Section: Biochemistry

Original Research Article

PREVALENCE OF THALASSEMIA TRAIT AMONG ANTENATAL WOMEN ATTENDING A TERTIARY CARE CENTRE.

M. Menaka Devi[1], R. Hemamalini[2], B.Rohini[3], M.P. Saravanan[4]

[1]*Assistant Professor, Institute of Biochemistry, Madras Medical college, Chennai, Tamil Nadu, India*
[2]*Senior Resident, Department of Biochemistry, Government Stanley Medical college, Chennai, Tamil Nadu, India*
[3]*Senior Resident, Department of Biochemistry, Madurai Medical College, Madurai, Tamil Nadu*
[4]*Professor and Head, Department of Biochemistry, Government Stanley Medical college, Chennai, Tamil Nadu, India*

Received *: 28/01/2025*
Received in revised form *: 05/04/2025*
Accepted *: 23/04/2025*

Corresponding Author:
Dr. M. Menaka Devi
Assistant Professor, Institute of Biochemistry, Madras Medical college, Chennai, Tamil Nadu, India
Email:menakadevisuresh@gmail.com

DOI: 10.70034/ijmedph.2025.2.180

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (2); 992-996

ABSTRACT

Background: Hemoglobinopathies represent the most prevalent hereditary illnesses worldwide, with thalassemia disorders being significant public health concerns, especially in Southeast Asia. In India, the beta-thalassemia trait (β-TT) has a prevalence of 3-4% nationwide, with regional variations. This study aimed to determine the prevalence of β-TT among pregnant women attending a tertiary care hospital in Chennai, Tamil Nadu.

Materials and Methods: This descriptive study included 150 antenatal women aged 18-40 years attending a tertiary care teaching hospital. Complete blood count, NESTROFT (Naked Eye Single Tube Red Blood Cell Osmotic Fragility Test), serum ferritin levels, and High-Performance Liquid Chromatography (HPLC) were performed to detect β-TT and distinguish it from iron deficiency anaemia (IDA).

Results: Among the 150 participants, 28.7% (43/150) had microcytic hypochromic anaemia. NESTROFT was positive in 15% (23/150) of participants, but only 2 (1.3%) were confirmed to have β-TT by HPLC (HbA2 >3.9%). An additional 3% (5/150) showed equivocal results with HbA2 between 3.5-3.9%, while 20% (30/150) had IDA. Comparative analysis showed that β-TT patients had lower haemoglobin (mean 7.5 g/dl), MCV (73 fl), and MCH (20.5 pg) values compared to normal subjects.

Conclusion: The 1.3% prevalence of β-TT among pregnant women in this study population represents a significant concern due to the risk of thalassemia major in offspring. The study demonstrates that NESTROFT and RBC indices alone are insufficient for accurate carrier detection. Mandatory HPLC screening for all pregnant women is recommended as part of a comprehensive thalassemia prevention program, along with education and awareness initiatives targeting healthcare personnel and women of reproductive age.

Keywords: Beta-thalassemia trait, Antenatal screening, NESTROFT.

INTRODUCTION

Among hereditary illnesses of man, haemoglobin (Hb) synthesis anomalies are the most prevalent.[1-4] It can be qualitative (different haemoglobins) or quantitative.[5] Thalassemia disorders fall under the category of quantitative defects. The most prevalent single-gene diseases in humans are caused by mutations in haemoglobin.[6-9] The transmission pattern of thalassemia is autosomal recessive.[10,11] Southeast Asia, the Indian subcontinent, the Mediterranean, the Middle East, and Africa are all home to endemic thalassemia variants. This is because thalassemia trait RBCs defend against the development of severe falciparum malaria,[12,13] Abnormalities of haemoglobin (Hb) impact 7% of the world's population.[14] A major variation is carried by 7% of pregnant women.[14]

