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Research Article

THE INCIDENCE OF BETA-THALASSEMIA MINOR IN PREGNANT FEMALES BY MEASURING HBA2 THROUGH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objectives: The aim of the study was to study the incidence of thalassemia minor by determining HbA2 levels in pregnant females attending Obstetrics and Gynaecology OPD and HbA2 levels of husbands of positive females for beta-thalassemia trait.

Methods: The prospective study spanning over 1½ years was conducted in the Department of Pathology in 1020 pregnant females who attended the Gynaecology and Obstetrics OPD of Government Medical College, Patiala for antenatal check-up. The pregnant females of any trimester without any specific sign and symptoms whose Hb level was <10 g/dL were screened in the study. Levels of HbA2 and HbF were determined by high performance liquid chromatography (HPLC) and the cases with raised HbA2 value above the cutoff limit (>3.5%) were labeled as BTT. Husbands of BTT positive females were also screened for the trait. Incidence of all these cases was calculated and analyzed statistically.

Results: The majority of the females were in the age group of 21–30 years. In present study, we found that total 134 (13.1%) patients were having beta thalassemia trait. Husbands of all these positive patients were also screened for BTT and only 2 (1.49%) of them were found to be positive.

Conclusion: HPLC has the advantage for screening and detection of various hemoglobinopathies by providing rapid and accurate results. HPLC can detect and measure HbF and HbA2 in a single system. Early diagnosis and management of thalassemia can help in reduction of burden on society as well as government.

Keywords: Beta thalassemia, Hemoglobinopathies, HbA2, HbF, High performance liquid chromatography.

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INTRODUCTION

Human hemoglobin consists of two pairs of globin chains with heme group attached to each of them. The individual globin chains synthesized in postnatal life are designated as α, β, γ, and δ. HbA has two α chains and two β chains (α2 β 2); HbF has two α chains and two γ chains (α2 γ 2) and HbA2 has two α chains and two δ chains (α2 δ2). Alpha chain synthesis is directed by two α genes, α 1 and α β, on chromosome 16, and β and δ chain synthesis by single β and δ genes on chromosome 11. γ chain synthesis is directed by two genes, G γ and A γ, which are also located on chromosome 11 [1]. HbF is the predominant hemoglobin of fetal life where as in children and adults, HbA is the major hemoglobin. HbA2 and HbF are found in small quantities in adult life (2–3.3% and 0.2–1.0%, respectively). The adult proportions of hemoglobin A, A2, and F are usually attained by 6–12 months of age [1].

The disorders of hemoglobin synthesis can be grouped into three main categories:

1. Due to structural variants of hemoglobin, such as HbS.
2. Due to failure of synthesis of one or more globin chains of hemoglobin at a normal rate, as in thalassemias.
3. Those due to failure to complete the normal neonatal switch from fetal hemoglobin to adult hemoglobin referred to as hereditary persistence of fetal hemoglobin (HPFH) [1].

Thalassemias refer to heterogeneous group of disorders in which there is decreased or absent synthesis of one or more polypeptide chains (α or β) as a result of missense/non-sense mutations (single-base substitutions) or frameshift mutations of the genes controlling the structure of the hemoglobin protein chains in one or both "allelic" globin genes causing decreased hemoglobin concentration, microcytosis, and anemia [2]. The two major categories are the α and β thalassemia while the others are rare forms. These subgroups have in common an imbalanced globin synthesis and the globin produced in excess is responsible for ineffective erythropoiesis and hemolysis. The thalassemias result from the effect of a number of different molecular defects leading to a variety of clinical and hematologic phenotypes. Functionally, thalassemia mutations that cause a complete absence of globin chain synthesis are called α0 or β0 thalassemias and that cause reduced rate of synthesis of globin chains are called α+ or β+ thalassemias [3]. Clinically, the thalassemia is classified according to their severity into major, intermediate, and minor forms [3].

Thalassemias are among the most common genetic disorders in the world and an estimated 1.5% of the worldwide population is carrier of β-thalassemia [4]. Thalassemia is mainly prevalent in South-east Asia and Mediterranean countries. Due to high rate of migration and inter racial marriage system, thalassemia is found all over the world in recent years. About 10% of the total world thalassemia patients belong to Indian subcontinent and among them 3–4% are carriers [5].

