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# Beta Thalassemia Carrier Detection Through RBC Metrics in Antenatal Females A Cross-Sectional Study

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**ABSTRACT**

Hemoglobin disorders represent a substantial cause of anemia worldwide, posing challenges during pregnancy. Thus, it is crucial to detect hemoglobin disorders during the prenatal period, as this not only helps anticipate the risk of thalassemia in offspring but also mitigates the complications associated with pregnancy-related anemia. Consequently, devising an affordable and reliable screening approach for hemoglobin disorders holds paramount importance. This study aimed to assess the utility of red blood cell (RBC) parameters, in identifying thalassemia carriers among healthy pregnant women receiving care at a tertiary hospital in Central India. Blood samples were obtained from 458 pregnant women aged 18 years or older, at less than 17 weeks of gestation and were subjected to complete blood count analysis, serum ferreting assessment and high-performance liquid chromatography for abnormal hemoglobin detection. Pregnant women diagnosed with iron deficiency anemia (serum ferreting <15 ng/ml) were excluded from the study. The prevalence of hemoglobinopathies was determined to be 12.23%, with ß-thalassemia trait (BTT) being the most prevalent subtype (7.86%). Individuals with BTT exhibited significantly lower levels of all RBC parameters compared to those with normal hemoglobin or other hemoglobinopathies. RBC parameters, including Hb, RBC count, MCV and MCH, can serve as a cost-effective and highly efficient screening method for identifying various hemoglobin disorders among pregnant women receiving antenatal care.

## INTRODUCTION

Anemia remains a significant contributor to morbidity and mortality during pregnancy. According to a survey conducted in 2015-2016, 58% of pregnant women in India were found to have anemia. Globally, iron deficiency accounts for the majority of anemia cases (63%), followed by thalassemia trait (5.4%). Thalassemia and other hemoglobinopathies represent the most prevalent single gene disorders affecting populations worldwide, characterized by reduced synthesis of hemoglobin (Hb) globin chains leading to varying degrees of anemia. The burden of thalassemia varies geographically, with 50% of cases concentrated in South-east Asian countries. India hosts the largest number of children with thalassemia major globally, with approximately 150,000 existing cases and 10,000-15,000 new cases detected annually. The prevalence of ß-thalassemia trait (BTT) in India is around 3.3%, although regional variations exist. Notably, higher prevalence rates have been observed in Punjab (6.5%) and Tamil Nadu (8.4%) compared to West Bengal (3.5%)[1-3]. A multi-center study conducted by the Indian Council of Medical Research (ICMR) across six cities revealed an overall incidence of thalassemia trait at 2.78%. Furthermore, different regions exhibit a predominance of specific hemoglobinopathies. For instance, HbE hemoglobinopathy is most prevalent in West Bengal (3.92%) and Assam (23.9%), while HbD-Punjab and HbS are prevalent among Sikhs in Punjab and individuals from Orissa, respectively. Effective screening methods during pregnancy are crucial for preventing the birth of children with hemoglobinopathies. Early detection through common screening methods can enable further molecular confirmation and selective termination of affected fetuses, thereby lessening the societal burden of thalassemia. Currently, high-performance liquid chromatography (HPLC) is considered the gold standard for detecting major and carrier states of hemoglobinopathies. However, the cost of complete blood count (CBC) with HPLC for mass screening poses challenges in countries with a high prevalence of microcytic hypochromic anemia. Automated cell counters are increasingly used, even in resource-limited areas, as a routine screening tool[4-6].

Various discrimination indices based on different parameters have been utilized to distinguish between BTT and iron deficiency anemia, although controversies exist regarding their cutoff values. These indices are primarily used for mass screening in populations. Red blood cell (RBC) parameters such as Hb%, RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red cell distribution width can be easily measured using automated cell counters and serve as effective screening tools for detecting different hemoglobinopathies[7]. This study aimed to assess the spectrum of hemoglobinopathies, with a focus on the role of RBC indices in detecting thalassemia among healthy pregnant women in a tertiary care hospital in Central India.

## MATERIALS AND METHODS

The study enrolled healthy pregnant women aged 18 years or older, with a gestational period of less than 17 weeks. Exclusion criteria included individuals with a recent history of blood transfusion within the preceding three months before sample collection or those beyond 17 weeks of gestation. Initial screening involved assessing participants for iron deficiency anemia (IDA) using a diagnostic criterion of serum ferreting levels below 15 ng/ml. Participants diagnosed with IDA were excluded from the study. CBCs were analyzed using the automated cell counter. Sample size calculation was performed using the formula 4pq/d^2, where p represents the prevalence of hemoglobinopathies in India, assumed as 13%. Utilizing this formula, the minimum required sample size was determined to be 174, yet 345 samples were included in this study. Statistical analysis was conducted using SPSS software. Venous blood samples were collected in two EDTA vials and HPLC was performed using an automated analyzer with the beta thalassemia program. HPLC operates on the principle of exchanging charged groups on an ion exchange material with charged groups on the hemoglobin (Hb) molecule. Identification of HbS was based on retention time, defined as the time in minutes from sample injection to the maximum point of the elution peak, with quantification of HbS achieved by determining the area under the corresponding peak in the elution profile. Retention times were used to define the manufacturer-assigned windows of the chromatogram.