Worldwide, 1.1% of couples are at risk of having children with a haemoglobin abnormality, with 2.7 out of every 1000 conceptions actually being affected. Every year, over 320,000 new-borns are

born with a serious haemoglobin condition. Eighty percent of births take place in poor nations.[15] Three lakh babies are born each year with a serious hemoglobinopathy.[16] Haemoglobin diseases are responsible for 3.4% of children under the age of five who die worldwide. Thalassemia in this condition and sickle cell disorders are serious public health issues. Southeast Asia is home to 50% of the world's β-TT population. In India, the average prevalence of beta-thalassemia trait is 3-4% (2,18), with a range of 3 to 17%.[17]

In North India, the frequency is 1.3%, while in South India, it is between 3% and 18%. In India, sickle cell trait and β thalassemia trait make up the most prevalent hemoglobinopathies. BTT is present in 6.5% of Punjab, 8.4% of Tamilnadu, 4.3% of south India, and 3.5% of Bengal, among other parts of India.[18,19] There are now 52 distinct BTT mutations known to exist in India.[20] among Gujarat, the frequency of BTT among pregnant women was 3.38 percent.[21]

The majority of patients cannot afford bone marrow transplants, the only treatment now offered for children with thalassemia. Therefore, having a child with thalassemia puts a physical and financial burden on the community as well as the affected child and family. Therefore, the focus needs to change from treating such births to preventing them in the future.

Numerous research have shown that screening schoolchildren does not have the intended effect. When they reached maturity, many of them were unaware of their carrier status. It is not practical to check for hemoglobinopathies prior to marriage. Pregnant women can be screened at prenatal clinics. A couple at risk is identified when a pregnant lady is diagnosed with thalassemia trait. Prenatal clinics should screen for disorders of haemoglobin synthesis in order to determine whether families would benefit from a prenatal test and to inform them of the significance of genetic counselling.

Neonatal screening can only provide secondary or tertiary prevention for the affected children. In a populous country like India, routine screening of antenatal cases can be routinely done as it will be cost effective in preventing the number of homozygous births and reducing the financial burden on the health care system.

Premarital counselling, antenatal screening, prenatal diagnosis, and mass screening for carriers are some of the preventative strategies. Prenatal screening and prenatal diagnosis are the most practical ways to prevent the delivery of homozygous infants in India, according to numerous studies. A screening program cannot be implemented without knowledge on the prevalence. The purpose of this study was to determine the prevalence of the thalassemia trait in pregnant patients who visited our hospital for a normal check-up. Additionally, all pregnant women should be screened for beta Thalassemia trait using RBC indices and the NESTROFT test. Serum ferritin levels in microcytic hypochromic anaemia should be measured to rule out iron deficiency anaemia, and the percentage of the Hb variation among those with positive screening test results should be determined by HPLC.

MATERIALS AND METHODS

The study was conducted at tertiary care teaching hospital, with support from the 24-hour Biochemistry and Pathology Laboratory over a 12-month period. This descriptive study employed a non-random, convenient sampling method to recruit 150 antenatal women aged 18 to 40 years attending the outpatient department, irrespective of their gestational age and parity.

Women with a history of recent blood transfusion (within 6 months) were excluded to avoid missing carrier states due to blood dilution and the possibility of transfusion from HbE carriers, which could affect HbA2 levels. Additionally, women with recent blood loss, known epilepsy patients on antiepileptics, alcoholics, and HIV patients on medication were excluded due to potential impacts on folate metabolism, haematological parameters, and HbA2 values.

After obtaining informed consent, 2ml of blood was collected in an EDTA tube and 3ml in a red top tube under aseptic conditions. The EDTA sample was analyzed for Complete Blood Count (CBC) using a Coulter automated cell counter on the same day. NESTROFT (Naked Eye Single Tube Red Blood Cell Osmotic Fragility Test) was also performed on the day of collection using 0.36% buffered saline. HbA2 was measured by High-Performance Liquid Chromatography (HPLC) using a Biorad D10 system within 4 days of collection.

Blood collected in the red top tube was allowed to clot and centrifuged at 2000-2500 rpm for 15 minutes for routine investigations including blood sugar, urea, and creatinine. Serum was immediately separated and refrigerated at -20°C for subsequent ferritin analysis by Electrochemiluminescence Immunoassay (ECLIA). Additional laboratory investigations included peripheral smear examination and HbA2 measurement by HPLC.

