



**UTILITY OF NESTROFT FOR β -THALASSEMIA SCREENING IN
THE AREA OF DADRA AND NAGAR HAVELI, INDIA**

**Kalyani Amit Jagadale^a, Deepali Vidhate^b, Suryakant Nagtilak^c,
Prem Barnabas A^d, Deepa Gupta^e**

^{a,b}Dept. of Biochemistry, School of Medicine, D. Y. Patil Deemed to be University, Nerul,
Navi Mumbai, India

^{c,e}Dept. of Biochemistry and Programme Officer, Sickle Cell Anemia Control Programme,
National Modern Medical Education & Research Institute attached with Shri Vinoba Bhave
Civil Hospital, Silvassa, Dadra & Nagar Haveli, India

^d Assistant Programme Officer, Sickle Cell Anemia Control Programme, Shri Vinoba Bhave
College of Nursing attached with Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra & Nagar
Haveli, India

Email: ^akalyani21021995@gmail.com, ^bdeepali.vidhate@dyatil.edu,
^cnagtilakbiochem@gmail.com

Abstract

Homozygous beta-thalassemia individuals are diagnosed during early years of life due to severity of clinical symptoms, but heterozygous beta-thalassemia cases being asymptomatic remains undetectable and increases the risk of beta-thalassemia major or double heterozygous hemoglobinopathy offspring if married to beta-thalassemia or any other hemoglobinopathy individual. Screening & counselling strategy may play important role in carrier diagnosis and awareness programme. Dadra and Nagar Haveli(DNH) is a former Union Territory situated on the western side of India. Majority of population in DNH is tribal and recent reports revealed presence of hemoglobinopathies in tribal population of DNH. To recognize the exact prevalence of beta-thalassemia in DNH, naked eye single tube red blood cells osmotic fragility test (NESTROFT) was standardized for screening population, confirmed by hemoglobin variant, high performance liquid chromatography (HPLC). Present study was commenced during September and December 2021 with total 290 nursing and paramedical students, predominantly Tribal natives of DNH as study participants. Whole blood samples of enrolled students were processed with both NESTROFT and HPLC. 196 samples showed negative reaction with NESTROFT, 74 showed turbidity considered as positive NESTROFT, 20 were partially turbid were also considered as positive NESTROFT. Total 13 (4.48%) found to have beta-thalassemia minor in present study shows the high prevalence of beta-thalassemia mutation in tribals of DNH and also suggest immediate need to detect exact prevalence and to conduct awareness sessions in present study area.

After comparing NESTROFT and HPLC results, NESTROFT showed 92% sensitivity, 71% specificity, 13% positive predictive value(PPV), 99% negative predictive value(NPV), 3.12 positive likelihood ratio(LR+) and 0.11 negative likelihood ratio(LR-) and 71.38% efficacy. Present study findings are suggestive of NESTROFT can be used for community screening for beta-thalassemia. Cost efficacy, handiness and fairly acceptable accuracy of NESTROFT make it a preferable screening technique in the remote areas like DNH.

Keywords: beta-thalassemia, Dadra and Nagar Haveli, HPLC, Mass Screening, NESTROFT

1. Introduction

Different genomic modifications resulting into disease in homozygous state like sickle cell anemia (HbS), glucose-6-phosphate dehydrogenase (G6PD) deficiency, *beta*-thalassemia was evolved in Mediterranean belt for survival against endemic malaria. India being malaria affected region, *beta*-thalassemia is one of the monogenic disorder distressing Indian population with day by day increasing burden on healthcare systems and families of affected individuals. The prevalence of heterozygous *beta*-thalassemia ranges from 0.00 to 37.90% while prevalence of homozygous *beta*-thalassemia ranges from 0.36 to 13.20% among different parts of India (1).

The major chunk in controlling inheritance of genetic disorder is to diagnose its carrier individuals. Screening strategy plays significant role in identifying population at risk. In case *beta*-thalassemia, homozygotes can be identified at their early years of age due to clinical symptoms but heterozygotes being asymptomatic cannot be identified directly without screening programme. *beta*-thalassemia carriers can lead almost healthy life but can cause actual threat if gets married with another *beta*-thalassemia carriers or heterozygote to any of the hemoglobinopathy, might passing homozygous or double heterozygous hemoglobinopathy to its offspring.

