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Original Research Article

Impact of Iron Deficiency on HBA2 Levels and its Implications in Thalassemia Trait Detection

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Abstract:

Background: Thalassemia trait screening often relies on the quantification of Hemoglobin A2 (HbA2) levels, which are typically elevated in beta-thalassemia carriers. However, iron deficiency, a highly prevalent condition in India, especially among females, has been postulated to reduce HbA2 levels, potentially leading to false-negative screening results. Understanding the impact of iron deficiency on HbA2 values is critical to ensuring accurate detection of thalassemia carriers, especially in population-level screening programs.

Objectives: To evaluate the effect of iron deficiency on HbA2 levels and determine whether iron deficiency can mask the diagnosis of beta-thalassemia trait in individuals undergoing screening.

Methods: This observational cross-sectional study was conducted in the Department of Pathology, Darbhanga Medical College and Hospital, Bihar, from August 2022 to July 2023. A total of 130 individuals aged 18–40 years undergoing anemia evaluation or thalassemia screening were included. Participants were grouped into iron-deficient and non-iron-deficient groups based on serum ferritin, transferrin saturation, and complete blood count findings. HbA2 quantification was done using high-performance liquid chromatography (HPLC). Statistical analysis was performed to assess the correlation between iron status and HbA2 levels.

Results: Out of 130 participants, 72 were iron-deficient while 58 had normal iron stores. The mean HbA2 level in the iron-deficient group was 2.3 ± 0.4%, significantly lower than the non-iron-deficient group which had a mean HbA2 level of 3.1 ± 0.5% ($p < 0.001$). Among iron-deficient individuals with suspected thalassemia trait, HbA2 levels were found to be within normal or borderline ranges, indicating the possibility of masked diagnosis. Upon iron repletion and re-evaluation in a subset, HbA2 levels rose significantly, unmasking latent beta-thalassemia trait in some cases.

Conclusion: Iron deficiency significantly lowers HbA2 levels and may obscure the diagnosis of beta-thalassemia trait in screening programs. Screening for thalassemia should either follow correction of iron deficiency or incorporate parallel iron status assessment to avoid misclassification. This is particularly relevant in high-prevalence regions like Bihar where iron deficiency anemia is common.

Keywords: HbA2, iron deficiency, beta-thalassemia trait, thalassemia screening, serum ferritin, HPLC, anemia, false-negative, hematology, Bihar

Introduction

Beta-thalassemia is one of the most common inherited hemoglobinopathies worldwide, with a particularly high carrier frequency in certain parts of India, including Bihar. Early and accurate detection of beta-thalassemia trait is essential for preventing the birth of children with thalassemia major through timely genetic counseling and partner testing [1]. One of the most widely accepted hematological markers for screening beta-thalassemia carriers is the level of Hemoglobin A2 (HbA2), which is typically elevated (>3.5%) in affected individuals. High-performance liquid chromatography (HPLC) has emerged as a standard and reliable method for quantifying HbA2 levels in population-based screening programs [2].

However, a critical challenge to the accuracy of thalassemia screening lies in the potential confounding effect of iron deficiency anemia (IDA), which is also highly prevalent in India, particularly among women of reproductive age [3]. Iron deficiency has been suggested to suppress HbA2 synthesis, thereby lowering HbA2 levels and potentially causing a false-negative result in beta-thalassemia trait detection. This phenomenon may lead to missed diagnoses during screening,

especially in resource-limited settings where both iron deficiency and hemoglobinopathies coexist [4].

Despite multiple studies suggesting a relationship between iron status and HbA2 levels, the extent to which iron deficiency can mask beta-thalassemia trait remains a topic of ongoing investigation and clinical relevance [5]. In regions like Bihar, where thalassemia carrier screening is increasingly being promoted as part of public health strategies, the need for clarity on this interaction is particularly urgent. Misdiagnosis or underdiagnosis of carriers can have serious implications for genetic counseling and prenatal diagnostic services [6].

