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# A Spectrum of Hemoglobinopathies and Thalassemia Trait Using HPLC: Hospital-Based Observational Study in Northern India

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**Abstracts** **Aim:** The publication deals with the prevalence of hemoglobinopathies among patients diagnosed with thalassemia based on High Performance Liquid Chromatography (HPLC) characteristics. We also compared the current study results to other published studies. Hemoglobinopathies, which can be quantitative (such as thalassemia syndrome) or qualitative (such as sickle cell disease), are a major public health concern in India (variant hemoglobinopathies). Every year, 10,000 infants are born in India with thalassemia major, accounting for 10% of all such children globally. According to the Thalassemia International Federation, around 0.2 million thalassemia major cases are alive and undergoing regular treatment globally. HPLC is regarded as a sensitive and specific method for detecting hemoglobin A2 (HbA2) levels greater than 3.5%, which is used to diagnose beta thalassemia trait (BTT). This study was conducted at a tertiary care hospital, where laboratory test reports of patients, who were diagnosed with thalassemia utilizing HPLC test from January 2022 to December 2022 were studied and analyzed. The age categories of 21 to 30 years were discovered to have the highest prevalence of beta-thalassemia features (36.9%), followed by 31 to 40 years (13.8%) and 11 to 20 years (11.3%). The data was displayed in the following formats: mean (average), median, standard deviation (SD), lowest and maximum range. HbF varied from more than 0.08 to 40.0, HbA2 from 1.5 to 27, and HbA0 from 52.5 to 95.5. The results of this investigation demonstrated the prevalence and range of hemoglobinopathies and thalassemia in patients. The coexistence of thalassemia and other hemoglobin variants was discovered to be frequent.

**Keywords** Hemoglobinopathies, B-Thalassemia, High Performance Liquid Chromatography, Anemia, Hemoglobin A2

## 1. Introduction

Hemoglobinopathies are disorders that affect hemoglobin composition, synthesis, or structure. The severity of these illnesses, the majority of which are hereditary, ranges from asymptomatic test abnormalities to catastrophic conditions like thalassemia major (Fig.1), which demand regular blood transfusions and careful medical attention. Among the many symptoms are hemolytic anemia, erythrocytosis, cyanosis, and vaso-occlusive stigmata [1]. Although additional hemoglobin illnesses such as Hemoglobin D (HbD), Hemoglobin S (HbS) and Hemoglobin E (HbE) can occur

alone or jointly in modern culture, beta-thalassemia remains the most frequent single-gene hemoglobin-associated disease. Sickle cell disease is one of the world's most frequent hereditary disorders. It is distinguished by the presence of homozygous hemoglobin S (Hb S) or Hb S coupled with other Hb variations. The quantification of Hb A2, family history, clinical data, and a complete blood count (CBC) are all important in the diagnosis of sickle cell disease [2]. Thalassemia major in newborns can be avoided by detecting a range of hemoglobin abnormalities, including thalassemia heterozygous, as soon as possible. Interactions between various hemoglobin mutations in the progeny are possible. The hematological deficit can also develop from being double heterozygous for specific alleles. The double heterozygous condition between certain alleles can also result in hematological deficits (Fig. 2) [3].