## RESULTS AND DISCUSSIONS

In this study, 458 healthy antenatal mothers participated, with a mean age of 25.63±5.31 years. Participants were categorized by religion, with Hindus being the most frequent and Christians the least frequent (Table 1). Out of the total participants, 56 clinically healthy antenatal mothers were identified with hemoglobinopathies, indicating a prevalence of 12.23% (Table 2). The most common type was Beta thalassemia trait, comprising 7.86% of the subjects.

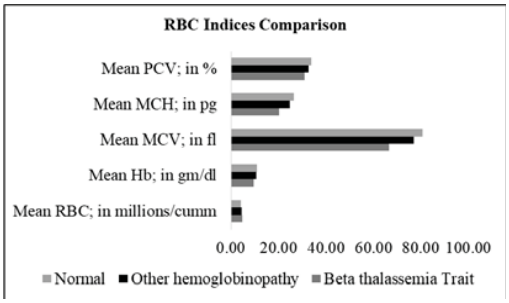


Fig 1: RBC indices among three study groups

**Table 1: Religion of antenatal mothers in study population**

| Religion | n | percentage |
|---|---|---|
| Bihari | 5 | 1.09 |
| Christian | 0 | 0.00 |
| Hindu | 379 | 82.75 |
| Marwari | 9 | 1.97 |
| Muslim | 57 | 12.45 |
| Punjabi | 5 | 1.09 |
| Others | 3 | 0.66 |

**Table 2: Type of Hemoglobinopathies detected in study participants**

| Type of Hemoglobinopathy | n | percentage |
|---|---|---|
| Beta thalassemia Trait | 36 | 7.86 |
| Other hemoglobinopathy | 20 | 4.37 |
| Normal | 402 | 87.77 |

**Table 3: Comparison of mean RBC indices among three study groups**

| Parameter | Beta thalassemia Trait | Other hemoglobinopathy | Normal | p-value |
|---|---|---|---|---|
| Mean RBC; in millions/cumm | 4.72 | 4.37 | 4.29 | <0.05 |
| Mean Hb; in gm/dl | 9.48 | 10.82 | 10.88 | <0.05 |
| Mean MCV; in fl | 66.45 | 77.15 | 80.65 | <0.05 |
| Mean MCH; in pg | 20.28 | 24.78 | 26.41 | <0.05 |
| Mean PCV; in % | 31.05 | 32.63 | 33.87 | <0.05 |

**Table 4: Values of HbA and HbA2 in Beta thalassemia Trait patients**

| Parameter | Value (Mean±SD) |
|---|---|
| HbA | 79.95±22.55 |
| HbA2 | 7.92±15.11 |

Other hemoglobinopathies accounted for 4.37% and included alpha thalassemia, Hb Lepore trait, D trait, D trait Punjab, E trait, E homozygous, Sickle cell trait, Hb D Punjab and Hb D Homozygous. RBC parameters such as mean Hb, mean RBC, mean packed cell volume, mean MCV and mean MCH were notably lower in the Beta thalassemia group compared to the other groups (Table 3, Fig: 1). In our study, the mean HbA2 value was 7.92% with a standard deviation of 15.11 among subjects with Beta thalassemia Trait (Table 4). Anemia poses a significant risk to maternal health, particularly among antenatal mothers in developing nations like India. Pandya *et al*. reported a high prevalence of anemia among Indian antenatal mothers, reaching 68.9%. Additionally, India witnesses approximately 15,000 births of children with thalassemia major annually, contributing to 10% of the global burden of thalassemia cases. Therefore, early screening for hemoglobinopathies in antenatal mothers not only prevents the birth of thalassemia children but also mitigates maternal morbidity and mortality[8-11].

In a previous study by Chowdhury and Talukdar in 2018, ß-thalassemia trait (BTT) emerged as the most prevalent hemoglobinopathy among antenatal mothers, with a prevalence of 5.1%[1]. Similarly, Sur and Mukhopadhyay reported a 5.22% prevalence of BTT among mothers attending antenatal clinics[3]. However, our study recorded a higher prevalence of BTT compared to these previous studies, possibly due to variations in racial and literacy status among the subjects. Nevertheless, like our study, previous research also identified BTT as the predominant hemoglobinopathy among antenatal mothers, albeit with varying prevalence rates. Dharmarajan *et al*. reported a 6.25% prevalence of BTT among pregnant women attending antenatal clinics, with a notable frequency (59.72%) of Hindu antenatal mothers participating in thalassemia screening programs[2]. These findings align with our study's observations. We also noted statistically significant lower values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) among individuals with BTT compared to healthy individuals, consistent with findings by Baliyan *et al*.[12].

However, it is crucial to note that both iron deficiency anemia (IDA) and BTT can manifest as microcytic hypochromic anemia and the presence of concurrent IDA may lead to a false low level of HbA2 on high-performance liquid chromatography (HPLC), potentially misinterpreting BTT as a normal hematogram. Yet, the study by Madan *et al.* demonstrated elevated levels of HbA2 ( = 3.5%) in individuals with BTT, regardless of IDA presence, with no significant differences between the groups. They also reported significantly lower MCV and MCH values in BTT patients compared to normal subjects, consistent with our study[13]. This study's strength lies in identifying readily available and cost-effective blood parameters for effective screening of hemoglobinopathies among antenatal mothers. However, a limitation of the study was the absence of molecular analysis in the participants, which could have provided additional insights.

## CONCLUSION

Red blood cell (RBC) parameters such as hemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) represent a cost-effective and highly efficient screening method for identifying various hemoglobinopathies among antenatal mothers.

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