RESULTS

The study included 150 antenatal women with ages ranging from 18 to 38 years, with a mean age of 25 years (SD = 4.27). The largest age group was 22-25 years (35.3%), followed by 26-29 years (26%). Most participants belonged to the higher socioeconomic classes according to the Modified BG Prasad's Social Classification, with 102 participants (68%) in Class I. In terms of demographics, the majority of participants were Hindu (105), followed by Muslim (33) and Christian (12). Regarding obstetric history, 54% were multigravida (2-3 pregnancies), 42.7% were primigravida, and 3.3% were grand multigravida (≥4 pregnancies). Most participants (71.3%) were in their third trimester at the time of the study.

Haematological analysis revealed that 28.7% (43/150) of participants had microcytosis (MCV <80 fl), while 71.3% had normal MCV values. Among those with microcytosis, 69.7% (29 participants) had iron deficiency anaemia (IDA), 27.9% (12 participants) had ferritin levels >12ng/ml, and 2.3% (1 participant) had β-thalassemia trait (β-TT).

The NESTROFT test was positive in 15% (23/150) of participants, including 12 women with microcytosis and, interestingly, 11 women with normal MCV values. Of the NESTROFT-positive participants, 8 had low ferritin levels (<12ng/ml) indicating iron deficiency.

Table 1: MCV & Ferritin level in NESTROFT positive cases

Parameter	NESTROFT POSITIVE	NESTROFT POSITIVE
MCV	12(<80)	11(≥ 80)
Ferritin	8(<12ng/ml)	15(≥ 12ng/ml)

HPLC analysis of the 23 NESTROFT-positive participants revealed that 2 had HbA2 levels >3.9% (diagnostic of β-TT), 4 had equivocal results with HbA2 between 3.5-3.9%, and 17 had normal HbA2 levels (<3.5%).

Table 2: Results of HPLC Study (In Nestroft Positive Persons)

HPLC result	β-TT (HbA2 >3.9%)	β-TT equivocal (HbA2 3.5-3.9)	Normal (HbA2 <3.5)
Number	2	4	17

The overall prevalence of β-TT in the study population was 1.3% (2/150), with one case of isolated β-TT (0.7%) and one case of β-TT with coexistent IDA (0.7%). Additionally, 3% (5/150) showed equivocal β-TT results with HbA2 between 3.5-3.9%, and 20% (30/150) had IDA. The remaining 8% (12/150) had microcytosis due to other causes.

Table 3: prevalence of beta thalassemia trait, iron deficiency anaemia in study population

β-TT	Coexistent β-TT with IDA	β-TTequivocal HbA2 (3.5-3.9)	IDA	Microcytosis (others)
1(0.7%)	1 (0.7%)	3(2%)	30(20%)	12(8%)

Comparative analysis of laboratory parameters showed that β-TT patients had lower hemoglobin (mean 7.5 g/dl vs. 10.9 g/dl in normal subjects), lower MCV (73 fl vs. 87 fl), lower MCH (20.5 pg vs. 27 pg), and higher RDW-CV (17.2% vs. 14.7%). When comparing β-TT with IDA, both conditions presented with similar hematological profiles, though β-TT cases showed slightly lower hemoglobin (7.5 g/dl vs. 8.8 g/dl) and MCV (71 fl vs. 73.45 fl) values.

Table 4: comparison of hematological parameters of β-tt with normal subjects

Hematological parameters	β –thalassemia trait	Normal subjects
Hb (g/dl)	7.5	10.9
RBC count (X/106/µl)	3.7	4.0
MCV (fl)	73	87
MCH (pg)	20.5	27
RDW –CV (%)	17.2	14.7

DISCUSSION

The Indian Council of Medical Research conducted the first multicenter study on the β-thalassemia trait in the middle of the 1980s, examining high school students from Delhi, Kolkata, and Mumbai. In this investigation, the β-thalassemia trait was found to be present in Mumbai at 2.7%, Delhi at 5.5%, and Kolkata at 10.2%. However, counselling youngsters while they were of school age did not have the anticipated effect, according to a follow-up of carriers conducted in Mumbai around 20 years after screening. When they were adults,[22] they forgot about their carrier status. The most receptive time for counseling is during pregnancy, and they will pay attention to their child's welfare. If the carrier state is identified, it is possible to do prenatal test and followed by termination of pregnancy.

