ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 7, Issue – 1, February - 2024, Page No. : 61 - 67

Practicality of time-worn Dithionate Tube Turbidity method for sickle gene screening in remote tribal areas of India.

[1]Mrs. Kalyani Amit Jagadale, Ph.D. Scholar, Dept. of Biochemistry, School of Medicine, D. Y. Patil Deemed to be University, Nerul, Navi Mumbai, Maharashtra. (Tutor, Dept. of Biochemistry, NAMO Medical Education & Research Institute, Silvassa, Dadra & Nagar Haveli, India.

[2]Dr.DeepaliVidhate, Professor & Head, Dept. of Biochemistry, School of Medicine, D. Y. Patil Deemed to be University, Nerul, Navi Mumbai.

[3]Dr.SuryakantNagtilak, Professor and Head, Dept. of Biochemistry, NAMO Medical Education & Research Institute Silvassa and Programme Officer, Sickle Cell Anemia Control Programme attached with Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra & Nagar Haveli, India.

[4]Dr.Deepa Gupta, Assistant Professor, Dept. of Biochemistry, NAMO Medical Education & Research Institute attached with Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra & Nagar Haveli, India.

[5]Mr. Prem Barnabas A., Associate Professor, Shri Vinoba Bhave College of Nursing and Assistant Programme Officer, Sickle Cell Anemia Control Programme, attached with Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra & Nagar Haveli, India.

[6]Dr.Amarendar Sura, Assistant Professor, Dept. of Pharmacology, NAMO Medical Education & Research Institute attached with Shri Vinoba Bhave Civil Hospital, Silvassa, Dadra & Nagar Haveli, India.

Corresponding Author: Mrs. Kalyani Amit Jagadale, Ph.D. Scholar, Dept. of Biochemistry, School of Medicine, D. Y. Patil Deemed to be University, Nerul, Navi Mumbai, Maharashtra. (Tutor, Dept. of Biochemistry, NAMO Medical Education & Research Institute, Silvassa, Dadra & Nagar Haveli, India.

How to citation this article: Mrs. Kalyani Amit Jagadale, Dr. Deepali Vidhate, Dr.Suryakant Nagtilak, Dr. Deepa Gupta, Mr. Prem Barnabas A., Dr. Amarendar Sura, "Practicality of time-worn Dithionate Tube Turbidity method for sickle gene screening in remote tribal areas of India", IJMACR- February - 2024, Volume – 7, Issue - 1, P. No. 61 – 67.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Introduction

Sickle cell anemia (HbS) is the most common example of single nucleotide base mutation causing severe health issues in homozygous state. According to estimates of Pie Fb et. al. Indian subcontinent will be ranked as 3rd country with highest cases of HbS by year 2050 (1). Though increasing day by day load on Indian healthcare systems, HbS is still neglected over noticeably important

disorders like diabetes, cancer, heart diseases etc. The lack of screening and awareness of hemoglobinopathy inheritance making its silent spread throughout communities of India. There are certain established communities all over the India with known legacy of hemoglobinopathies but the extent at which its frequency increasing is also one of the important factors for effective control over the disease.

The verified HbS inheriting ethnic group is tribal/Adivasi population in India (2). Dadra and Nagar Haveli (DNH) (Former Union Territory of India before merger with Daman & Diu) with almost 51.95% tribal population may have significant number of HbS cases but devoid of any scientific publications or research documentation (3).

The area DNH is situated at the foothills of Western Ghats comprising hilly areas covered with deciduous forest. The main inhabitants of DNH are tribal, who prefers to live in segregated localities specially in woods, making it difficult to access their health status. Many localities i.e. Faliya or Padas are far away from direct road connectivity can be reached by walk only. Also the houses of DNH natives are scattered, not too close to each other. To overcome these remote localities, present study was planned with timeworn though simplest diagnostic technique i.e. Dithionate Tube Turbidity (DTT)Test for screening of HbS in DNH and also to assess effectiveness and practical use of DTT with the most limited resource set up.

