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# FREQUENCY OF BETA THALASSEMIA TRAIT IN A MEDICAL SCHOOL OF KARACHI, PAKISTAN.

1. **AFSHAN SUMERA** MBBS, DTCD
2. **SULAIMAN AHMED** MBBS M.Phil
3. **AKBAR AGHA** MBBS, DCP,DIH
4. **SM ADNAN ALI** Ali MSc, PhD
5. **RAFIQ KHANANI** MBBS, M.Phil, PhD

*1* ***Lecturer, Pathology,*** DOW INTERNATIONAL MEDICAL COLLEGE (DIMC), DOW UNIVERSITY OF HEALTH SCIENCES, KARACHI.
*2* ***Trainee Pathology,*** INSTITUTE OF BASIC MEDICAL SCIENCES, DUHS
*3* ***Assistant Professor Pathology,*** DOW INTERNATIONAL MEDICAL COLLEGE (DIMC), DUHS
*4* ***Assistant Professor Pathology,*** DOW INTERNATIONAL MEDICAL COLLEGE, DOW UNIVERSITY OF HEALTH SCIENCES, KARACHI.
*5* ***Associate Professor,*** **HOD, Pathology,** DOW INTERNATIONAL MEDICAL COLLEGE, KARACHI.

***Correspondence:***
**DR AFSHAN SUMERA**
**Department of Pathology,**
DIMC, OJHA CAMPUS,
UNIVERSITY ROAD, KARACHI.
Contact # +92-333-2457389,
email. afshanmehrab@hotmail.com

**ABSTRACT**

OBJECTIVE: *To study the frequency of ?- Thalassemia trait (BTT) in a medical students of Karachi, Pakistan and to confirm the validity of Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) to qualify for a mass screening test for BTT detection.*
SETTING: *An analytical cross sectional study, performed from Jan 2010 to May 2010 was conducted at Dow Medical College, Dow University of Health Sciences Karachi, Pakistan.*
MATERIAL & METHODS: *An analytic cross sectional study of 266 medical students (mean age 23.9 years) was conducted at a medical school in Karachi via medical camp. With written informed consent, EDTA anti-coagulated whole blood samples were collected for on-site NESTROFT testing, and later tested for Complete Blood Count (CBC) and serum Ferritin concentration at Dow Diagnostics Reference & Research laboratory, DUHS, Karachi. Screening for BTT was done on Naked Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) with 0.36% freshly prepared saline. The diagnosis of BTT was confirmed on automated Hemoglobin Electrophoresis at cellulose acetate - alkaline pH. Hemogram indices (automated Hematology cell counter cell-tac alpha) were assessed along with peripheral smear morphology (Leishman's stained slides) as enhanced tool for BTT case finding.*
RESULTS: *Females were 78.2% and males were 21.8%. Mean hemoglobin was 13.02g/dl, Red blood cell (RBC) count was 4.77 x $10^{12}$/L, Hematocrit 39.12, Mean corpuscular volume (MCV) 82.04, Mean corpuscular hemoglobin (MCH) 27.44 and Mean corpuscular hemoglobin concentration (MCHC) 33.30. The overall frequency of ?-Thalassemia Trait (BTT) observed in students was 5.3%.*
*The sensitivity, specificity, positive and negative predictive values and efficiency of NESTROFT were 85%, 97%, 61%, 99% and 96.6% respectively.*
CONCLUSION: *With a 5.3% rate of BTT, studies on national level are needed to know the actual prevalence in premarital people. NESTROFT is economical, sensitive and specific screening test can be used for screening for BTT in resource limited settings.*

**KEY WORDS:** ß- Thalassemia trait, NESRTROFT, Medical school

**INTRODUCTION:**

Thalassemia is most common single gene disorder worldwide with carrier rate of 1.7% and high prevalence in Asia including Pakistan comprising of nine million carrier results in more than 5000 transfusion dependent children per year. [1-3]