India offers a number of screening programs, including prenatal, cascade, and mass screening. The prevalence in the area determines the kind of screening program. There aren't many studies on the prevalence of β-TT in pregnant women in Tamilnadu, as far as we know. The purpose of this hospital-based study was to ascertain the prevalence among them.

Beta thalassemia trait (β-TT) and iron deficiency anaemia (IDA) are the most frequent causes of microcytic hypochromic anaemia. As recommended by Ferrara et al., screened participants with microcytosis and/or hypochromia had their complete clinical histories taken, their serum ferritin levels were measured, and their red blood cell (RBC) indices were determined using an electronic cell counter. The next phase involved using HbA2 quantification to diagnose β-TT.[23]

In the current study, 150 pregnant women were chosen for β-TT during a one-year period. Their entire hemogram was examined right away, and NESTROFT was performed on every sample. In order to validate the β thalassemia trait, samples that tested positive for NESTROFT, MCV<80fl, and MCH<27pg were further examined for HbA2 using CE-HPLC.

150 pregnant women in all were screened. There were forty-three individuals (28.7%) with microcytic hypochromic anaemia. Two individuals (4.7% of microcytic hypochromia) in this group exhibited beta thalassemia phenotype. Iron deficiency anaemia was present in 30 people (69.8%).

Study conducted by Raghavan et al,[24] showed that NESTROFT had sensitivity (95.5%) and specificity (87%). 23 participants in the current study tested positive for NESTROFT; only two pregnant women had HbA2 >3.9% (beta thalassemia trait), and seven individuals with iron deficiency anaemia also tested positive, but their HbA2 levels were within normal ranges. Thus, it had a 91.3% false positive value. However, NESTROFT outperformed MCV and MCH. Similar results were also noted by Mamtani et al,[25] in their investigation. They stated that NESTROFT was better than MCV and HbA2, despite missing 7% of cases of beta thalassemia trait. NESTROFT (0.36% buffered saline) has a very high negative predictive value (90.9%), according to a study by Singh and Gupta et al. In light of this, NESTROFT positive samples underwent HPLC analysis.[26]

Additionally, our investigation reveals equivocal β-TT outcomes for four individuals (HbA2 3.5-3.9). Those with equivocal β-TT results who also reacted favourably to NESTROFT can be classified as having beta thalassemia trait because numerous studies indicate that the HbA2 level should be greater than 3.5%. Ghosh et al.'s guidelines on the prevention and control of hemoglobinopathies in India, including sickle cell disease, thalassemias, and other variable haemoglobins, explicitly state that a thorough review is required before classifying an ambiguous result as a beta thalassemia characteristic.[27] Husbands of women whose HbA2 was between 3.5 and 3.9 refused to participate in molecular analysis and HPLC. Molecular analysis is necessary for patients with borderline HbA2 levels that cannot be explained by family history or iron status. Molecular analysis is the final confirmatory diagnosis and this was not carried out in our study as the antenatal women were not willing.

HbA2 levels are lowered in iron deficient anaemia. One individual with beta thalassemia trait equivocal in the current study had a ferritin value of 7.34. Given the possibility of a lower HbA2 level from iron deficient anaemia, this individual may have had β-TT. In their investigation, Usman et al. conducted HbA2 analysis both before and after iron therapy in order to investigate the impact of iron therapy on HbA2 levels.[7] According to the current study, 1.3% of pregnant women had it. The general prevalence of β thalassemia in Tamilnadu is 1-3%, according to the 2016 Guidelines on Prevention and Control of Hemoglobinopathies (India).

