statistical software MiniTab 17.0 for calculating the p values. Comparison of mean between two groups was done using Unpaired 't' test, association between two non-parametric variables was done using Pearson Chi-square test and sensitivity, specificity, positive predictive value, negative predictive value and diagnostic value of each formula was calculated against the NESTROFT Results. Youden's index = (Sensitivity + Specificity) - 100.

3. Results

Distribution of patients according to age, sex, religion and admission to various OPDs were evaluated. The evaluated patients were divided into two groups i.e Beta thalassaemia trait- NESTROFT Positive and non beta thalassaemia trait- NESTROFT Negative. Beta thalassaemia group includes positive NESTROFT and the standard reference values for various mathematical indices and peripheral smear showed microcytic hypochromic anemias. Non beta thalassaemia trait group includes negative NESTROFT and iron deficiency anaemias, anaemia of chronic diseases and sideroblastic anemias.

Sensitivity, specificity, positive predictive value and negative predictive value of NESTROFT and the various mathematical indices were evaluated and the significant

Table 1:

	Hematological index	Formula	β thal trait	IDA
1	Mentzer index (MI)	MCV/RBC	<13	>13
2	England and Fraser (E and F)	MCV − (5 × Hb) − RBC − 3.4	<0	>0
3	Srivastava	MCH/RBC	<3.8	>3.8
4	Shine and Lal (S and L)	MCV × MCV × MCH/100	<1530	>1530
5	RDWI	MCV × RDW/RBC	<220	>220
6	Ricerca	RDW/RBC	<4.4	>4.4
7	Green and King (G and K)	MCV × MCV × RDW/Hb × 100	<65	>65
8	MDHL	(MCH/MCV) × RBC	>1.63	<1.63
9	MCHD	MCH/MCV	>0.3045	<0.3045
10	Ehsani	MCV − (10 × RBC)	<15	>15
11	RBC count		>5	<5

difference between them was tabulated.

In 5000 cases analysed, age ranged from 1- 82 yrs. Majority of cases were seen in 21 – 60 yrs age group (75%) with a female preponderance (M:F- 1:1.8). According to religion, majority cases were hindus followed by muslims and boudh. Among hindus; Gujratis, sindhis, punjabis and other hindus were seen. According to speciality OPD's; majority of patients were referred from Medicine OPD(40.2%) followed by Surgery OPD(23.8%) and Chemotherapy OPD(12%).

Out of 5000 cases, 152 were NESTROFT Positive. Among these 130 were further subjected to HPLC which was positive in 124 cases.

Majority of the patients in NESTROFT positive cases were in age group of 21-40 yrs with a female preponderance (M:f- 1:3.2). Maximum cases were gujratis followed by muslims and other hindus with maximum referral from Medicine OPD. On comparing various hematological parameters with NESTROFT test results as shown in Table 2, the mean RBC is found to be significantly higher in the NESTROFT Positive Cases, while Hemoglobin, HCT, MCV, MCH, and RDW were significantly lower in the NESTROFT Positive Cases. Further applying various mathematical formulae on hematological parameters, diagnostic accuracy for beta thalassemia trait was highest with Srivastava index (92.16%) and lowest with RICERCA index (25.24%) as shown in Table 3.

According to Sensitivity, Specificity, Youden's index; good mathematical indices are in decreasing order as Mentzer index > Ehsani index > RDWI > RBC count > Green and King > MDHL > Shine and Lal > England Fraser > Srivastava > Ricerca > MCHD were ideal for screening tools to identify the beta -thalassemia trait cases a shown in Table 4. It also shows that while the association of NESTROFT was evaluated with Pearson Chi-square test (χ^2), there was a statistically significant association seen between all mathematical indices and NESTROFT test except MCHD, showing that NESTROFT test is dependent on these indices.

In the NESTROFT positive group, peripheral smears of β-thalassaemia trait cases were evaluated and showed mild to moderate microcytosis, hypochromic red cells with mild anisocytosis. Target cell were seen in 70 (46%) cases.

4. Discussion

Worldwide, underlying causes of anaemia are many, varied and largely preventable. These include nutritional deficiencies, infections and haemoglobin disorders. Worldwide frequency of beta thalassemia trait is about 3% whereas in India it ranges from 3 – 18 %. Certain communities such as Sindhis, Gujaratis, Kutchis, Lohanas, Punjabis, Mahars, Agris, Bhanushalis, Gaud, Saraswasts, Gowdas etc., have a higher frequency of beta thalassemia.[1]

Prenatal diagnosis using genotyping techniques and various other confirmatory diagnostic tests are expensive to be made available in poorly resourced countries.