Pakistan is under developed and resource limited country, this high burden of disease causes economic problems for affected families which also reflect on nation. The management of thalassemic child costs about more 90000 to 100,000 per year for blood transfusions and costly iron chelation therapy which is associated with repeated transfusions. [4]

There is lack of proper awareness and health education regarding disease of this magnitude which results in increasing number of consanguinity and increased disease burden. Thalassemia can easily be prevented by awareness, education, screening, genetic counselling and prenatal diagnosis. There is high need of primary preventive strategies to prevent further births of thalassemia major children. [2] The control programs can reduce birth of child with thalassemia major as in Sardinia have reduced the birth of these children from 1:250 to 1:4000. [5] Prospective prevention strategies, which include population education, mass screening, genetic counselling and prenatal diagnosis can efficiently reduce new number of thalassemia major patient. [4]

The screening of students of high school, colleges for BTT detection along with education and awareness of disease is important to prevent marriages of thalassemia minors. Screening

programmes for high school students are currently being used and recommended. [6] The gold standard test for diagnosis of the carrier state is haemoglobin electrophoresis [7] but because of its high cost, it is not suitable for mass screening in developing countries. The need, therefore, is for a simple, low cost, rapid and reliable test which can be applied for mass screening. [4] Recent studies have shown that the Naked Eye Single Tube Red cell Osmotic Fragility Test (NSTROFT) can be a very useful screening tool for ?-thalassemia trait. [4, 8, 9]. The 0.36% saline is the most sensitive and effective solution since it could detect 96 to 100% of heterozygotes with ?-thalassemia. [10] Routine use of haematological data from automated cell counters may complement the results of the NESTROFT.[9] The test proved to be simple, cheap, easy to perform and adaptable for mass screening coming close to an ideal screening test for ?-Thalassemia trait.

The aims of this study were to screen the medical students for frequency of BTT and to evaluate the efficiency of NESTROFT as screening test.

**TABLE. I.**
**DESCRIPTIVE STATISTICS OF SCREENING SUBJECTS**

| Characteristics | All subjects |
|---|---|
| Males (%) | 58 (21.8) |
| Females (%) | 208 (78.2) |
| Total hemoglobin (g/dl; mean ± SD) | 13.02 (1.64) |
| RBC count ( x $10^{12}$/L; mean ± SD) | 4.77 (0.58) |
| Hematocrit (%; mean ± SD) | 39.12 (5.41) |
| MCV (fl; mean ± SD) | 82.04 (7.63) |
| MCH (pg; mean ± SD) | 27.44 (3.14) |
| MCHC (g/dl; mean ± SD) | 33.30 (1.35) |

**TABLE. II.**
**SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES AND EFFICIENCY OF NESTROFT IN PREDICTION OF BTT.**

| Sensitivity (%) | Specificity (%) | Positive Predictive Value (%) | Negative Predictive Value (%) | Efficiency of Test (%) |
|---|---|---|---|---|
| **85** | **97** | **61** | **99** | **96.6** |

**TABLE III.**
**$HbA_2$ HbF in ß-Thalassemia Trait Subjects.**

| | Mean | SD |
|---|---|---|
| $HbA_2$ | 5.35 | 0.83 |
| HbF | 0.31 | 0.08 |

## MATERIAL AND METHODS:

This analytical cross sectional study was carried out at Dow Medical College, Karachi on a sample of 266 medical students through a medical camp. After obtaining the necessary permission from the head of medical school, one day screening camp was organised at the premises in April 2010. Awareness regarding thalassemia was created in the vicinity of camp site by distributing pamphlets, personal meetings and pasting up posters around the camp site.