Dadra and Nagar Haveli (DNH) situated on the western parts of India between Maharashtra and Gujrat states has almost 52% tribals of its total population (2). Very few studies reported frequency of hemoglobinopathies in DNH (3,4,5,6). Recent study reported high prevalence of HbS specifically in tribal population of DNH (7). Another study demonstrates the presence of *beta*-thalassemia mutation diagnosed as double heterozygotes (HbS-*beta*-thalassemia) during HbS screening and also emphasized on initiation of *beta*-thalassemia screening in DNH (8).

With this review of literature present study was planned to develop and assess utility of naked eye single tube red blood cells osmotic fragility test (NESTROFT) reagent for *beta*-thalassemia screening in the population of DNH.

2. Material and Method:

A cross-sectional study was conducted at Central Clinical Biochemistry Laboratory, Shri Vinoba Bhave Civil Hospital (SVBCH) attached with NAMO Medical Education and Research Institute Silvassa. Present study was conducted during September to December 2021. The study was initiated after approval from Institutional Ethics Committee. The study participants enrolled in present study were students from B.Sc. Nursing and Paramedical Diploma courses from Shri Vinoba Bhave College of Nursing, Silvassa and A.P.J. Abdul Kalam College of Paramedical Sciences, Silvassa respectively. All enrolled students, Principle and involved faculties of both Colleges were explained about present study and permission for screening programme was taken from Principle of both colleges. Informed consents were taken from all enrolled study participants.

2 ml blood samples were withdrawn from antecubital vein into ethylene diamine tetra-acetic acid (EDTA) vacutainer with all aseptic precautions. Blood samples were analyzed by NESTROFT reagent (0.36% buffered saline) for screening *beta*-thalassemia and by hemoglobin variant high performance liquid chromatography (HPLC) D-10 Bio-Rad: HbA2/HbF Method as a confirmatory test for hemoglobinopathy.

Reagent composition of NESTROFT

Stock Solution: 10 % buffered saline (BS).

Dilution of Stock Solution: 1% BS = 1:10 dilution of stock solution with distilled water(D/W)

Working NESTROFT Reagent: 0.36% BS= 36 ml of 1% BS + 64 ml D/W (9)

Principle of NESTROFT:

Whole blood of *beta*-thalassemia individuals when added into test tube containing NESTROFT reagent (0.36% buffered saline), red blood cells (RBCs) begins to lyse and develop turbidity in the test tube. Whereas RBCs of normal healthy individual does not lyse at this concentration, gives transparent solution, thus can be differentiated from each other.(10)

3. Result

Present study planned to rule out beta-thalassemia carriers has screened total 292 subjects of which 59 were 1st year B.Sc. Nursing students, 57 were 2nd year B.Sc. Nursing and 178 Paramedical Diploma students, out of which 2 were excluded from study due to clotted and hemolysed blood sample respectively. Out of 290 net total subjects 38 were male and 252 were female with Mean \pm SD age 19.88 \pm 1.93. In present study, out of total screened subjects 254 (87.59%) were natives of DNH with Scheduled Tribes (ST) (78.28%) as a main social community screened, with small groups of Scheduled Caste (SC), Other Backward Classes (OBC) and General (GEN) category (Fig. No. 1)

The main tribes of subjects in present study was Varli (40.53%), Kukana (39.65%), Dhodi (18.06%), Halpati (1.32%) and Koli (0.44%) (Table No. 1).

Out of 290 samples 196 showed negative NESTROFT with transparent solution in a test tube, 74 were turbid and considered as positive NESTROFT. 20 samples showed partial turbidity were also considered as positive NESTROFT. All samples processed on HPLC analyzer. sickle cell trait (SCT) was detected in 23 (7.93%), *beta*-thalassemia minor in 13 (4.48%), HbD Punjab trait in 1(0.34%) and rest 253 (87.24%) were having normal hemoglobin. Results obtained after NESTROFT and HPLC analysis were compared for assessing utility of NESTROFT as a screening test. (Table No. 2).

