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Abnormal Hemoglobin Variants – a Single Center Hospital-Based Study: Comparison of Sensitivity of Three Red Cell Indices for the Diagnosis of Beta Thalassemia Trait

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Abstract

Background and Aim: The frequency of the beta thalassaemia gene in India is 3.3 %, with considerable regional variations. There is limited data regarding the prevalence of beta thalassaemia in the state of Kerala. We studied the clinical and haematologic profile of cases with haemoglobinopathy, with special emphasis on beta thalassaemia trait (BTT). The sensitivity of the Mentzer Index (MI), Ricerca Index (RI), and Green & King Index (GKI) in diagnosing BTT was also evaluated.

Methods: Cases with a new diagnosis of haemoglobinopathy at a single center in Kerala between November 2023 and October 2024 were included in this study based on a chart review. High-performance liquid chromatography was used to detect abnormal haemoglobin variants. Clinical presentation, the red cell parameters – haemoglobin, RBC count, mean corpuscular volume, and red cell distribution width – were recorded. MI, RI and GKI in BTT cases were calculated.

Results: There were 1021 new registrations in the Haematology clinic at the time of the study. 72 cases had abnormal haemoglobin variants. Among them, 60 cases (83.33 %) had BTT. Sickle haemoglobin was present in 7 cases (9.7 %). The median age of the BTT cases was 37.5 years (range 3 – 79), and 60 % were female. Fatigue was the predominant symptom, reported in 65 % of cases. Anaemia was present in 86.67 % (52/60) of patients. The median Hb level was 10 g/dL (range 7 – 14.1). Hemolytic anaemia was observed in 15 % of patients. The sensitivity of MI, RI, and GKI in diagnosing BTT was 51.67 %, 86.67 %, and 53.33 %, respectively.

Conclusion: BTT is a significant cause of anaemia in our population. RI exhibited higher sensitivity than the MI and GKI for the diagnosis of BTT.

Keywords: Beta-Thalassemia; Erythrocyte Indices; Hemoglobinopathies; Screening tool; Sensitivity.

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1. Introduction

The global frequency of beta thalassaemia gene is 1.5 %. However, the carrier frequency of the gene in Indian population is 3.3 % (1). Each year, 10,000 infants with beta thalassaemia major are born in India, which constitutes 10 % of the global burden (2). In India, there are regional variations in beta thalassaemia carrier frequency. This includes 0.25 % in the Valsad region of Gujarat, 6.5 % among the Bhutyan tribe of Orissa, 3.07 % among the Munda tribes of Assam, and 13.99 % among the Malayali tribes of Jawadhu hills in Tamil Nadu (3-6). In the state of Kerala, where the present study was conducted, there is a lack of population-based studies on the frequency of beta thalassaemia gene. However, in a study published in 2018, the presence of beta thalassaemia gene is documented (7). We therefore decided to study the clinical and hematological profile of beta thalassaemia trait cases attending the Haematology clinic of our Institute, a tertiary hospital in the district of Kozhikode in north Kerala.

Iron deficiency anaemia (IDA) is a major global public health problem, affecting 4 -5 million people every year (8). In India, the prevalence of nutritional iron deficiency anaemia is also very high, with 85 % of adolescent girls and 88 % of pregnant women reported to be anaemic (9). Both IDA and beta thalassaemia trait may present with microcytic anaemia. Different indices including Mentzer index, Shine-Lal index, RDW-based index, Green & King index, Ricerca index, England Fraser index, and Srivastava index have been used as a simple screening test to differentiate beta thalassaemia trait (BTT) from IDA using complete blood count parameters (10). In a study from Eastern Uttar Pradesh, the Ricerca index and Green & King index performed better than the Mentzer index in differentiating BTT from IDA (11). Hb electrophoresis also helps in differentiating BTT from other anemias, such as anemia of chronic disease and sideroblastic anemia. In this study, we decided to evaluate the sensitivity of the latter three indices in diagnosing BTT.

2. Methods

Study design and setting

We analysed the electronic health records of the patients with BTT attending Haematology clinic of a tertiary healthcare institute in north Kerala between November 2023 and October 2024. The diagnosis of beta thalassaemia was made by haemoglobin variant analysis using cation exchange high-performance liquid chromatography (HPLC) method on the BIO RAD D-10 platform. An HbA2 level > 3.5 % was taken as a cut-off value for the diagnosis BTT. The demographic details, clinical features, haemoglobin (Hb), and red blood cell (RBC) indices were recorded. The red cell parameters – haemoglobin (Hb), RBC count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width (RDW) – were generated using Sysmex XN 1000 -2 automated cell analyser. The Prevalence of haemolytic anemia was noted.

The three RBC indices - MI, RI and GKI - were calculated as follows:

MI was calculated by dividing the value of mean corpuscular volume (MCV) in femtoliters (fL) by the RBC count in millions/microliter.