With informed consent, a detailed questionnaire was filled by each student. Whole blood samples were drawn in two tubes (K2-EDTA anti-coagulated) for Complete blood counts (CBC), NESTROFT & Hb Electrophoresis. Clotted blood sample was drawn for serum Ferritin level in SST Gel tube. After performing on-site NESTROFT, all blood samples were transported to DDRRL, maintaining the optimal transportation temperature. NESTROFT was done with freshly prepared 0.36% buffered saline from stock solution. [13]

For NESTROFT testing, 20 ?L volume of EDTA anti-coagulated whole blood was pipetted out into a clean glass test tube (10x100 mm) containing 4 mL of 0.36% freshly prepared buffered saline solution. Contents of tubes were mixed and left at room temperature for 20 minutes. After mixing again, tubes were read in a standardized light against three sharp black lines drawn behind the tube at a standardized distance. The results were recorded as "Negative" with clearly visible lines and "Positive" when lines were not visible and "Doubtful" when partially visible lines seen. The doubtful cases were also interpreted as positive result.

The Preliminary NESTROFT test result cards were issued to all participating subjects. Subjects with positive NESTROFT were counselled for follow up confirmation of BTT on Hb Electrophoresis at 8.6 pH ($HbA_2$ ? 3.5 %). CBC were performed on all the samples on Automated Hematology System (Celltac-?) within 6 hours of collection. Hemogram values were recorded and peripheral smear examination (stained with Leishman's stain) was done. Samples with MCV < 80fl were tested for serum Ferritin (solid phase Automated Enzyme linked Immunoassay Test Kit – Bio Check, Inc. USA) to establish and confirm the co-existent iron deficiency anemia. Peripheral blood smear morphology consistent with Hypochromia, Microcytosis and Anisocytosis with abnormal forms was assessed for correlating with Hemogram values and segregating the cases into suspected group viz. IDA, Non IDA and BTT. Ferritin was done on all suspected IDA, NESTROFT positive and random non IDA cases.

Hb Electrophoresis on cellulose acetate at alkaline pH was performed on *Genio S* (Fully Automated Electrophoresis System for all NESTROFT positive, doubtful and randomly selected NESTROFT negative samples. $HbA_2$ ? 3.5 % was considered diagnostic for BTT. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Values and efficiency of test were calculated for NESTROFT.

The characteristic data for each student and test results were recorded and analysed on SPSS program version 16.0.

## RESULTS:

Total 266 samples were screened for BTT. Male to female ratio was 3.5:1, (female 78.2% & males 21.8%). Mean age was 23.9 ± 5.4 years. Mean Total Hb was 13.02 g/dl (±1.64). Mean RBC count 4.77 x $10^{12}$/L (±0.58) and mean MCV was found out to be in the range of 82.04 (±7.63). Overall frequency of BTT in medical students was found out to be 5.3%. Peripheral blood smear morphology revealed a fairly hypochromic microcytic red cell picture with anisocytosis with presence of target cells in BTT positive cases. Serum Ferritin level < 15ug/dl was taken as cut off for IDA. Ferritin levels

were found normal in BTT cases.

Descriptive statistics of all subjects are summarized in Table. I. Based on CBC, the mean values of red blood cell indices are also summarized in Table. I.

Out of 266 samples, NESTROFT was Positive in 18 (6.7%) and Negative in 248 (93.2%). Out of all NESTROFT positive cases, 11 (4.1%) were true positive ($HbA_2$ >3.5%), whereas remaining 7 (2.6%) were false positive. False negative result was observed in 2 (1%) subjects only. Sensitivity (85%), specificity (97%), positive Predictive Value (61%) and Negative predictive value (99%). Efficiency of test was calculated to be 96.6%; also shown in table II.