Material & Methods

A cross sectional-community based study conducted under sickle cell anemia control programme (SCACP), Govt. of India, at Shri Vinoba Bhave Civil Hospital (SVBCH), Silvassa (4). The SCACP team composed of a programme officer, assistant programme officer, a counselor, technical staff and researchers used to go to the field every day for mass screening in the area of Dadra from January-2020 to March-2022 (withheld during COVID-19 Pandemic). The study was initiated with due permission of Institutional Ethics Committee. The major focus of present study was to screen tribal population for presence of HbS gene. The study was conducted with the help of SCACP team. All involved health facilitators along with village heads were informed 2 to 3 days prior to the study visit for gathering people at one place in the village or pada on the day of screening. The consents/assents were obtained from subjects/parents/family head/Sarpanch i.e. village head on behalf of their family or village. Subjects of both genders were screened in their villages, schools, Sub-Health Centre, Community Health Centre (CHC).

Technical procedures

Capillary blood samples by finger-prick were collected by the technical staff into test tubes containing DTT reagents. All DTT positive (DTT +ve) subjects had venepuncture with 4 ml blood taken into tubes containing ethylene diamine tetracetic acid (EDTA), stored in the insulating box with ice packs until delivered to the Biochemistry Laboratory, SVBCH. High performance liquid chromatography (HPLC) for hemoglobin variants was performed on D-10 Bio-Rad Analyzer using HbA2/HbF method on all DTT +ve samples along with known controls. DTT negative (DTT –ve) samples obtained from Antenatal Care (ANC) females of study area were also performed on HPLC as a control. All diagnosed cases were counselled and extended family screening was done for all of them.

Principle of DTT Test: In presence of a reducing agent i.e. sodium dithionate, blood sample containing Sickle Hemoglobin forms turbidity when added to a test tube of

DTT solution, while blood sample with normal hemoglobin does not form any turbidity in the DTT solution.

Preparation of DTT Reagent

DTT Buffer Stock Solution: 143gmof Potassium dihydrogen orthophosphate (KH_2PO_4), 250gm of Di-potassium hydrogen orthophosphate (K_2HPO_4), 2gm of Saponin and 2.5 of gm Benzoic Acid were added initially into volumetric flask (1000ml capacity) containing 300 ml distilled water, mixed properly and then diluted with distilled water to final volume of 1 Liter. To dissolve all chemicals properly the solution is mixed thoroughly by using electric shaker for 1 to 2 hours.pH of DTT buffer solution was measured and adjusted to 7. This stock solution was stored at room temperature when not in use and it was stable and had constant pH up to 15 days from the date of preparation. Stability of DTT stock buffer solution was assessed on daily basis by processing known positive and negative samples as a control along with test samples.

DTT Buffer Working Solution: Sodium dithionate when exposed to air decomposes rapidly thus it was added just before starting the test procedure. 30gm of sodium dithionate per 1 liter of DTT stock solution were added to prepare working DTT solution.

DTT Test Procedure: 2 ml working DTT reagent taken into a test tube and 20µl of EDTA blood sample to be tested added into the test tube. Blood sample with sickle hemoglobin showed turbidity in the test tube, while sample with normal hemoglobin remained clear (5-6).

Results

A programme planned to identify the HbS, has screened 3592 subjects ranging 8 Months to 90 years of age amongst the villages in covered under CHC, Dadra between January 2020 and March 2022. Of total screening1540 (43%) were male and 2052 (57%) were female (Figure No. 1). 43.99% of total screened population belonged to Scheduled Tribes (ST) with Dhodi was prominent tribe in Dadra, followed by Halpati, Varli & Kokana. The total number of sickle gene (HbS) in present study population was 229 (6.38%). The sickle cell trait (HbAS) was found in 219(6.10%) and sickle cell disease (HbSS) in 6(0.17 %), HbS-β-thalassemia observed in 4 (0.11%). One sample showed no result on HPLC analyzer (Table No. 1).