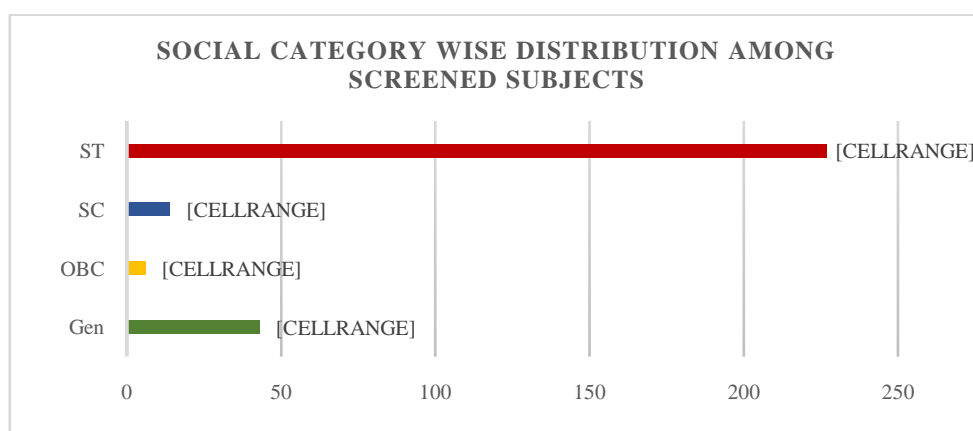


Figure No.1 Social category wise distribution among NESTROFT screened subjects

Table No.1 Tribe wise distribution among NESTROFT screened ST subjects

Tribe	No. of subjects
Varli	92 (40.53%)
Kukana	90 (39.65%)
Dhodi	41 (18.06%)
Halpati	3 (1.32%)
Koli	1 (0.44%)

Table No. 2 NESTROFT results in comparison with gold standard hemoglobin HPLC analysis.

		Results of HPLC analysis and genotype distribution				
		Total	SCT	B-thal	HbD Punjab Trait	Negative
Results of NESTROFT test	Total	290	23	13	1	253
	Negative	196	9	1	0	186
	Partially turbid	20	3	0	1	16
	Turbid	74	11	12	0	51

Table No.3 Diagnostic importance of NESTROFT in terms of statistical terminologies in comparison with previous Indian studies.

Study	Sensitivity (Sn) %	Specificity (Sp) %	Positive Predictive Value (PPV) %	Negative Predictive Value (NPV) %	Positive Likelihood Ratio (LR+)	Negative Likelihood Ratio (LR-)	EFFIC ACY of Test %
Present Study (2022)	92.31	70.40	12.77	99.49	3.12	0.11	71.38
Sharma et. al (2016) (12)	93.22	88.71	88.71	93.22	8.26	0.08	90.91
Shewale et. al. (2014) (13)	95.40	82.10	84.20	94.69	5.33	0.06	88.75
Piplani et. al. (2013) (10)	100.00	85.47	66.00	100.00	6.88	0.00	88.67
Singh et. al (2008) (9)	97.70	83.33	95.51	90.91	5.86	0.03	94.59
Mangalani et. al. (1997) (14)	94.37	64.24	35.26	98.22	2.64	0.09	69.40
Raghavan et al (1991) (15)	95.45	38.10	88.98	61.54	1.54	0.12	86.26

Present study reported 12 true positive, 82 false positive, 195 true negative & 1 false negative subject with 92% sensitivity, 71% specificity, 13% positive predictive values (PPV), 99% negative predictive values (NPV), 3.12 positive likelihood ratio (LR+) & 0.11 negative likelihood ratio (LR-). All statistical values were calculated for assessing characteristics of NESTROFT based on standard formula (11).

4. Discussion

Beta-thalassemia gene frequency in DNH was not reported in any of the earlier studies. Recently Nagtilak et. al. (2020) reported high prevalence of HbS in DNH (7). Jagadale et al. (2023) reported some cases of HbS-*beta*-thalassemia in the population of DNH and suggested screening of population for *beta*-thalassemia (8). Although *beta*-thalassemia can directly have detected by HPLC analysis, for mass screening HPLC will not be an economic and handy test. Hilly areas, forest cover, scattered colonies of tribals makes it difficult for health facilities of DNH to reach them. Huge numbers of sample collection and transportation is one of the main issue in remote areas like DNH. NESTROFT is portable, convenient and low-priced test thus it was standardized in present study which shows that it is a reliable test for community screening. Table No. 3 shows sensitivity, specificity and supplementary statistical findings of present study in comparison with previous Indian studies.

5. Conclusion

Beta-thalassemia was found prevalent in DNH. Community screening needs to be introduced at the earliest and can be initiated with NESTROFT as a screening test.

Conflicts of interest

The authors declare that they have no conflict of interest.

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