The Thalassemia International Federation believes that there are 0.2 million thalassemia major cases alive and undergoing regular treatment worldwide, based on World Health Organization (WHO) data and statistics. It is prevalent in the Mediterranean region, India, Central Asia, south China, the Middle East, and the Far East, as well as countries along South America's northern coast and in Africa [4]. Hemoglobinopathies are a key cause of many diseases in South Asia's poor population, and they can be quantitative (such as thalassemia syndrome) or qualitative (variant hemoglobinopathies) [1]. Every year, 10,000 infants are born in India with -thalassemia major, accounting for 10% of all such children globally [5]. In India, the frequency of beta-thalassemia is 3-4%, and 10,000 to 12,000 newborns are born with the condition each year [6]. According to the state population, the prevalence of Beta-thalassemia trait (BTT) is 6.5% in Punjab, 8.4% in Tamil Nadu (A south Indian state), 4.3% in South India, and 3.5% in Bengal. The prevalence of BTT in Bengal's male population is 3.6%, whereas it is 5.95% in pregnant mothers [7]. Low or absent amounts of the b-globin chain are one of the key components of hemoglobin A and one of the characteristics of beta-thalassemia (HbA, a2b2). Several hundred mutations in the b-globin gene or its regulatory components have now been found, resulting in decreased or nonexistent b-globin production [8]. The severity of the condition is determined by the type of beta gene allele (0, +, +++). The excess globin is reduced by the interaction of -globin chains with globin chains, resulting in varied degrees of disease severity. Extra or unpaired free-globin chains precipitate in the erythroblast cells, hemolyzing red blood cells. Thus, the degree of anemia and illness condition are negatively related to the free form of chains [9].