Figure 1: Gender wise distribution among screened subjects

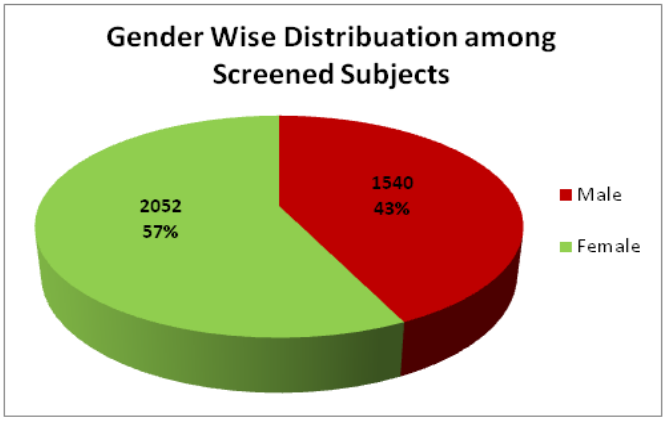

Table 1: Sickle hemoglobin screening report from villages covered under Community Health Centre Dadra, Dadra and Nagar Haveli, India.

Name of Sub-Centre	Name of Village	Total Population	Total ST Population	Total Population Screened	Total ST Screened	Total DTT +ve	Total HPLC Tested	HbAA	HbAS	HbSS	HbS-β-Thalassemia	No Result
Dadra	Dadra	13039	2207	2583	1010	103	102	3	95	1	3	0
	Vaghdhara	699	No Record Obtained (31 from Screening record)	178	31	27	27	0	22	4	0	1
Demani	Demani	3642	781	534	335	61	61	1	59	1	0	0
	Tighra	2657	2430	297	204	46	46	2	43	0	1	0
Total		**20037**	**5449**	**3592**	**1580**	**237**	**236**	**6**	**219**	**6**	**4**	**1**
Frequency (%)			**27.19**	**17.93**	**43.99**	**6.60**	**6.57**	**0.17**	**6.10**	**0.17**	**0.11**	**0.42**

Table 2: Dithionate tube turbidity test results in comparison with gold standard hemoglobin HPLC analysis.

DTT Test Results		HPLC Result		
Total DTT	292	HbAA	Sickle Gene	No Result
DTT +ve	236	06	229	01
DTT -ve	56	56	00	00

Table 3: Diagnostic importance of Dithionate Tube Turbidity test in terms of statistical terminologies.

True Positive	False Positive	True Negative	False Negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Positive Likelihood ratio	Negative Likelihood ratio
229	06	56	0	1	0.90	0.97	1	10.33	0

A total 237 subject found to have positive DTT test of which 1 subject denied giving venous blood sample thus only 236 samples were processed with hemoglobin variant HPLC analysis. Out of total 236 DTT +ve subjects 230 (97.46%) found to have sickle hemoglobin (HbS) and 6 (2.54%) found to have normal adult hemoglobin (HbAA), 1 sample showed no result on HPLC analysis. A total of 56 DTT –ve samples were also analysed with hemoglobin variant HPLC Machine, of which none of the sample shows sickle hemoglobin on HPLC report (Table No. 2)

Number of true positive or negative and false positive or negative samples for DTT test were calculated based on the results of Gold Standard hemoglobin variant HPLC analysis. Table No. 3 shows all statistical values for DTT obtained after analysing data (7). In Present study DTT shows 100% sensitivity (Sn) and 90% specificity (Sp) with 97.44% positive predictive value (PPV) and 100 negative predictive value (NPV). The positive likelihood ratio (LR+) for DTT was 10.33 and negative likelihood ratio (LR-) was 0.

Discussion and Conclusion

Although DTT is old technique over gold standard HPLC, present study demonstrates its use & feasibility in remote areas for mass screening. DTT is economic and most importantly easy to do test requires least technical expertise, can be done anywhere with minimum resources, so it has got all merits to be the good screening test and decreasing load on highly sophisticated HPLC or any other confirmatory techniques.

In present study DTT as a screening test has fulfilled all the criteria for practically effective diagnostic test. Similar studies reported more or less difference in sensitivity, specificity, PPV, NPV, LR+ and LR- and % efficacy of DTT test as shown in Table No.4:

Table 4: Diagnostic importance of DTT test 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-)	Efficacy of test %
Present Study (2022)	100.00	90.32	97.45	100.00	10.33	0.00	97.94
Jain et. al (2020) (14)	96.86	29.63	87.96	64.00	1.38	0.11	86.20
Shewale et. al. (2014)(15)	94.80	87.80	88.60	94.41	7.77	0.06	91.30
Vasaikar et. al. (2012)(16)	100.00	99.93	99.72	100.00	1451.15	0.00	99.94
Surve et. al (2000) (17)	93.76	99.87	99.66	97.55	724.10	0.06	98.12

Present study diagnosed 4 cases of HbS-β-Thalassemia which indicates presence of β-Thalassemia mutation in study population. As DTT cannot diagnose β-Thalassemia mutation, present study limits to screen population for β-Thalassemia. There may be more frequency of β-Thalassemia mutation in present study area which is required to be screened at the earliest.