β thalassemia is the most common single gene disorder in India. More than 30 million people worldwide carry thalassemia causing defective gene. Carrier frequency varies from 3 to 17% in different populations [6].

Definite diagnosis of thalassemia can be made by a step-wise algorithmic approach, starting with a detailed clinical history, through hematologic evaluation complete blood count (CBC)], reticulocyte count, red blood cell (RBC) morphology, Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), protein based analytic methods [Hb electrophoresis or isoelectric focusing (IEF), cation exchange high performance liquid chromatography (HPLC), reversed phase HPLC]

to nucleic acid based methods such as polymerase chain reaction (PCR), reverse transcribed (RT)-PCR, sequencing of genomic DNA, and sequencing of RTPCR amplified globin cDNA of the gene of interest [7].

Individuals with trait are healthy and unaware of their carrier status. If both parents are carrier of hemoglobinopathy trait, there is a one in four chance with each pregnancy that their child will inherit a major hemoglobinopathy [8]. Women who are carrier for β thalassemia minor appear perfectly healthy, other than a mild anemia leading to their misdiagnosis as iron deficiency anemia [9]. Antenatal diagnosis especially during first pregnancy in high incidence areas was found to be very useful. Management becomes easier and more effective if the couple already know their carrier status and come for counseling in time [10].

Carriers of β-thalassemia have levels of hemoglobin A2 and F >3.5% and 2% of the total hemoglobin, respectively. HbA2 is one of the most important marker of β-thalassemia heterozygous carriers so its determination plays a key role in screening programs for β-thalassemia [10]. When the MCH is below 27 pg and the Hb A2 is above 3.5%, a diagnosis of heterozygous β thalassemia is made [11].

Quantitative evaluation of HbA2 can be made by either electrophoresis or by high-pressure liquid chromatography (HPLC). HPLC has the additional advantage of quantifying HbF at the same time. Carriers of the β-thalassemia trait demonstrate increased values of HbA2 and HbF [3].

Paucity of published data regarding prevalence of β thalassemia in pregnant females in region of Patiala and adjoining areas intended us to carry out this study.

METHODS

This study was a prospective study conducted at Pathology Department, Government Medical College and Rajindra Hospital, Patiala, during the period of 1½ year. The study included antenatal cases of any trimester without any sign and symptoms just like a normal screening procedure in all pregnant females. Out of total antenatal patients attending obstetric clinic, 1020 patients were randomly selected in present study fulfilling the inclusion criteria (Hb<10 g/dl). Husbands of BTT positive females were also screened for the disease. For all these cases, the results of hemoglobin and other red cell indices were taken into consideration. Mentzer index for thalassemia was calculated for all the results.

1. Informed and written consent was taken from all patients for using their samples for study purpose.
2. A 2 ml venous blood sample was collected in EDTA anticoagulant.
3. Hb and red cell indices were measured on an automated hematology analyzer (Sysmex XP100).
4. Mentzer index for thalassemia was calculated for all the results. Index of <13 was considered indicative of beta-thalassemia trait.
5. HbA2 and HbF were studied by HPLC method used for chromatographic separation of human hemoglobin. The cases with raised HbA2 value above the cutoff limit (>3.5%) were labeled as BTT (Figs. 1-3).

HPLC chromatograms

Incidence of all these cases was calculated and analyzed statistically.

Ethical issues

Ethical approval was taken from the Institutional Ethic Committee of Government Medical College, Patiala, before starting the study.