RI was calculated by dividing the value of red cell distribution width- coefficient of variation (RDW-CV) by the RBC count in millions/microliter.

GKI was calculated by formula: GKI = MCV x MCV x RDW-CV/Hb (g/dL) x 100 with the units for various parameters same as above.

Sensitivity was calculated using the following formula:

$$\text{Sensitivity} = \frac{True\ positive\ cases}{True\ positive + False\ negative}\ x\ 100$$

3. Results

Out of 891 new registrations in the Haematology outpatient department during the study period, there were 72 cases with haemoglobinopathy (Table 1). Of these, 60 (83.33 %) were beta thalassaemia trait (BTT). Among them 36(60 %) were females and the rest were males.

Table 1. Distribution of cases

Type of haemoglobinopathy	Number of patients
Beta thalassaemia minor	60
Thalassaemia intermedia	2
Sickle-beta+ thalassaemia	1
Hb AS	3
Hb SS	1
Hb SD	2
Hb AD	2
HPFH heterozygous	1

Hb- haemoglobin; Hb A – adult haemoglobin; Hb S – sickle haemoglobin; Hb D – D-Punjab haemoglobin; HPFH – hereditary persistence of foetal haemoglobin

The median age was 37.5 years (range 3 – 79), with 13 patients (21.67%) aged ≥ 60 years. Of the 36 females, 7 (19.44%) were pregnant. Twenty patients were newly diagnosed during the study period. Third-degree consanguinity among parents was documented in only one case. Twelve cases (20 %) had family members with beta thalassaemia. Table 2 summarizes the characteristics of patients with BTT.

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Table 2. Characteristics of beta thalassemia trait cases

Sex	
Females	36 (60 %)
Males	24 (40 %)
Age	
≤ 15 years	7 (11.67 %)
16 – 30 years	16 (26.67 %)
31 – 59 years	24 (40 %)
≥ 60 years	13 (21.67 %)
Positive family h/o beta-thalassaemia	12 (20 %)
Symptoms:	
Fatigue	39 (65 %)
Breathlessness on exertion	7 (11.67 %)
Overt haemolysis	9 (15 %)
Lab parameters:	
Median Haemoglobin(g/dL)	10 (range 7 – 14.1)
Median MCV(fL)	64.45 (range 55.1 – 76.1)
Median RBC count(millions/µL)	5.1 (range 3.2 – 7.6)
Median Hb A2 (%)	5.1 (range 3.6 – 6.6)

g/dL= grams/deciliter; fL= femtoliter; µL= microliter

Fatigue was the most predominant symptom, reported by 65 % (39/60) of patients. Seven patients (11.67%) experienced breathlessness on exertion. One patient had coexisting myelodysplastic syndrome/myeloproliferative neoplasm. Another had antiphospholipid antibody syndrome with documented renal cortical ischemia. Two patients were prospective renal transplant donors. One patient was diagnosed during work-up for prospective renal transplantation for chronic kidney disease. Two patients had splenomegaly. Nine patients (15 %) had features of haemolytic anemia. Two patients had coexisting iron deficiency anaemia, as evidenced by low ferritin levels.

The median Hb level was 10 g/dL (range 7– 14.1). 86.67 % (52/60) of patients had anaemia, with 12 % (5/60) having severe anaemia (Hb < 8 g/dL). Microcytosis was present in all cases. The median MCV was 64.45 fL (range 55.1 – 76.1). The median RBC count was 5.1 million/microliter (range 3.2 – 7.6). The median HbA2 level was 5.1 (range 3.6 – 6.6).

Table 3 shows the performance of various RBC indices as screening tools for BTT. The median MI was 12.61 (range 6.48 – 23). An MI less than 13 indicates diagnosis of BTT. 31 out 60 BTT cases had MI < 13. The sensitivity of MI in diagnosing BTT was 51.67 %.

Table 3 . Performance of the three RBC indices as a screening tool for the diagnosis of BTT

RBC index	Number of cases detected	Sensitivity (%)
Green & King Index	32/60	53.33
Mentzer index	31/60	51.67
Ricerca index	52/60	86.67

Median RI was 3.08 (range 2.03 – 6.09). RI less than 4.4 indicates a diagnosis of BTT. 52 out of 60 BTT cases had RI < 4.4. The sensitivity of RI in diagnosing BTT was 86.67 %. Median GKI was 63.85 (range 43.93 – 138.99). GKI less than 65 indicates a diagnosis of BTT. 32 out of 60 BTT cases had GKI less than 65.The sensitivity of GKI in diagnosing BTT was 53.33 %

4. Discussion

In our hospital-based study, BTT accounted for 83.3 % of cases with abnormal haemoglobin. Sickle haemoglobin (HbS) was present only in 11.67 % (7/60) of cases. This contrasts with the condition in Spain, where the prevalence of HbS was reported to be higher than that of BTT. The incidence of HbS in this country is 0.03 per 1000 live births compared to 0.002 thalassaemia cases per 1000 live births (12). In India, beta thalassaemia major patients depend on government hospitals for regular transfusions and iron chelation therapy, as they cannot afford to pay for these services at private institutions. This accounted for the lack of beta thalassaemia major cases in our study. In Kerala, the HbS gene was found mostly in the tribal population, with a gene frequency ranging from 0.019 to 0.196 among various tribes and sects (13). In contrast to HbS, there was a uniform distribution of the beta thalassaemia gene among the

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people of Kerala.