Present study reported 6.38% frequency of HbS gene in the area of Dadra, DNH. a few studies reported sickle gene in the area of DNH. Joshi et.al. (1978) reported 19.3% frequency of sickle gene in DNH which is higher

than present study findings (8).R.Samtani et. al. (2008) reported 12.7% HbAS in Varli population of DNH (9), NA Devi et. al. (2009) reported 2.98% HbAS frequency in the Rajput population of DNH (10). Mohd. Asghar et. al. (2009) reported 18.10% hemoglobin S frequency (3.34% homozygous and 14.76% heterozygous) in Dhodi population of DNH (11). Immunohematology Bulletin-NIIH (ICMR, 2013) reported 15.2% sickle gene frequency in the population of DNH (12). All studies reported earlier have higher frequency than frequency reported by present study. There may be multifactorial reasons behind the difference in HbS frequency in earlier studies as compared to present study.There may be difference in study design, sampling method and selection of particular tribe as a target group. However recent report presented by S. Nagtilak (2020) testified 6.07% frequency of sickle gene in DNH which is quite similar to present study findings (13).

This may be the first research documentation reporting HbS gene frequency using accurate techniques and with adequately large sample size. None of the study conducted before depicted double heterozygosity (HbS/β-Thalassemia) frequency in DNH.

The tribal population of DNH is mostly underprivileged, socio-economically disadvantaged and live in distant areas primarily in forest and hilly areas, strictly following their culture as well as social system. Due to isolated existence & endogamy over centuries, these tribes have their distinctive genomic identity. Being malaria endemic region, hemoglobinopathies like HbS are common in DNH. Apart from endemicity to malaria, inbreeding among tribes, lack of knowledge & awareness, illiteracy also contribute to the high frequency of HbS in tribal population. The two divisions observed among population were rural–urban & poor-rich. Most economically dominant as well as urban people of DNH prefer private health facilities and have misconceptions regarding hemoglobinopathies and hide their hemoglobinopathy status. There is need to emphasize on awareness in study population.

With the strengths and restrictions discussed above, the present study has screened over 3592 subjects detecting HbS & some double heterozygous conditions among the tribal people of Dadra. Prerequisite to success of present study is effective patient education and more attention towards prevention of hemoglobinopathy. Genetic, premarital counseling & prenatal diagnosis are the only possible ways to prevent the inheritance of the disease. Management of hemoglobinopathies is a challenging task due to numerous associated factors but the health department of DNH giving best efforts to take initiatives in the development of practices which will be beneficial to hemoglobinopathy patients as well as may be executed all through the affected inhabitants of India.

Acknowledgments: Authors are thankful to the SCACP Team and Administration of Dadra and Nagar Haveli for Technical assistance and cooperation.

Abbreviations

1. HbS: Sickle cell anemia
2. DNH: Dadra and Nagar Haveli
3. HbAS: sickle cell trait
4. HbSS: sickle cell disease
5. DTT: Dithionate Tube Turbidity
6. HPLC: High performance Liquid Chromatography
7. CHC: community health centre
8. ST: Scheduled Tribes
9. SCACP: Sickle cell anemia control programme
10. SVBCH: Shri Vinoba Bhave Civil Hospital
11. DTT +ve :DTT positive
12. EDTA: Ethylene diamine tetracetic acid

13. DTT -ve : DTT negative
14. ANC: Antenatal Care
15. KH_2PO_4: Potassium dihydrogen orthophosphate
16. K_2HPO_4: Di-potassium hydrogen orthophosphate
17. HbAA: adult hemoglobin
18. Sn: sensitivity
19. Sp: specificity
20. PPV: positive predictive value
21. NPV: negative predictive value
22. LR+: positive likelihood ratio
23. LR-: negative likelihood ratio
24. ICMR: Indian Council of Medical Research
25. NIIH: National Institute of Immunohematology