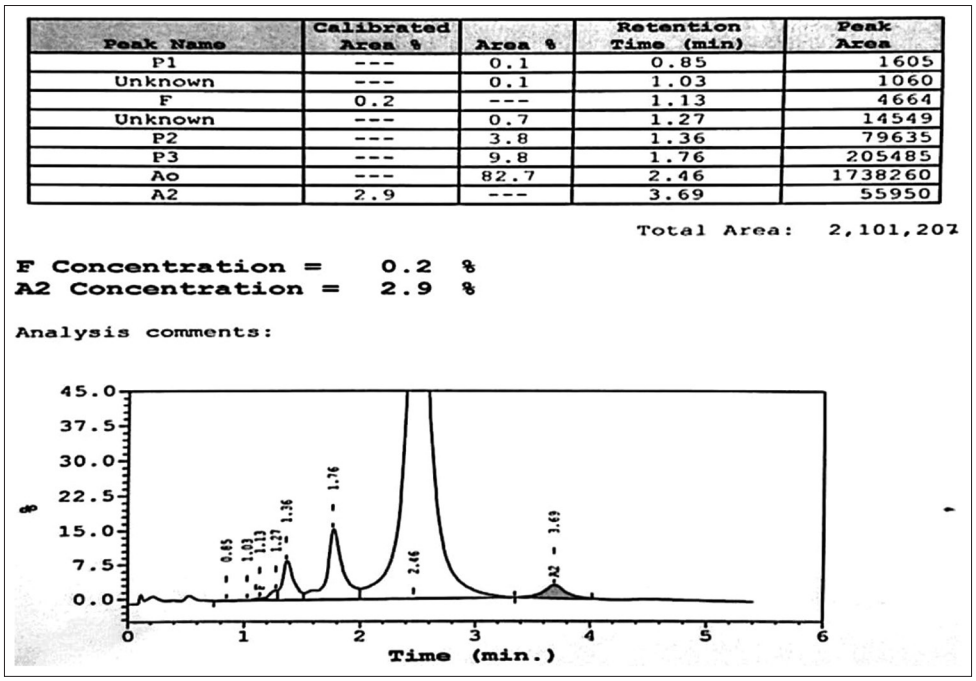
RESULTS

In the present study included 1020 patients based on inclusion criteria, we found that total of 134 (13.1%) patients were of beta-thalassemia trait. Husbands of all these positive patients were also screened for BTT and only 2 (1.49%) of them were positive.

Out of total 1020 cases, 134 were positive for BTT (13.1%), 886 were negative for BTT (86.9%) (Table 1).

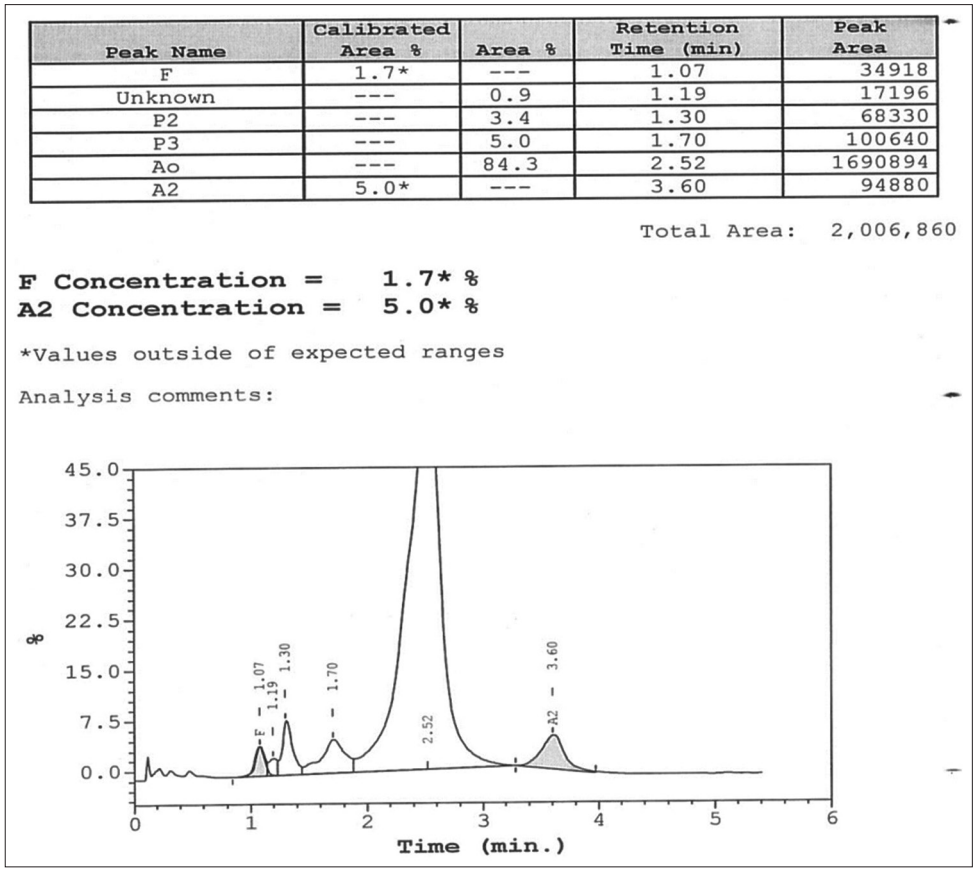
Out of total 1020 patients, the majority of the patients diagnosed in second trimester, that is, 529 patients (56.8%) in the present study. In both BTT and NBTT, the majority of patients fall in second trimester (Table 2 and Figure 4).