Comparison with other studies

Sachdev et al. conducted hospital-based research on samples referred for Hb variant analysis in HPLC, whereas Balgir did a study on anaemic patients referred from peripheral hospitals. In the current study, we discovered that the NESTROFT test and RBC characteristics are not accurate ways to determine carrier status. All pregnant women should ideally be screened with HPLC.[5,28]

Thalassemia prevention program is the need of the hour in India. The strategy for addressing the thalassemia issue is to stop and manage the emergence of new instances.

For instance, appropriate thalassemia measures have significantly decreased the incidence and prevalence of the disease in Cyprus, Italy, the United States, and more recently the United Kingdom, as well as other countries of Europe and Africa. Cyprus, where the incidence has decreased by 96%, is a noteworthy example. In Iran, the annual birth rate of afflicted infants and associated medical costs have decreased by 70% as a result of premarital screening to identify carrier couples and following counselling.

The prevalence of infants born with thalassemia major has significantly decreased in a number of nations across the world as a result of the implementation of mandated national premarital screening programs and screening young, single mothers for carriers.

The trait of thalassemia our communities accept carrier screening through prenatal screening, prenatal diagnosis, and medical pregnancy termination. There are no serious negative effects on the mother, foetus, or society with this method. Raising awareness among the general public and medical professionals would help the screening program in India succeed by enabling them to provide screening tests at the appropriate times and increase detection rate.

CONCLUSION

Given the likelihood of giving birth to a kid with thalassemia major and the expense of treating that child, the 1.3% incidence of beta thalassemia trait in pregnant women is a serious concern. In order to prevent the birth of children with thalassemia major, we propose that HPLC screening be made mandatory for all pregnant women. To avoid the birth of a child with thalassemia, health personnel, pregnant women, and women of reproductive age groups should all get education and awareness-raising.

REFERENCES

1. Piplani ML. Nestroft - a Valuable, Cost Effective Screening Test for Beta Thalassemia Trait in North Indian Punjabi Population. JCDR [Internet]. 2013 [cited 2019 Jul 23]; Journal of Clinical and Diagnostic Research. 2013 Dec, Vol- 7(12): 2784-2787