Hence various simpler, cost effective, easy to apply screening tools at outpatient clinics are beneficial to identify thalassaemia traits. These include NESTROFT and various mathematical formulae.

A total of 5000 outpatient clinic patients coming for treatment in various specialties with low haemoglobin formed the study group in which 152 were NESTROFT positive thereby suggestive of beta thalassaemia trait.

In the present study, NESTROFT positivity was seen in 152 cases with maximum number of patients in 21 – 40 years age group (80 patients; 52.6%) and a female preponderance (116; 76.3%). Maximum number of cases were seen in Gujratis (31.6%) followed by Muslims (27.6%) and other Hindus (19.7%). The biggest group was seen in patients referred from Medicine OPD (57.9%) followed by Surgery OPD (18.4%) and Paediatric OPD (18.4%). Similar observations were seen with Piplani S et al[6] in which the maximum number of patients belonged to 21 – 30 years of age group (53 patients; 35.53%) and a female preponderance. Rao G B et al (2017) also showed male: female ratio of 1:3, with a female preponderance.[7]

Out of the total 5000 cases studied 152 were NESTROFT Positive. Among these 130 subjected to HPLC further

Table 2: 2 : Comparison of hematological parameters in relation to NESTROFT test results(n=5000)

Hematological Parameter	NESTROFT Positi[Mean ± SD] (n=152)	NESTROFT Negative [Mean ± SD] (n=4848)	T value	P value
RBC	5.36 ± 0.42	4.33 ± 0.95	13.330, df=4998	0.000*
Hemoglobin	9.74 ± 1.16	10.13 ± 1.29	-3.675, df=4998	0.000*
HCT	30.50 ± 4.01	32.12 ± 3.86	-5.086, df=4998	0.000*
MCV	63.07 ± 5.83	77.97 ± 10.99	-16.644, df=4998	0.000*
MCH	22.16 ± 4.25	24.71 ± 3.64	-8.474, df=4998	0.000*
MCHC	31.12 ± 1.79	31.79 ± 9.79	-0.861, df=4998	0.389, NS
RDW	14.71 ± 1.15	15.19 ± 2.11	-2.858, df=4998	0.004*

Table 3: Diagnostic accuracy of mathematical index in NESTROFT positive identified cases

Formula	Positive number of cases % age		Diagnostic accuracy
NESTROFT Test	152	3.0	100
Mentzer	664	13.3	89.44
England Fraser	406	8.1	91.72
Srivastava	352	7.0	92.16
Shine and Lal	2442	48.8	54.12
RDWI	1138	22.8	80.12
RICERCA	3886	77.7	25.24
Green and King	554	11.1	90.28
MDHL	816	16.3	85.12
MCHD	3580	71.6	29.84
Ehsani	680	13.6	89.12
RBC >5	1128	22.6	79.92

Table 4: Shows Sensitivity, Specificity, Positive predictive value, Negative predictive value, Youden's index

INDICES	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Youden's Index (%)
Mentzer	94.74	89.27	21.69	99.82	84.01
Ehsani	94.74	88.9	21.18	99.81	83.64
RDWI	97.37	79.58	13.01	99.90	76.95
RBC count	90.79	79.58	12.23	99.64	70.37
Green and King	72.37	90.84	19.86	99.06	63.21
MDHL	73.68	85.48	13.73	99.04	58.85
Shine and Lal	98.68	52.72	6.14	99.92	51.4
England Fraser	47.37	93.11	17.13	98.26	40.48
Srivastava	36.84	93.89	15.19	97.93	30.73
Ricerca	98.68	22.94	3.86	99.82	21.62
MCHD	73.68	28.47	3.13	97.18	2.15

confirmed positivity in 124 cases. The diagnostic accuracy of NESTROFT Test was found to be 100.0%. Regarding the usefulness of the NESTROFT as a screening test for the detection of beta thalassaemia trait, present study corroborated well with other studies as shown below Table 5.

Hence, the NESTROFT Test was used as an alternative test to HPLC in the present study.

Further on correlation of the RBC indices and other mathematical indices with other similar studies, the mean haemoglobin values of our study group was 9.74 ± 1.16 g/dl which was lower than that of observation made by Chakraborty et al,[11] Vehapoglu A et al[9] and Piplani S et al[10] who reported haemoglobin of 10.09±0.65 g/dl, 10.39±0.69 g/dl and 10.7±0.8 g/dl respectively.

The mean red blood cell count values of our study was 5.26 ± 0.42, 10^6/cmm which was almost similar to that of the observation of ICMR Study.[12] Higher mean RBC count has been reported by Vehapoglu A et al[10] (5.56±0.4) and Piplani S et al[10] (5.79±0.76). Lower mean RBC count has been reported by Chakraborty et al[11] (10.09±0.65).