On Hb Electrophoresis the mean HbA2 in cases was 5.35% ±0.83% and HbF was 0.31± 0.08%

**DISCUSSION:**

?- thalassemia is the commonest inherited hemoglobinopathy worldwide with about 1.7% of world population carrying the mutated gene.[3] Pakistan is resource limited country having prevalence of ?- thalassemia trait that varies from 5-6% in various regions.[2]

It is difficult to suspect BTT on clinical examination as the classic heterozygote carrier of BTT is fairly asymptomatic. [1] Usually the possibility of BTT is suspected on evaluation of positive family history, a suspected CBC Hemogram or during population screening. [14] The correct identification of carriers with BTT is essential part of any screening programme since significant number of persons do not have family history of affected homozygote individuals. [1]

Regional data on frequency of BTT has reported carrier rate of about 6% in Pakistani population. [13, 14] In the present study, frequency of BTT was 5.3% which is fairly comparable to local data in Pakistan. [3, 15, 16]

Screening for BTT is very difficult mainly because of heterogeneity of ?- thalassemia and there is no single test to detect all variants. Despite these difficulties many researchers have made attempts to establish an effective screening protocol to answer this problems viz. estimation of HbA, $HbA_2$, HbF & determination of red cell indices. However, all these techniques are time consuming and expansive for mass screening programmes. [11]

A positive test of NESTROFT indicates lower than normal Red Cell Osmotic Fragility, which is suggestive of BTT. *Kettamis et al* (1981) studied 893 cases and found sensitivity, specificity, PPV, NPV to be 98.4%, 90.9%, 91.3%, 98.3% respectively. [17]

*Raghavan et al* (1991) found NESTROFT sensitivity, specificity, PPV and NPV to be 95.5%, 87%, 70.5% and 98.3%.[18] *Thomas et al* in 996 studied 137 cases and found NESTROFT sensitivity, specificity, PPV and NPV to be 98.7%, 66.6%, 87% and 96.5%.[19] *Manglani et al* (1997) studied 1695 cases and found NESTROFT sensitivity, specificity, PPV and NPV to be 94.4%, 64.2%, 35.3% and 97.6%.[7] In Pakistan, *Yazdani et al* (2008) screened 515 subjects for BTT and found NESTROFT sensitivity, specificity, PPV and NPV to be 92.5%, 95.2%, 85.38% and 97.66% respectively. [10]

According to a study (2008) sensitivity of NSTROFT was 97.7% and specificity was 83.3% for detection of ?-thalassemia trait. NESTROFT, when done with 0.36 % buffered saline solution, provides more accurate results compared to the other concentrations tested. [11]

In present study the sensitivity was 85%, specificity was 97%, PPV was 61% NPV was 99% as shown in Table. II, similar findings were studied previously. [20-23] NESTROFT showed very high negative predictive value. Hence, the negative NESTROFT is efficient enough to rule out the possibility of BTT.

**CONCLUSION:**

Overall frequency of BTT in medical students was found to be fairly significant. Considering our presence in resource limited, thalassemia prevalent belt on the world map, NESTROFT is an inexpensive, easy to operate, fairly sensitive and highly specific screening test for detection of BTT.

**RECOMMENDATIONS:**

Among the highly prevalent and low resource areas, this test may be used in population based studies. BTT screening programs are needed at national level in schools/colleges to prevent further disease burden.

**LIMITATIONS OF THE STUDY:**

Molecular studies were not carried out to assess for genetic mutation consistent with ß- Thalassemia, prevalent in Pakistan. Larger sample size was not inducted due to financial constraints.