References

1. Piel Fb, Hay Si, Gupta S, Weatherll DJ, Williums TN. Global burden of sickle cell anemia in children under five, 2010-20250: modelling based on demographics, excess mortality and interventions. PLos Med. 2013;10: e1001484
2. RB. Colah, MB. Mukherjee, S. Martin, K. Ghosh. Sickle cell disease in a tribal population of India. Indian J. Med. Res. 2015;141:509-515.
3. Census of India 2011. Office of the registrar General and Census Commissioner. Ministry of Home Affairs, Govt. of India. Available from : http://www.censusindia.gov.in, accessed on February 06,2023.
4. Directorate Medical and Health Services, Dadra and Nagar Haveli-Silvassa, India.; Sickle Cell Anemia. (updated: 2023February 1st) Available from: vbch.dnh.nic.in/content/sickle –cell-anemia-0, accessed on February 06,2023.
5. Patel JS, Naik JP. Optimization of DTT buffer volume in Dithionite Tube Test (DTT) with respect to hemoglobin concentration used for field screening of sickle hemoglobin (HbS). Indian J App Res, 2016; 6(2): 63-66.
6. Patel JS, Naik JP. Sickle hemoglobin (effective) diagnosis interfered by false positive and negative results, during field screening by DTT test, can be improved by finding the optimum concentration of saponin and Na-dithionite. World Journal of Pharmaceutical Research, 2017; 6(16): 966-977.
7. Shreffler J, Huecker MR. Diagnostic Testing Accuracy: Sensitivity, Specificity, Predictive Values and Likelihood Ratios. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557491/
8. Joshi SR, Mehta MM, Mehta DM, Bapat JP, Baxi AJ and Bhatia HM Genetic Studies in three tribal groups of Dadra and Nagar Haveli regions in Western India. Ind. J. Phy. Anth. Hum. Genet.,1978;4:133-140.
9. R. Samtani, PR. Mondal, KN. Saraswathy. Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle cell hemoglobin among the Warli Tribe of Dadra and Nagar Haveli. Anthropologist. 2008;10(4):301-303.
10. NA. Devi, MP. Sachdeva. Sickle cell hemoglobin and Glucose-6-Phosphate Dehydrogenase Deficiency among the Rajputs of Dadra and Nagar Haveli. Anthropologist.2009;11(1):45-47.
11. Mohd. Asghar, MP. Sachdeva. Glucose-6-Phosphate Dehydrogenase Deficiency and Sickle cell hemoglobin among the Dhodias of Dadra and Nagar Haveli. Anthropologist. 2009;11(1):61-62.
12. Immunohematology Bulletin of the Indian Council of Medical Research (ICMR); Contribution of

National Institute of Immunohematology (NIIH) on Tribal Health research. 2013;44(2)1-12

13. S. Nagtilak, M. Naphade, Hemoglobinopathy and Thalassemia Screening Programme in Dadra & Nagar Haveli (UT). Organized by Department of Biochemistry, Zydus Medical College and Hospital, Dahod Proceedings of "The Sickle Cell Diagnosis: Conventional to Molecular":2020 Feb. 20th.
14. Jain R, Saxena S. The efficacy and reliability of Solubility test followed by High-Performance Liquid Chromatography (HPLC) for sickle cell disorders in Gujarat- An original research article. Trop J PatholMicrobiol. 2020;6(2):198-203.
15. Shewale SP, Meshram DP, Sameer MA et. al. Study of effectiveness of NESTROFT and solubility test as a screening test for the detection of haemoglobin disorder at Nanded region of Maharashtra. Int J Health Sci Res. 2014;4(9):49-54.
16. Vasikar et al 2012. Efficacy of Solubility Test in Diagnosis of Sickle Cell DisordersJournal of Research in Medical Education & Ethic Vol. 2, No. 3, November, 2012, pp-214-216.
17. Surve RR, Mukherjee MB, Kate SL, Nagtilak SB, Wadia M, Tamankar AA, Ghosh K, Colah RB, Mohanty D. Detection of the beta s gene: an evaluation of the solubility test against automated chromatography and haemoglobin electrophoresis. Br J Biomed Sci. 2000;57(4):292-4. PMID: 11204858.