The mean MCV of our study group was 63.07±5.83 fl which was almost similar to that of the observations made by Chakraborty et al[11] and Piplani S et al.[10] Higher mean

Table 5:

Authors	Year	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Sirichotivakul et al[8]	2004	97.6	72.9	33.6	99.5
Chakraborty et al[9]	2012	94.12	95.23	41.02	99.78
Piplani s et al[10]	2013	100	85.47	66	100
Present study	2019	100	100	100	100

MCV has been reported by Madan et al[8] (64.7 ± 4.8).

On comparison of various mathematical indices in beta thalassemia trait positive cases in present study with some earlier studies showed that in the present study, Mentzer Index exhibited a reasonably high sensitivity, specificity, good Youden's index and diagnostic accuracy of 94.74%, 89.27%, 84% and 89.4% respectively. Similar observations were proposed by Ehsani et al[13] in which sensitivity, specificity, Youden's index and diagnostic accuracy were as 94.6%, 95.5%, 94.71% and 90.1% respectively. Almost similar results have been documented by Piplani S et al,[10] Rahim et al[14] and Vehapoglu A et al[9] who recorded sensitivity and specificity of 94.4% and 86.9% respectively.

In the present study, Ehsani Index also exhibited high sensitivity, specificity, good Youden's index and diagnostic accuracy of 94.74%, 88.94%, 83.64% and 89.12% respectively. Similar observations were proposed by Ehsani et al[13] in which sensitivity, specificity, Youden's index and diagnostic accuracy of 90%, 95.5%, 85.5% and 92.9% respectively which also corroborated well with Vehapoglu A et al[5] and Piplani s et al.[2]

In the present study, RDWI also showed high sensitivity, specificity, good Youden's index and diagnostic accuracy of 97.37%, 79.58%, 76.95% and 80.12% respectively which are comparable with the findings of Rahim et al,[14] Niazi M et al,[15] Adlekha S et al[1] and Tong L et al.[16]

In the present study, RBC count >5 showed sensitivity, specificity, Youden's index and diagnostic accuracy of 90.79%, 79.58%, 76.37% and 79.92% respectively which are comparable with the findings of Rahim et al[14] who found these to be 94%, 84%, 72% and 90% respectively. Almost similar results have been documented by Vehapoglu A et al[9] and Jameel T at al.[17]

Alfadhli S M et al[18] and Ferrara M et al[19] documented not only high Youden's index but also high specificity for England and Fraser index. But in the present study though the specificity was 93.11%, this index showed a Youden's index of just 40.48%. In the present study, the Srivastava index showed a sensitivity, specificity and low Youden's index of 36.84%, 93.89% and 30.73% respectively. Niazi M et al[15] also found a low Youden's index of 27%.

England and Fraser (1979) after evaluation of 1500 peripheral smears of beta thalassaemia traits concluded that microcytic hypochromic red cells and significant target cells are an important finding.[20] The present study also showed mild to moderate microcytic hypochromic red cells, with mild anisocytosis in majority of beta thalassemia trait cases and 46% cases showed presence of target cells.

The best laboratory index of a screening test for beta thalassaemia trait should have a very high sensitivity as well as a reasonably high specificity so that the false positivity can be decreased.

In present study, NESTROFT positive results were correlated well with mathematical indices and we found Mentzer index > Ehsani index > RDWI > RBC count > Green and King were ideal for screening cases of beta thalassemia trait as they showed statistically significant results. Other indices like MDHL, Shine and Lal, England Fraser, Srivastava, Ricerca though statistically significant were not of much use in our study. MCHD also did not show statistically significant results. We observed a low positive predictive value in our study which may be due to non-targeted population of study group.

Haematological parameters in the present study group were on lower side as the cases were included from lower socioeconomic status, coexistence of iron deficiency anemia and referrals from various speciality OPD's like chemotherapy and anti-retroviral therapy OPD.

Though all NESTROFT positive patients were counselled and referred to haematology OPD to undergo HPLC, only 130 cases followed up. Remaining 22 cases were lost to follow up due to unawareness and not understanding significance about disease, illiteracy and lower socioeconomic status.

To conclude, in the present study, various mathematical indices were used but none of those indices shows sensitivity and specificity of 100%, while NESTROFT shows sensitivity and specificity of 100%. Hence, both haematological parameters and NESTROFT have to be used in conjunction as primary screening tools for thalassemia trait in general population. Based on reliability, cost effectiveness and non-requirement of hi-tech equipments, it makes it an ideal contender to be adopted in rural settings of developing countries, for early diagnosis and thereby decreasing the morbidity associated with thalassemia.

5. Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

6. Source of Funding

None.

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