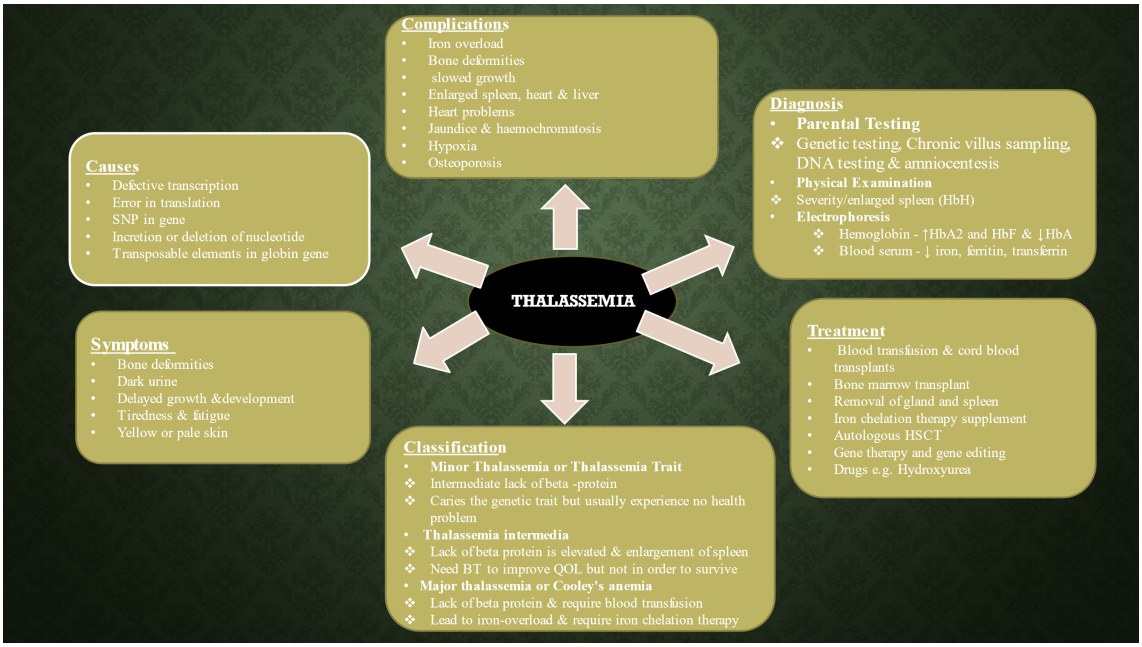


**Figure 1.** Study characterization of thalassemia

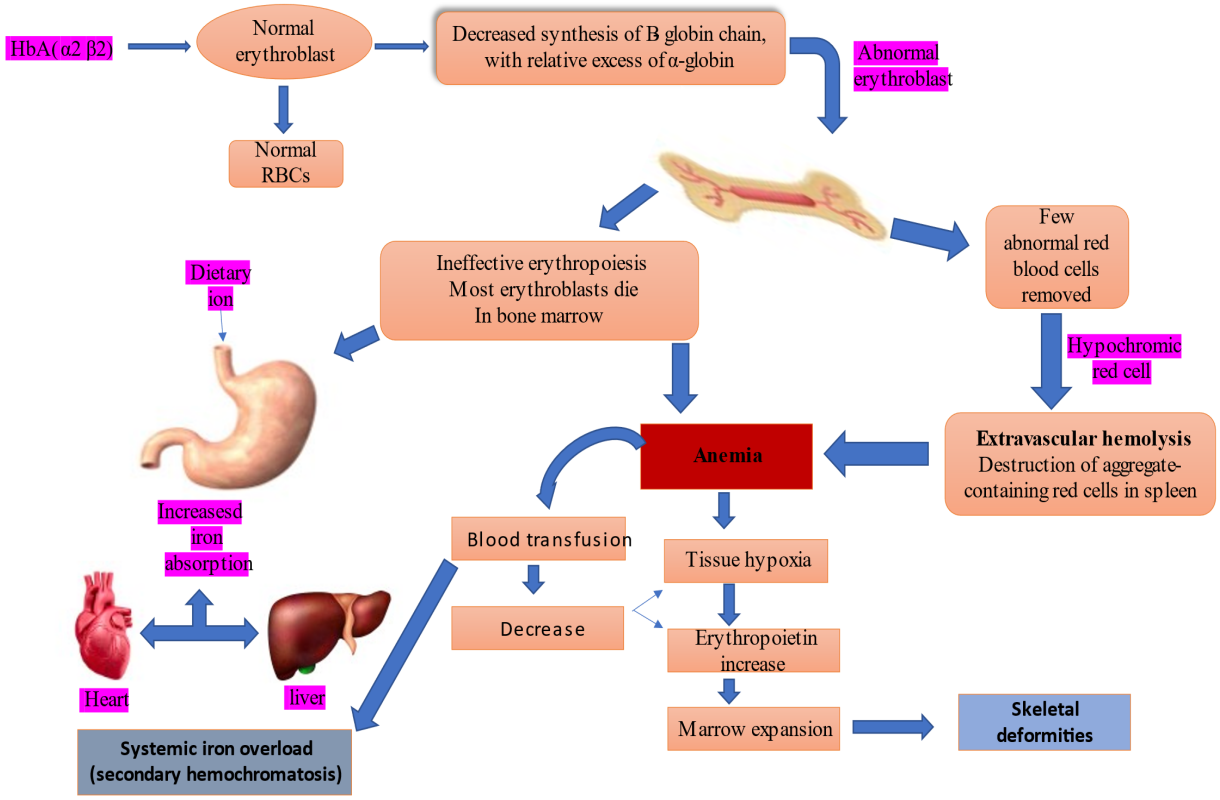


**Figure 2.** Pathogenesis of Thalassemia- explains how the deficiency of some crucial factors required for erythropoiesis affects the production of hemoglobin leading to hematological deficiencies in the body systemically

For the first diagnosis of beta and alpha thalassemia and iron deficiency anemia, hemoglobin electrophoresis and CBC blood tests are used. MCV 80, MCH 27, and HbA2 3.5 are all considered minor thalassemia. The fundamental distinction between alpha and beta thalassemia indexes is that the HbA2 level is less than 3.5. The indices in iron deficiency anemia are identical to those in alpha minor thalassemia, with the exception that the hemoglobin index is less than 10 and there is less hemoglobin overall [10].

Electrophoresis, CBC, molecular diagnostic procedures such as Polymerase chain reaction (PCR) genotyping, and HPLC are the most often used thalassemia detection methods [11–13]. A laboratory diagnostic of hemoglobin abnormalities is essential to validate the basic diagnosis of severe sickling diseases and beta-thalassemia major, explain hematological irregularities, and allow prospective patients to receive genetic counseling. HPLC has long been regarded as one of the methods of choice for accurately quantifying Hb A2 and has been the subject of controversy in the medical literature for several decades [2].

The main aim is the study of hemoglobinopathies prevalence among patients diagnosed with thalassemia based on HPLC characteristics. We also compared the current study results to other published studies.