Out of 1020 cases, the majority of the patients have Hb 8–10g/dl (783 cases=76.8%) and same scenario is seen in both BTT (69.4%) and NBTT (77.9%) patients. Mean Hb for (134) cases of BTT was (8.37±1.06%) and it differed significantly from NBTT cases (8.56±1.07%) (Table 3 and Figure 5).



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.1	0.85	1605
Unknown	---	0.1	1.03	1060
F	0.2	---	1.13	4664
Unknown	---	0.7	1.27	14549
P2	---	3.8	1.36	79635
P3	---	9.8	1.76	205485
Ao	---	82.7	2.46	1738260
A2	2.9	---	3.69	55950

Fig. 1: Normal high performance liquid chromatography chromatograms



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	1.7*	---	1.07	34918
Unknown	---	0.9	1.19	17196
P2	---	3.4	1.30	68330
P3	---	5.0	1.70	100640
Ao	---	84.3	2.52	1690894
A2	5.0*	---	3.60	94880

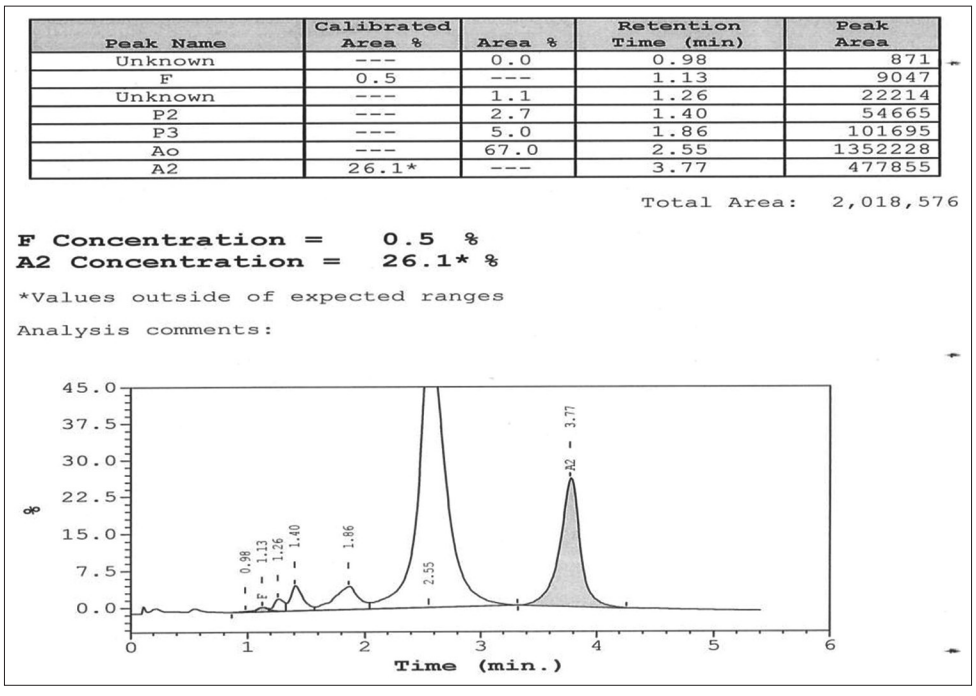
Total Area: 2,006,860

F Concentration = 1.7* %
A2 Concentration = 5.0* %

*Values outside of expected ranges

Analysis comments:

Fig. 2: Beta-thalassemia trait



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.98	871
F	0.5	---	1.13	9047
Unknown	---	1.1	1.26	22214
P2	---	2.7	1.40	54665
P3	---	5.0	1.86	101695
Ao	---	67.0	2.55	1352228
A2	26.1*	---	3.77	477855

Total Area: 2,018,576

F Concentration = 0.5 %
A2 Concentration = 26.1* %

*Values outside of expected ranges

Analysis comments:

Fig. 3: HbE heterozygous

MI is one of the most important indicators to differentiate between BTT and NBTT. A value of MI<13 is indicative of BTT.