2. Philip J, Kushwaha N, Sarkar R. Microcytic hypochromic anemia: Should high performance liquid chromatography be used routinely for screening anemic and antenatal patients? Indian J Pathol Microbiol. 2013;56(2):109.
3. Baruah MK, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: high performance liquid chromatography studies in 9000 patients. Indian J Pathol Microbiol. 2014 Jun;57(2):236–43.
4. Lavee O, Kidson-Gerber G. Antenatal haemoglobinopathy screening: Patterns within a large obstetric service. Working towards a standard of care. Obstet Med. 2015 Dec;8(4):184–9.
5. Sachdev R, Dam A, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol. 2010;53(1):57.
6. Nagar R, Sinha S, Raman R. Haemoglobinopathies in eastern Indian states: a demographic evaluation. J Community Genet. 2015 Jan;6(1):1–8.
7. Usman M, Moinuddin M, Ahmed SA. Role of iron deficiency anemia in the propagation of beta thalssemia gene. Korean J Hematol. 2011;46(1):41.
8. Matos JF, Dusse LMS, Stubbert RVB, Ferreira MR, Coura-Vital W, Fernandes APSM, et al. Comparison of discriminative indices for iron deficiency anemia and β thalassemia trait in a Brazilian population. Hematology. 2013 May;18(3):169–74.
9. Payandeh M, Rahimi Z, Zare ME, Kansestani AN, Gohardehi F, Hashemian AH. The prevalence of anemia and hemoglobinopathies in the hematologic clinics of the kermanshah province, Western iran. Int J Hematol Oncol Stem Cell Res. 2014;8(2):33–7.
10. Mehdi S, Al Dahmash B. A comparative study of hematological parameters of α and β thalassemias in a high prevalence zone: Saudi Arabia. Indian J Hum Genet. 2011;17(3):207.
11. Aggarwal R, Aggarwal M, Prakash A. Thalassemia: An overview. J Sci Soc. 2014;41(1):3.
12. Kotila TR. Thalassaemia is a tropical disease. Ann Ib Postgrad Med. 2012 Dec;10(2):11–5.
13. Majeed T, Akhter MA, Nayyar U, Riaz MS, Mannan J. Frequency of beta thalassemia trait in families of thalassemia major patients, Lahore. J Ayub Med Coll Abbottabad. 2013 Dec;25(3–4):58–60.
14. Wajcman H, Moradkhani K. Abnormal haemoglobins: detection & characterization. Indian J Med Res. 2011 Oct;134:538–46.
15. Hossain MS, Raheem E, Sultana TA, Ferdous S, Nahar N, Islam S, et al. Thalassemias in South Asia: clinical lessons learnt from Bangladesh. Orphanet J Rare Dis. 2017 Dec;12(1):93.
16. Mukhopadhyay D, Saha K, Sengupta M, Mitra S, Datta C, Mitra PK. Spectrum of Hemoglobinopathies in West Bengal, India: A CE-HPLC Study on 10407 Subjects. Indian J Hematol Blood Transfus. 2015 Mar;31(1):98–103.
17. Singh SP, Gupta SC. Effectiveness of red cell osmotic fragility test with varying degrees of saline concentration in detecting beta-thalassaemia trait. Singapore Med J. 2008 Oct;49(10):823–6.
18. Gorakshakar AC, Colah RB. Cascade screening for beta-thalassemia: A practical approach for identifying and counseling carriers in India. Indian J Community Med. 2009 Oct;34(4):354–6.
19. Rathod DA, Kaur A, Patel V, Patel K, Kabrawala R, Patel V, et al. Usefulness of Cell Counter–Based Parameters and Formulas in Detection of β-Thalassemia Trait in Areas of High Prevalence. Am J Clin Pathol. 2007 Oct;128(4):585–9.
20. Sinha M, Panigrahi I, Shukla J, Khanna A, Saxena R. Spectrum of anemia in pregnant Indian women and importance of antenatal screening. Indian J Pathol Microbiol. 2006 Jul;49(3):373–5.
21. Bhukhanvala DS, Sorathiya SM, Sawant P, Colah R, Ghosh K, Gupte SC. Antenatal Screening for Identification of Couples for Prenatal Diagnosis of Severe Hemoglobinopathies in Surat, South Gujarat. J Obstet Gynecol India. 2013 Apr;63(2):123–7.
22. Colah R, Surve R, Wadia M, Solanki P, Mayekar P, Thomas M, et al. Carrier Screening for β-Thalassemia during Pregnancy in India: A 7-Year Evaluation. Genetic Testing. 2008 Jun;12(2):181–5.
23. Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between beta thalassemia trait and iron deficiency in children. Hematology. 2010 Apr;15(2):112–5.
24. Raghavan K, Lokeshwar MR, Birewar N, Nigam V, Manglani MV, Raju NB. Evaluation of naked eye single tube red cell osmotic fragility test in detecting beta-thalassemia trait. Indian Pediatr. 1991 May;28(5):469–72.
25. Mamtani M, Jawahirani A, Das K, Rughwani V, Kulkarni H. Bias-corrected diagnostic performance of the naked eye single tube red cell osmotic fragility test (NESTROFT): An effective screening tool for β-thalassemia. Hematology. 2006 Aug;11(4):277–86.
26. Singh SP, Gupta SC. Effectiveness of red cell osmotic fragility test with varying degrees of saline concentration in detecting beta-thalassaemia trait. Singapore Med J. 2008 Oct; 49(10):823–6.
27. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N, et al. Guidelines for screening, diagnosis and management of hemoglobinopathies. Indian J Hum Genet. 2014 Apr; 20(2):101–19.
28. Balgir RS. Spectrum of hemoglobinopathies in the state of Orissa, India: a ten years cohort study. J Assoc Physicians India. 2005 Dec; 53:1021–6.