This study aims to evaluate the HbA2 levels in individuals with and without iron deficiency and to investigate whether iron deficiency significantly impacts the accuracy of beta-thalassemia screening. By doing so, it seeks to provide evidence-based recommendations on whether screening should be deferred until iron stores are replenished or whether parallel iron status evaluation should be integrated into standard protocols.

Methods

This observational cross-sectional study was carried out in the Department of Pathology, Darbhanga Medical College and Hospital, Darbhanga, Bihar, over a period of one year from August 2022 to July 2023. The study included 130 individuals aged between 18 and 40 years who were referred for evaluation of anemia or for routine thalassemia screening. Written informed consent was obtained from all participants prior to inclusion in the study. Individuals with recent blood transfusion (within the past three months), known hemoglobinopathies, chronic illnesses, or ongoing iron supplementation were excluded to avoid confounding factors.

Each participant underwent a detailed clinical evaluation along with laboratory investigations, including complete blood count (CBC), peripheral smear examination, serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation. Iron deficiency was diagnosed based on serum ferritin levels less than 15 ng/mL and/or transferrin saturation below 16%. HbA2 quantification was performed using high-performance liquid chromatography (HPLC), which is a standardized and sensitive method for hemoglobin fraction analysis.

Participants were categorized into two groups based on their iron status: iron-deficient and non-iron-deficient. The mean HbA2 levels of both groups were compared to assess the influence of iron deficiency on HbA2 values. In a subset of iron-deficient individuals who were initially found to have borderline or low-normal HbA2 levels, oral iron supplementation was given for 8–12 weeks. These individuals were reassessed after iron repletion to observe any change in HbA2 levels. Statistical analysis was conducted using SPSS software. Continuous variables were expressed as mean ± standard deviation and compared using the independent t-test. A p-value of <0.05 was considered statistically significant.

Results

In this study of 130 participants, 72 individuals were diagnosed with iron deficiency, while 58 had normal iron stores. The mean HbA2 level was found to be significantly lower in the iron-deficient group as compared to those with adequate iron levels. A subset of 20 iron-deficient individuals was reassessed after iron therapy, and a marked rise in HbA2 levels was noted, unmasking beta-thalassemia trait in some cases. The results support the hypothesis that iron deficiency can indeed mask thalassemia screening outcomes.

Table 1: Gender Distribution of Study Participants

Gender	Iron Deficient (n=72)	Non-Iron Deficient (n=58)	Total (n=130)
Male	28 (38.9%)	26 (44.8%)	54 (41.5%)
Female	44 (61.1%)	32 (55.2%)	76 (58.5%)

Table 2: Age Group Distribution

Age Group (years)	Iron Deficient	Non-Iron Deficient	Total
18–20	10	7	17
21–30	40	33	73
31–40	22	18	40

Table 3: Comparison of Mean Hemoglobin Levels

Group	Mean Hb (g/dL) ± SD
Iron Deficient	9.2 ± 1.1
Non-Iron Deficient	12.1 ± 1.3
p-value	<0.001

Table 4: Comparison of Mean Serum Ferritin

Group	Mean Ferritin (ng/mL) ± SD
Iron Deficient	9.5 ± 2.8
Non-Iron Deficient	42.3 ± 5.6
p-value	<0.001

Table 5: Comparison of Mean HbA2 Levels

Group	Mean HbA2 (%) ± SD
Iron Deficient	2.3 ± 0.4
Non-Iron Deficient	3.1 ± 0.5
p-value	<0.001

Table 6: Distribution of HbA2 Levels

HbA2 Level (%)	Iron Deficient	Non-Iron Deficient
<2.5	34	5
2.5–3.4 (Normal)	32	28
≥3.5 (Elevated)	6	25

Table 7: Comparison of Red Cell Indices

Parameter	Iron Deficient (Mean ± SD)	Non-Iron Deficient (Mean ± SD)	p-value
MCV (fL)	68.4 ± 4.5	76.2 ± 5.0	<0.001
MCH (pg)	22.1 ± 1.3	26.4 ± 1.7	<0.001

Table 8: HbA2 Levels Before and After Iron Supplementation (n=20)

Time Point	Mean HbA2 (%) ± SD
Before Treatment	2.4 ± 0.3
After Treatment	3.5 ± 0.4
p-value	<0.001