In the present study, out of 134 cases of BTT, 101 cases had MI <13% which is highly suggestive of BTT. p value was calculated using Chi-square method which was statistically significant (Table 4 and Figure 6).

Incidental detection of the of other hemoglobinopathies was found to be percentage-wise as follows HbE 0.3%, P3 window 0.3%, S window

Table 1: Incidence of BTT and NBTT (n=1020)

Diagnosis	No of cases	%
BTT	134	13.1
NBTT	886	86.9
Total	1020	100.0

Table 2: Trimester wise distribution

Period of Gestation (weeks)	BTT		NBTT		Total	
	No. of cases	%	No. of cases	%	No. of cases	%
1st trimester	25	18.7	133	15.0	158	15.5
2nd trimester	80	59.7	499	56.3	529	56.8
3rd trimester	29	21.6	254	28.7	283	27.7
Total	134	100.0	886	100.0	1020	100.0
Chi-square	3.304					
p value	0.192					
Significance	NS					

Table 3: Hemoglobin value (g/dL) in BTT and NBTT

Hb (g/dl)	BTT		NBTT		Total	
	No. of cases	%	No. of cases	%	No. of cases	%
<4	0	0.0	0	0.0	0	0.0
4–6	3	2.2	28	3.2	31	3.0
6–8	38	28.4	168	19.0	206	20.2
8–10	93	69.4	690	77.9	783	76.8
Total	134	100.0	886	100.0	1020	100.0
Mean±S.D	8.37±1.06		8.56±1.07		8.54±1.07	
p value	0.048					
Significance	S					

Table 4: Distribution of Mentzer index (MI) of BTT and NBTT

MI	BTT		NBTT		Total	
	No of cases	%	No of cases	%	No of cases	%
<13	101	75.4	8	0.9	49	4.8
>13	33	24.6	878	99.1	971	95.2
Total	134	100.0	886	100.0	1020	100.0
Mean±S.D	12.86±0.77		18.59±2.48		18.05±2.79	
Chi-square	676.30					
p value	<0.001					
Significance	HS					

Table 5: Incidental detection of other hemoglobinopathies

Hemoglobinopathy	No. of cases	Percentage
HbE	4	0.3%
P3 window	4	0.3%
S window	4	0.3%
D window	3	0.2%
C window	2	0.1%
HbD Iran	1	<0.1%
HbQ trait	1	<0.1%

0.3%, D window 0.2%, C window 0.1%, HbD Iran <0.1%, and HbQ trait <0.1% (Table 5 and Figure 7).

In the present study of antenatal screening for BTT by HbA2 measurement through HPLC, 134 females came out positive. As per the inclusion criteria of the present study, husband of all the females with HbA2 suggestive of BTT was tested for HbA2 levels. Husband of two females had HbA2 >3.5%.