In a multicenter study which included six cities across India, the beta thalassaemia gene was the most prevalent abnormal haemoglobin gene, affecting 1.48 % to 3.64 % of the population in different cities (14). The study also revealed some regional variations in the prevalence of abnormal haemoglobin genes in different parts of India. In Vadodara, the prevalence of HbS trait was higher than that of BTT (2.94 % vs 2.68 %), and in Dibrugarh, Assam, HbE trait was much more prevalent than BTT (23.9 % vs 1.48 %). Similar to our study, population-based research from Eastern India reported that BTT was the most common haemoglobinopathy, present in 4.7 % (15) of the population. In this study, the second most common abnormal haemoglobin was HbE trait, present in 3.02 % of the population. This indicated, the wide prevalence of HbE trait in the eastern parts of India. In our study, we did not find cases with HbE. This is consistent with findings of another study from Kerala, where HbE frequency was low and most positive cases were migrants from eastern parts of India (7). Data from a study in Spain by Bardon-Cancho et al. showed that HbE was extremely rare there (16).

87 % of our BTT cases had anaemia. Iron deficiency was excluded/corrected prior to the diagnosis of BTT by HPLC. Furthermore, 12 % of our cases had Hb levels < 8 g/dL. This indicates that BTT is a major cause for anaemia and associated morbidity, given the high prevalence of the beta thalassaemia gene in the population. The very high prevalence of anaemia in our study may be due to referral bias, as patients with anaemia are more likely to be referred to a tertiary care center. A study from Delhi showed a 71.5 % prevalence of anaemia among iron replete patients with BTT (17). When there was concomitant iron deficiency among the BTT patients, the prevalence of anaemia increased to 90.5 %. In contrast, a study from Islamabad, Pakistan showed a lower (though still high) prevalence of anaemia at 51.5 % among BTT cases (18).

We also examined the efficacy of red cell indices—MI, RI and GKI— as screening tools for diagnosing BTT. In a large-scale public health screening program, performing HPLC for abnormal Hb variants may not be feasible for all cases due to cost constraints. Hence, efficient screening tools are required to select cases with high probability of abnormal haemoglobin variants, so that samples from these cases alone were subjected to HPLC studies. MI is the red cell index widely used to differentiate between IDA and BTT. In the current study, RI exhibited better sensitivity compared to MI and GKI. Our findings are consistent with the results of Bhargava et al(11). Similarly, Niazi et al. also reported that RI index had superior sensitivity to diagnose BTT compared to MI and GKI (10). Mustafa et al, also examined the sensitivity of various indices in patients from Islamabad, Pakistan (19). They found the Shine and Lal index to have 100 % sensitivity. MI, RI, Srivastava index, and Ehsani index also performed well while detecting BTT cases, with sensitivity of 92.56 %, 96.6 %, 91.7 % and 92.58 %, respectively.

Our study has certain limitations. Since the study population was drawn from a tertiary care center, the prevalence of haemoglobinopathy may not reflect that in the general population. To better assess the efficacy of the red cell indices, a larger sample size was desirable. Furthermore, we could not evaluate alpha thalassemia, as molecular studies are needed to accurately diagnosis it, which were not implemented due to resource constraints. However, the present study provides valuable insights into the prevalence of abnormal haemoglobin variants among the people of Kerala.

5. Conclusion

The Beta thalassaemia gene is prevalent among the people of north Kerala. Beta thalassaemia trait is a significant cause for anaemia and associated morbidity in our population. Physicians need to be aware of this, as both BTT and IDA present with microcytic anemia, and individuals with BTT may be inadvertently treated with iron supplements if BTT is not diagnosed promptly. The Ricerca index is a more sensitive screening tool for the diagnosis of BTT compared to the Mentzer index in our population. Finally, we need comprehensive population-based studies to identify the exact prevalence of abnormal haemoglobin variants in our region. This will guide policymakers make informed decisions to better implement the National Thalassaemia Prevention Program.

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Ethical Considerations and Compliance with Ethical Guidelines

The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. The Institutional Ethics Committee gave exemption from ethics approval due to retrospective and non-interventional nature of the study.

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Conflict of interest

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AI Using Declaration

The authors declare no artificial intelligent chatbot use.

Author's contributions

RSP & RSN conceptualized and designed the study, RSN acquired the data, RSP & RSN analysed and interpreted the data, RSP drafted the manuscript. Both authors reviewed it critically for important intellectual content and gave the final approval. Both authors agree to be accountable for all aspects of the work.

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