## 2. Material and Method

This is an institutional, retrospective observational study that was carried out with total laboratory test reports of 88 samples, which have been clinically and hematological suspected cases of hemolytic anemia from January 2022 to December 2022, Maharishi Markandeshwar Institute of medical science and research (MMIMSR), Mullana. All of the necessary information was gathered from the biochemistry and hematology departments of the multi-specialty hospital in Haryana, North India. After receiving written informed consent from patients or guardians, each diagnosed patient withdrew 2-3 ml of blood and collected it into Ethylenediamine tetra-acetic acid (EDTA) anticoagulant bottles by lab technicians. For the diagnosis of BTT, a reference level of HbA2 more than 3.5 was regarded as the gold standard. We studied the reports of the patient's hematological profile.

### 2.1. Inclusion

Report of patient's hematological profile reports prepared through HPLC test for the diagnosis of thalassemia and other hemoglobinopathies was considered in the study of both genders.

### 2.2. Exclusion

Reports of hematological tests done other than HPLC and other illnesses were excluded.

### 2.3. Statistical Methods

Microsoft Excel 2019 was used to generate descriptive statistics. Mean, Median, and Standard Deviation was computed for quantitative data. The Student's t-test (independent) was utilized to determine the significance of the mean values of the laboratory parameters.

## 3. Result

For the detection and screening of beta-thalassemia, hemoglobin fraction and total RBC indices are utilized. Beta thalassemia is an inherited blood condition that is passed down through a parent's DNA (Fig. 2). Thalassemia major is inherited, when two parents with thalassemia minor pass on the disease to their child. Each child of two carriers has a 25% chance of inheriting the predominant form of beta-thalassemia. Minor thalassemia is inherited, when two carrier parents pass it on to their child. Each kid born to two carrier parents has a 50% chance of inheriting beta thalassemia minor.

In our investigation, a total of 155 patient reports were collected, 59 of which were discarded, because they fit our exclusion criteria, and 8 - because the reports were inadequate. Male (32.2 years) and Female (30.11 years) patients (mean) ages were determined for the final examination of 88 case reports. 36.9% of patients were between the ages of 21 and 30, followed by 13.8% of them - between 31 and 40 years and 11.3% of them - between 11 and 20 years. Table 1 presents numerous laboratory parameter data and statistical analysis of those data. The values are shown in mean, median, standard deviation (SD), minimum, and maximum range formats. HbF varied from >0.08 to 40.0, HbA2 from 1.5 to 27, and HbA0 from 52.5 to 95.5. According to Table 1, the mean SD values for HbF, HbA2, and HbA0 were respectively 1.715, 3.532, and 82.384.

Table 2 displays the distribution of male and female hemoglobinopathies and TBB values. Males and females had statistically equivalent HbA2, HbA0, PCV, MCV, and RDW HPLC spectra. Furthermore, there was no association between the distributions of thalassemia and hemoglobinopathies and different genders. Females are more likely to carry the beta-thalassemia trait than males.

**Table 1.** Distribution of laboratory parameter

| PARAMETER | HbF | HbA0 | HbA2% | Hb | RBC | PCV | MCV | MCH | MCHC | RDW |
|---|---|---|---|---|---|---|---|---|---|---|
| **Mean** | 1.7 | 82.38 | 3.53 | 9.84 | 5.98 | 33.88 | 75.2 | 24.61 | 31.9 | 19.28 |
| **Median** | 27 | 0.8 | 2.8 | 9.9 | 4.24 | 32.8 | 76.1 | 23.5 | 29.7 | 17.3 |
| **ST.DEV ±** | 15.86 | 4.75 | 2.86 | 2.61 | 7.12 | 10.9 | 19 | 10.83 | 27.29 | 8.36 |
| **Minimum** | >0.08 | 52.5 | 1.5 | 3.41 | 1.04 | 16.2 | 23.2 | 11.8 | 16.9 | 11.5 |
| **Maximum** | 40.8 | 95.5 | 27 | 15.8 | 41.3 | 89.7 | 155.8 | 85.8 | 286 | 58.1 |