Table 9: Diagnosis of Beta-Thalassemia Trait After Iron Correction

Parameter	Number of Cases
Initially Suspicious (HbA2 < 3.5%)	20
Confirmed After Re-evaluation	6
Not Confirmed	14

Table 10: Risk of False-Negative Thalassemia Trait Diagnosis

Group	At Risk of False-Negative (%)
Iron Deficient (n=72)	47.2%
Non-Iron Deficient	8.6%

Discussion

The findings of this study provide compelling evidence that iron deficiency significantly alters HbA2 levels, thereby posing a risk of false-negative results in beta-thalassemia trait screening. Our results are consistent with several previous studies that have suggested a suppressive effect of iron deficiency on HbA2 synthesis. In this cohort of 130 individuals, we observed that those with iron deficiency had substantially lower mean HbA2 levels (2.3 ± 0.4%) compared to individuals with normal iron status (3.1 ± 0.5%), with a statistically significant p-value of <0.001. This difference has crucial clinical implications, particularly in areas like Bihar where both iron deficiency and hemoglobinopathies are prevalent [7,8].

The pathophysiological basis for this phenomenon lies in the altered globin chain synthesis in iron-deficient erythropoiesis. Iron is essential for the production of hemoglobin, and its deficiency leads to a general suppression of hemoglobin synthesis, including the delta chain that contributes to HbA2 formation [9]. Thus, even individuals with beta-thalassemia trait may exhibit deceptively normal or low HbA2 levels if they are concurrently iron deficient. In our study, nearly half of the iron-deficient participants were at risk of being misclassified during initial screening. This highlights a significant limitation in current thalassemia screening protocols that do not routinely account for iron status [10].

Furthermore, the subset analysis in which iron-deficient participants were given iron therapy revealed a marked increase in HbA2 levels post-treatment, from 2.4 ± 0.3% to 3.5 ± 0.4%, confirming the reversible nature of this masking effect [11,12]. Importantly, six individuals who were initially suspected but not confirmed as beta-thalassemia carriers were accurately diagnosed after iron repletion. This reinforces the importance of including iron studies as part of any thalassemia

screening program, especially in iron-deficient populations [13,14].

Our findings also emphasize the necessity for clinicians to interpret HbA2 levels with caution in the presence of microcytic anemia. The overlap of clinical and hematological features between iron deficiency anemia and beta-thalassemia trait further complicates diagnosis. Red cell indices such as MCV and MCH were also found to be significantly lower in the iron-deficient group, consistent with previous literature, but they are not sufficient alone to distinguish between these two conditions.

The study does have certain limitations, including its cross-sectional design and relatively modest sample size. However, the significant results and the observed post-treatment responses lend strong support to our conclusions. Future research with larger, multi-centric populations and follow-up designs could further validate these findings and contribute to policy changes in screening guidelines.

In summary, iron deficiency has a clinically significant impact on HbA2 levels and may obscure the diagnosis of beta-thalassemia trait [16]. In regions like Bihar where both conditions are prevalent, thalassemia screening should be integrated with iron status assessment, or deferred until iron deficiency is corrected [17,18]. This strategy can enhance the diagnostic yield and prevent missed opportunities for early intervention and genetic counselling [19].

Conclusion

This study clearly demonstrates that iron deficiency significantly lowers HbA2 levels, potentially leading to false-negative results during beta-thalassemia trait screening. In a region like Bihar, where both iron deficiency anemia and thalassemia are commonly encountered, this interaction poses a serious public health concern. Our findings underscore the need for clinicians and laboratories to interpret HbA2 values in the context of iron status. Correcting iron deficiency before screening or concurrently evaluating iron parameters can greatly enhance the diagnostic accuracy for thalassemia trait. Incorporating this approach into standard screening protocols, especially in community and antenatal programs, can reduce the risk of missed diagnoses and improve preventive strategies for thalassemia. Ultimately, integrating iron assessment into thalassemia screening practices is a simple yet critical step toward ensuring reliable carrier detection and effective genetic counseling.

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