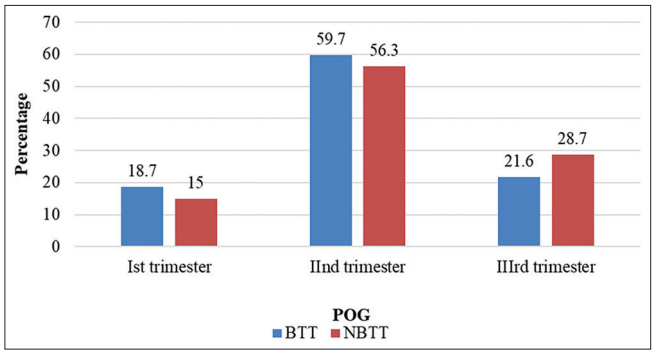


Fig. 4: Trimester-wise distribution

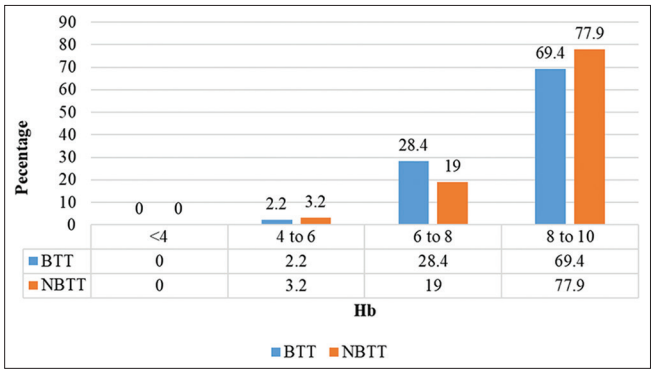


Fig. 5: Bar graph showing hemoglobin value in BTT and NBTT

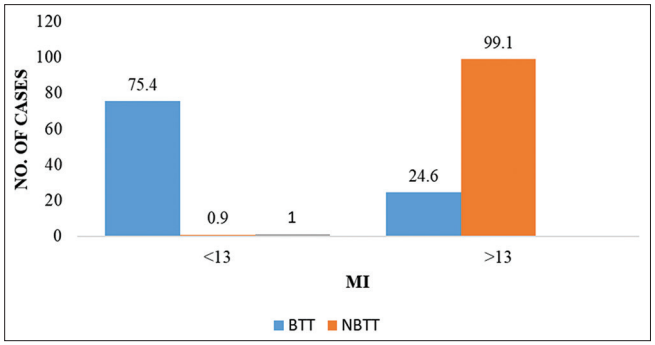


Fig. 6: Bar chart showing distribution of mentzer index of BTT and NBTT cases

Fig. 7: Bar chart showing other hemoglobinopathies

DISCUSSION

Thalassemia is a quantitative disorder of hemoglobinopathy in which the majority of the patients are asymptomatic so pre-natal counseling and screening is of utmost importance in such cases. In the present study, all the antenatal females who attended Obstetric OPD at Rajindra Hospital, Patiala were enrolled after consent for screening of BTT through HPLC, which is the gold standard method to determine Hb variants. HbA2 levels were measured and females with HbA2 above the cutoff value (>3.5%), their spouses were also screened so as to prevent the risk of beta-thalassemia major children. During our course of work, while studying for BTT, various other Hb variants were also found incidentally.

In the present study, the incidence of BTT was found out to be 13.1% by measuring the levels of HbA2 through HPLC (Table 1). The majority of the cases were found out to be in the age group 21–30 years in both BTT (85.1%) and NBTT (78.9%) which might be due to the fact that marriageable age group in India is in 20s and to prevent high-risk pregnancy above 30 years of age. These findings were similar to the findings in the study done by Khadija *et al.* [12] and Baliyan *et al.* [13]. On the other hand, major chunk of the patients falls in second trimester in both BTT (59.7%) and NBTT (56.3%). These findings were similar to the findings in the study done by Colahet *et al.* [14] and Mendiratta *et al.* [15]. General awareness among masses regarding thalassemia is the need of hour to get screened before marriage or as early as possible after getting conceived so that the pregnancy can be timely terminated in affected patients. On correlation of blood groups with thalassemia, the majority of the patients were of A+ blood group in BTT (46.3%) and of O +ve blood group in NBTT (45.9%) Sinha *et al.* found most common blood group O +ve in ß-thalassemia patient which may be due different geographical area [16].

In the present study, inclusion criteria for patients with Hb <10 g/dl were taken, so all the antenatal females with Hb <10 g/dl were enrolled. Among these females, the mean Hb for BTT was found out to be 8.37 g/dl (Table 3). (p=0.048%, which was statistically significant.) However, no relevant literature where Hb <10 g/dL as inclusion criteria was present.

The incidence of thalassemia is variable in different regions of the country and in particular in different ethnic groups. A few studies done earlier have shown that certain communities such as Sindhi's, Kutchhi, Bhanushali's, and Punjabis from Western and Northern India have high prevalence of BTT (5–15%) [17]. In our study, the incidence of BTT was found out to be 13.1% (Table 6).

Saxena *et al.* in 2020 carried out a study on 1236 patients out of which 741 (59.9%) patients were of iron deficiency anemia and 495 (40.1%) patients were of β-thalassemia trait. They calculated Mentzer index and it was found to be more reliable to detect true positive cases for β-thalassemia trait with a sensitivity of 89.0% and specificity of 87.9%. They concluded that iron deficiency anemia and thalassemia have different effects on blood indices. In doubtful cases, the diagnosis was confirmed by HPLC [25].