**Table 2.** Distribution of HPLC findings based on gender and various hematological parameters

| Parameter | Male (n=21) | Female (n=67) | *P* |
|---|---|---|---|
| Mean age | 32.2 ±25.19 | 30.1 ±10.83 | 0.844 |
| HbF | 3.77 ±9.30 | 1.05 ±0.66 | 0.022 |
| HbA0 | 82.16 ±1.98 | 82.45 ±6.61 | 0.825 |
| HbA2 (%) | 3.53 ±1.76 | 3.63 ±3.13 | 0.583 |
| Hb (g/dL) | 11.1 ±2.92 | 9.42 ±2.35 | 0.011 |
| RBC(*$10^{12}$/L) | 5.01 ±0.96 | 6.29 ±8.16 | 0.491 |
| PCV | 35.2 ±10.58 | 33.45 ±11.04 | 0.531 |
| MCV (fL) | 73.68 ±13.07 | 75.71 ±20.81 | 0.623 |
| MCH (pg) | 22.70 ±6.25 | 25.24 ±11.89 | 0.315 |
| MCHC | 40.59 ±53.76 | 29.04 ±2.74 | 0.085 |
| RDW (%) | 17.166.66 | 19.98 ±8.73 | 0.184 |

**Table 3.** Comparison of BTT (HbA2 and HbA0) from various existing studies

| Author [Ref] | Year | No of cases | HbA2% | HbA0% |
|---|---|---|---|---|
| Bodarya et al. [5] | 2016 | 41 | 5.10±1.57 | |
| Bhuvana et al. [9] | 2022 | 387 | 5.42±0.71 | 81.53±2.4 |
| Titilope et al. [11] | 2014 | 19 | 5.14±1.27 | 83.6±2.7 |
| Rachna et al. [12] | 2014 | 62 | 5.7±0.9 | 83.6±2.7 |
| Mondal et al. [13] | 2016 | 5488 | 2.6±0.4 | 86.6±2.2 |
| Krishnamurti et al. [14] | 2016 | 133 | 5.22±0.79 | |
| Rauf et al. [15] | 2017 | 100 | 5.54±0.59 | |
| Nitu et al. [16] | 2020 | 64 | 3.19±1.01 | 84.78±3.55 |
| Enass et al. [17] | 2020 | 70 | 6.440±9.08 | 92.53±3.41 |
| Sushanta et al. [18] | 2022 | 104 | 5.54±0.8 | 89.1±2.7 |
| Present study | 2022 | 88 | 3.53±2.86 | 82.38±4.75 |