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**REFERENCES:**

1. Madan N, Meera S, Satendra S, Usha R, Kusum K. Red cells Indices and Discriminant functions in the detection of Beta – Thalassemia Trait in a population with High prevalence of Iron Deficiency Anemia. Indian J Pathol Microbiol 1999; 42 (1): 55-61.
2. Shahid M, Ayesha A , Hammad H, Jamshaid M, Muhammad A et al Prenatal diagnosis of ?-thalassemia in Southern Punjab,Pakistan. Prenat Diagn 2006; 26: 903–5.
3. Urrechaga. E. Discriminant value of % microcytic/% hypochromic ratio in the differential diagnosis of microcytic anemia. Clin Chem Lab Med 2008;46(12):1752–8
4. Manglani M, Lokeshwar M, Vani V, Bhatia N. 'NESTROFT'—an effective screening test for beta-thalassemia trait: Indian Pediatr. 1997 Aug;34(8):702-7.
5. Cao A, Rasatelli M, Gallanello R. Control of beta-thalassemia by carrier screening genetic counselling and prenatal diagnosis: The Sardinian experience. Ciba Found Symp 1996;197:137-51.
6. Madan N, Sharma S, Sood S, Colah R, Bhatia H. Frequency of B-thalassemia trait and other hemoglobinopathies in northern and western India: Indian Journal of Human Genetics 2010;16(1):16-25.
7. Altay C, Yilgor E, Beksac S, Gurgey A. Premarital screening of hemoglobinopathies: a pilot study in Turkey. Hum Hered 1996 Mar-Apr; 46(2): 112-4
8. Annals of the New York Academy of Sciences. Volume 1054 Issue Cooley's Anaemia: Eighth Symposium, Pages 18 – 24 Published Online: 6 Jan 2006. © 2008 New York Academy of Sciences
9. Mamtani. M, Das. K, Jawahirani. A, Is NESTROFT sufficient for mass screening for ?-thalassemia trait? J Med Screen 2007;14:169–73
10. Yazdani M, Ahmed S, Bhatti F, Azim W. One tube osmotic fragility test: a screening test for microcytic red cells: Pak Armed Forces Med J Mar 2008;58 (1):45-50.
11. Singh S, Gupta S, Effectiveness of red cell osmotic fragility test with Varying degrees of saline concentration in detecting ? thalassaemia trait Singapore Med J. 2008 Oct; 49(10):823-6.
12. Caterina B, Galanello R. Thalassemia and Related Disorders: Quantitative disorders of Hemoglobin Synthesis. John Gree (editor). Wintrobes Clinical Hematology, 11th edn, Lippincott Williams and Wilkins, 2004; 1319-66
13. Ahmed S et al. Prenatal diagnosis of beta-thalassaemia in Pakistan: experience in a Muslim country. Prenatal Diagnosis, 2000, 20(5):378–83.
14. Hafeez M et al. Regional and ethnic distribution of beta thalassemia mutations and effect of consanguinity in patients referred for prenatal diagnosis. JCPSP, 2007, 17(3):144–7.
15. http://www.experiencefestival.com/a/Thalassemia_Prevalence/id/5512485
16. Rubina G, Mehdi A, Nikhat A. hemoglobinopathies among five major ethnic groups in karachi, Pakistan. southeast

asian j trop med public health 2002; 33(4): 855-61.
17. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting beta-thalassemia trait. J Med Genet 1981;18:266–70.
18. Raghavan K, Lokeshwar M, Birewar N, Nigam V, Mangalani M, Raju. Evaluation of naked eye single tube red cell osmotic fragility test in detecting ?-thalassemia trait. Indian Pediatr 1991; 28: 469-72.
19. Susana T, Srivastana A, Jayaseelan L, Dennison D and Chandy C. NESTROFT as a screening test for the detection of thalassemia and common haemoglobinopathies: An evaluation against a high performance liquid chromatographic method. Indian J Med Res 1996; 104: 194-7.
20. Gorakshaker A, Colah R, Desai S. Evaluation of the single tube osmotic fragility test in detection of b-thalassemia trait Natl Med J India 1990;3:171–3.
21. Deng J, Liu X, Liu Y. Evaluation of red cell mean corpuscular volume and simple tube red cell osmotic fragility quantitative test in detection of thalassemia. Zhonghua Fu Chan Ke Za Zhi 2000;35:610–12.
22. Maram E, Amiri Z, Haghshenas M. Effectiveness of osmotic fragility screening with varying saline concentration in detecting b-thalassemia trait. Irn J Med Sci 2000;25:56–8.
23. Winichagoon P, Thitivichianlert A, Lebnak T, Piankijagum A, Fucharoen S. Screening for the carriers of thalassemias and abnormal hemoglobins at the community level. Southeast Asian J Trop Med Public Health 2002;33(Suppl. 2):145–50.