In the present study, various other hemoglobin variants were detected accidently. We found HbE 0.3%, HbS 0.3%, and HbD 0.2% (Table 5 and Figure 7). Dolai *et al.* on screening of 35,413 individuals from rural areas in West Bengal showed that the prevalence of HbE carriers was 4.3% while β-thalassemia carriers were 10.38% and HbS and HbD carriers were 1.12% and 0.37%, respectively, which may due large sample size and different geographical area [26].

Although antenatal diagnosis is of paramount importance to the control of thalassemia, screening programs form an important and integral part in the prevention of thalassemia. Several Mediterranean and Western countries have achieved a significant change in the homozygote population since the past two decades. In India, several antenatal screening programs are effective. Still more screening centers are required. In these programs, the population essentially screened with follow-up of awareness of their thalassemic status and need for reduction in the birth of beta-thalassemic homozygotes through antenatal diagnosis. The screening programs play a role in increasing awareness and education regarding hemoglobinopathies [22].

Table 6: Comparison of incidence of BTT in various studies

Study	Incidence of BTT
Sachdev *et al.* [18] (2010)	8.9%
Mohanty *et al.* [19] (2013)	2.78%
Bhukhanvala *et al.* [20] (2013)	3.38%
Hanprasertpong *et al.* [21] (2013)	3.7%
Kumar *et al.* [22] (2015)	3.3%
Mondal *et al.* [23] (2016)	4.60%
Konar *et al.* [24] (2018)	17%
Present study (2021)	13.1%

Screening may be voluntary or mandatory. Beta-thalassemia carriers in a family are now easily detected using well-calibrated automated hematology cell counters and automated HPLC systems. However, the prevention program including early screening of pregnant women and spouse of thalassemic pregnant women for antenatal diagnosis and termination of a homozygote fetus has been slow in India, due to several factors. These include late reporting of pregnancy and the lack of widespread awareness and facilities for screening and antenatal diagnosis.

A multidisciplinary approach including screening of high school/college students, premarital screening, and of the extended family of thalassemic along with antenatal diagnosis needs to be considered for this vast and ethnically diverse country so that burden of disease can be minimized efficiently and effectively.

CONCLUSION

HPLC is simple, accurate, and superior technique combined with complete automation makes it an ideal method for diagnosis of thalassemia and other hemoglobinopathies. However, there were certain limitations of HPLC which can be overlooked with molecular profiling of BTT patients.

AUTHORS CONTRIBUTION

All the authors have contributed toward the preparation, review preparation, and editing of the manuscript. Dr. Anita Chaudhary: Collection of data. Dr. Ninder Kumar: Writing of manuscript and interpretation of data. Dr. Ritu Kundal: Collection of data. Dr. Ramesh Kumar and Dr. Preet Kamal Sibia: Proof reading of manuscript and analysis of data.

CONFLICT OF INTEREST

All the authors have none to declare.

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REFERENCES

1. Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Haematology. 12th ed. London: Churchill Livingstone; 2017.
2. Petrakos G, Andriopoulos P, Tsironi M. Pregnancy in women with thalassemia: Challenges and solutions. Int J Womens Health 2016;8:441-51. doi: 10.2147/IJWH.S89308, PMID 27660493
3. Pignatti CB, Galanello R. Thalassemias and related disorders. Quantitative Disorders of hemoglobin synthesis. Solutions. In: Wintrobe's Clinical Hematology. 14th ed. Netherlands: Wolters Kluwer; 2019.
4. Modell B, Bulyzhenkov V. Distribution and control of some genetic disorders. World Health Stat Q 1988;41:209-18. PMID 3232409
5. Gu X, Zeng Y. A review of the molecular diagnosis of thalassemia. Hematology 2002;7:203-9. doi: 10.1080/1024533021000024102,