**Table 4.** Assessment and comparison of hematological parameters with various existing studies

| Study Authors [Ref] | No of patients | Year | Hb(g/dl) | RBC(x1012/l) | MCV (Fl) | MCH(Pg.) | MCHC% | RDW% |
|---|---|---|---|---|---|---|---|---|
| Bodarya et al. [5] | 41 | 2016 | 10.80±0.82 | 5.62±0.73 | 66.71±5.62 | 22.39±4.27 | - | 19.37±9.77 |
| Bhuvana et al. [9] | 387 | 2022 | 10.68±1.64 | 5.60±0.96 | 65.56±8.02 | 19.27±2.23 | 29.45±1.89 | 17.29±2.97 |
| Titilope et al. [11] | 19 | 2014 | 6.76±1.25 | - | 69.68±5.32 | 21.59±7.1 | 28.18±1.03 | - |
| Rachna et al. [12] | 62 | 2015 | 9.3±2.7 | 5.18±2.7 | 62.7±7.7 | 19.56±2.5 | 31.4±2.3 | 19.5±4.4 |
| Mondal et al. [13] | 5488 | 2016 | 9.5±1.7 | 4.1±1.30 | 70.1±5.8 | 21.0±3.2 | 27.8±1.6 | 33.6±6.9 |
| Krishnamurthi et al. [14] | 133 | 2016 | 11.65±1.71 | 4.94±0.48 | 69.01±7.37 | 23.62±3.27 | 34.17±1.89 | 5.22±0.79 |
| Rauf et al. [15] | 100 | 2017 | 11.4±1.6 | 6.06±0.87 | 64.7±4.10 | 18.9±1.4 | 29.3±1.4 | 37.8±2.4 |
| Enass et al. [17] | 70 | 2020 | 9.58±1.49 | - | 62.23±7.16 | 20.533±2.72 | 26.8±3.12 | - |
| Sushanta et al. [18] | 104 | 2022 | 10.2±2.3 | 4.7±0.9 | 70.1±11.7 | 29.6±2.6 | 29.6±2.6 | 19.4±4.5 |
| Rao et al. [19] | 145 | 2009 | 10.3±2.1 | 5.06±0.9 | 68.8±7.4 | 20.54±2.03 | 28.3±1.8 | |
| Alam et al. [20] | 62 | 2016 | 10.6±1.8 | 6.30±0.94 | 67.5±5.8 | 20.5±2.2 | - | 16.9±4.0 |
| Sandeep et al. [21] | 941 | 2020 | 10.48±1.63 | 4.87±1.63 | 69.11±3.96 | 21.58±1.85 | 31.23±0.83 | 16.89±1.95 |
| Neelum et al. [22] | 2422 | 2022 | 8.02±2.7 | 5.2±0.9 | 72.6±17.6 | 23.1±6.4 | 32.1±6.2 | 21.5±9.7 |
| Present study | 88 | 2022 | 9.84±2.61 | 5.98±7.12 | 75.2±19 | 24.61±10.83 | 31.9±27.29 | 19.28±8.36 |

Table 3 exhibits data for HbA2 and HbA0 [5,9,11-18], whereas Table 4 contains data for HbA2, Hb, RBC, PCV, MCV, MCH, MCHC, and RDW ranging from 2009 to 2022 and corresponding to studies by multiple authors [5,11-15,17-22]. In our study, the mean levels of HbA2 and Hb0 were determined to be 3.532.8 and 82.384.75 for all of our patients with beta-thalassemia, respectively. When HbA2 and HbA0 were compared to each of the related studies, both sets of data revealed decreased results and were comparable to the study conducted by Nitu et al. [16] HbA2 and HbA0 readings of 3.19% and 84.78%, respectively, in all previous research indicate a high prevalence of beta-thalassemia.

## 4. Discussion

B-thalassemia is one of the inheritable heterozygous illnesses in India. It leads to a reduction or absence of Hb chain synthesis. In addition to carrier identification and prenatal analysis, its variants are required for its management and to avoid marriages between carriers of changed genes, including consanguineous types. The bulk of India's tribal population suffers from a variety of diseases and afflictions. In northern India, Sachdev et al. [23], Sandeep et al. [24], and Seema et al. [19] reported prevalence rates of 8.9%, 11.21%, and 10.38% for the beta-thalassemia trait, respectively. Although our analysis revealed that the HbA2 trait is more widespread than the beta-thalassemia trait, the beta-thalassemia trait appears to be the most common hemoglobinopathy. With prevalence rates of 34.4%, 25.3%, 17.8%, 15.1%, 3.4%, and 1.5%, respectively, homozygous E, beta thalassemia trait, sickle cell thalassemia, and thalassemia major are the most prevalent hemoglobinopathies [25]. Another study indicated that the thalassemia trait was the most prevalent hemoglobinopathy in the Indian state of Gujarat, followed by thalassemia major and HbS [3].

Multiple investigations have demonstrated that Cellulose acetate electrophoresis (CAE) may diagnose BTT reliably and precisely. Historically, it has been regarded as the most efficient approach for discovering and measuring various hemoglobin variations [15]. Similar findings were reported by Tyagi et al. [25] in India, who identified all BTT cases using both electrophoresis and HPLC. In a comparison of CAE and HPLC for the quantitative determination of HbA2 levels by Khosa et al. [26], found that both procedures exhibited 100 percent sensitivity in 40 BTT cases. As shown in Table 4, a comparative analysis of hematological parameters from several existing research over the past 23 years has been conducted. Upon comparing the HPLC results and their statistical significance to the findings of our inquiry, we discovered distinct deviations. Hemophilia is recognized globally as one of the most severe and prevalent hereditary illnesses. Among the hemoglobinopathies, sickle cell disease and -thalassemia, which affect millions of individuals worldwide, have the greatest influence on morbidity and mortality [27]. Enass et al. [17], Rachna et al. [12], and Mondal et al. [13] found comparable levels of hemoglobin; however, Rauf et al. [15] and Krishnamurthi et al. [14] detected higher levels of Hb, as shown in Table 4.

HbA2 4% is the standard diagnostic threshold for -thalassemia trait, however, levels between 3.6% and 3.9% are questionable. As part of prenatal counseling, women with these scores are encouraged to check their partners. There were few patients with borderline HbA2 levels. Several authors have proposed different HbA2 cutoff values, ranging from 3.5% to 4%, for the diagnosis of beta thalassemia trait. Every laboratory should establish its normal ranges, as stated [19]. 3.6-4.0% was the range we utilized for borderline HbA2. Rangan et al. [28] followed the term borderline for HbA2 levels between 3.0 and 4.0%, although 32% of patients with HbA2 levels between 3.4% and 3.9% had mutations. Colah et al. [29] made comparable observations. In addition, it is known that individuals with concurrent iron shortage had lower HbA2 levels, hence it is recommended that iron deficiency anemia be treated before HPLC [21]. Even though HbA2 levels may be low in iron deficiency anemia, Khera et al. [12] discovered in 2015 that they were sufficiently discriminatory to identify true carriers. Important for sickle cell disorders is the accurate detection of Hb abnormalities. To determine the clinical significance of Hb A1 and Hb A2 and/or Hb F in conjunction with clinical and laboratory data for the diagnosis of Hb S/- thalassemia, this study examined Hb A2 values by HPLC in conjunction with Hb S and Hb C, and AT in a population with a low estimated prevalence of Beta-thalassemia [2].

MCH can be used as a screening test, followed by HbA2 measurement, to diagnose BTT. The value of MCH in patients with BTT is displayed in Table 4. Table 4 indicates that BTT cases have MCH 27 Pg, which is frequently recommended as a preliminary screening test for BTT. The current study reveals that individuals with multiple hemoglobinopathies, including MCHC, RBC, and RDW, had significant changes in several hematological parameters [17].

## 5. Conclusions

The current study's findings and all previous laboratory research point to a higher prevalence and incidence of the B-Thalassemia Trait among hemoglobinopathies in northern India. Furthermore, females (76.13%) have a higher incidence of BTT than men (23.86%). When compared to previous studies, we discovered that the values of some hematological parameters determined by HPLC, such as HbA, HbF, MCV, MCH, and RDW, were similar to ours, which is critical for diagnosing a patient with a hemoglobinopathy disorder. Finally, HPLC is a sensitive, focused, and accurate method for identifying and

quantifying various Hb fractions. However, it should be noted that the limitation of HPLC is its inability to differentiate between thalassemia and normal HbA2 thalassemia. There was no link found between the distributions of thalassemia and hemoglobinopathies and gender types. Females are more likely than males to have the beta-thalassemia trait. Because of the high prevalence of thalassemia traits, antenatal and age-appropriate screening is required. This will aid in the prevention of thalassemia major in children.

## Abbreviations

A non-glycated fraction of human hemoglobin (HbA0)
Fetal Hemoglobin (HbF)
Fetal Hemoglobin (HbF), Hemoglobin (Hb)
Hemoglobin (Hb)
Hemoglobin Subunit Alpha 2 (HbA2)
Mean Cell Hemoglobin Concentration (MCHC)
Mean Cells Hematocrit (MCH)
Mean Cells Volume (MCV)
Packed Cells Volume (PCV)
Red Blood Cell (RBC)
Red Cell Distribution Width (RDW)

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## Conflicts of Interest

There are no conflicts of interest.

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