PMID 14972782

6. Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A2 value in screening for hemoglobinopathies. Clin Biochem 2009;42:1786-96. doi: 10.1016/j.clinbiochem.2009.06.026, PMID 19591816
7. Kutlar F. Diagnostic approach to hemoglobinopathies. Hemoglobin 2007;31:243-50. doi: 10.1080/03630260701297071, PMID 17486507
8. Leung TY, Lao TT. Thalassaemia in pregnancy. Best Pract Res Clin Obstet Gynaecol 2012;26:37-51. doi: 10.1016/j.bpobgyn.2011.10.009, PMID 22079388
9. Baxi A, Manila K, Kadhi P, Heena B. Carrier screening for β thalassemia in pregnant Indian women: Experience at a single center in Madhya Pradesh. Indian J Hematol Blood Transfus 2013;29:71-4. doi: 10.1007/s12288-012-0165-8, PMID 24426339
10. Shirlyn B, McKenzie IS, editors. Textbook of Hematology. 2nd ed. Philadelphia, PA: William and Wilkins; 1996. p. 1-9.
11. Whipple GH, Bradford WL. Mediterranean disease-thalassemia (erythroblastic anaemia of Cooley): Associated pigment abnormalities simulating hemochromatosis. J Pediatr 1936;9:279-311. doi: 10.1016/S0022-3476(36)80021-3
12. Khadija, Arain SP, Alahmadi YM, Alolayan SO, Ahmer A, Ahmed T. Frequency of beta thalassemia in anemic pregnant women. JPRI 2021;33:1-5. doi: 10.9734/jpri/2021/v33i531175
13. Baliyan M, Kumar M, Nangia A, Parakh N. Can RBC indices be used as screening test for beta-thalassemia in Indian antenatal women? J Obstet Gynaecol India 2019;69:495-500. doi: 10.1007/s13224-019-01220-8, PMID 31844363
14. Colah R, Gorakshakar A, Surve R, Wadia M, Ghosh K, Mohanty D. Feasibility of antenatal screening of beta-thalassemia in Mumbai, India. Acta Haematol 2001;105:252. doi: 10.1159/000046576, PMID 11528103
15. Mendiratta SL, Bajaj S, Popli S, Singh S. Screening of women in the antenatal period for thalassemia carrier status: Comparison of NESTROFT, red cell indices, and HPLC analysis. J Fetal Med 2015;2:21-5. doi: 10.1007/s40556-015-0036-0
16. Sinha PA, Mulkutkar SH, Bhavani JB. Study of distribution of ABO blood groups in ß-thalassemia patients. Int J Res Med Sci 2017;5:3479-83. doi: 10.18203/2320-6012.ijrms20173545
17. Balgir RS. Spectrum of hemoglobinopathies in the state of Orissa, India: A ten years cohort study. J Assoc Physicians India 2005;53:1021-6. PMID 16572956
18. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol 2010;53:57-62. doi: 10.4103/0377-4929.59185, PMID 20090224
19. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, *et al*. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: A multicentre study. J Community Genet 2013;4:33-42. doi: 10.1007/s12687-012-0114-0, PMID 23086467.
20. 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 Gynaecol India 2013;63:123-7. doi: 10.1007/s13224-012-0271-4, PMID 24431619
21. Hanprasertpong T, Kor-anantakul O, Leetanaporn R, Suntharasaj T, Suwanrath C, Pruksanusak N, *et al*. Pregnancy outcomes amongst thalassemia traits. Arch Gynecol Obstet 2013;288:1051-4. doi: 10.1007/s00404-013-2886-9, PMID 23681496
22. Kumar R, Gupta S, Jindal A, Kakkar S, Kaur A. Screening of beta thalassemia trait and other hemoglobinopathies among blood donors in Punjab. Int J Med Public Health 2015;5:1-4
23. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci 2016;10:105-10. doi: 10.4103/0973-6247.175424, PMID 27011683
24. Konar (Sanyal) K, Karmakar A, Mondal B. Clinico-hematological pattern of thalassemias and hemoglobinopathies in children presenting with microcytic anaemia: An outdoor-based study at Burdwan, West Bengal. Int J Curr Res Rev 2018;10:27-34. doi: 10.31782/IJCRR.2018.10104
25. Saxena S, Jain R. Evaluation of the diagnostic reliability of mentzer index for beta thalassemia trait followed by HPLC. Trop J Pathol Microbiol 2020;6:124-9. doi: 10.17511/jopm.2020.i02.03
26. Dolai TK, Dutta S, Bhattacharyya M, Ghosh MK. Prevalence of hemoglobinopathies in rural Bengal, India. Hemoglobin 2012;36:57-63. doi: 10.3109/03630269.2011.621007, PMID 22004064