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Original Research Article

A study of RBC morphology in patients with β-thalassemia

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ABSTRACT

Introduction: Red blood cells (RBCs) with β-thalassemia may show certain distinctive morphological alterations. Red blood cells structure and function are affected, which leads to aberrant hemoglobin formation and ineffective erythropoiesis. **Aims and objectives:** The purpose of this study was to determine how frequently patients with β-thalassemia minor had red blood cell abnormalities. **Material and Method:** RBC abnormalities in patients with β-thalassemia minor were systematically investigated in this study using strict criteria and proper diagnostic techniques. The 66 diagnosed patients that made up the study population were carefully evaluated using hemoglobin electrophoresis and High-Performance Liquid Chromatography. The frequency and extent of RBC abnormalities were presented along with a thorough analysis of the data. **Result:** The study's findings shed important light on the respondents' laboratory characteristics and gender distribution, with an emphasis on the complex erythrocyte morphological abnormalities seen in people with β-thalassemia minor. The findings are made easier to understand by the tables that are provided, which can be used for additional research and analysis in clinical and academic contexts. **Conclusion:** A study show that erythrocyte morphological abnormalities are frequently seen in peripheral blood smears (PB smears) from patients with β-thalassemia minor. As a result, all of the slides under analysis showed anisocytosis, poikilocytosis, and target cells. Additionally, most of the slides

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showed ovalocytes, elliptocytes, cells with basophilic stippling, dacryocytes, stomatocytes, and irregularly contracted cells. This finding might help with the differential diagnosis of anemia in routine clinical laboratory work.

Key words: Red blood cells, Morphology, β-thalassemia, Hemoglobin

INTRODUCTION

Since red blood cells (RBCs) carry oxygen to bodily tissues, they are the most prevalent blood cells in the body. When determining a patient's pathological state, counting these essential cells is frequently the first step.(1), (2) Discocytes are anucleated red blood cells that have a central pallor and a biconcave disc shape. (3) Blood RBCs with abnormal shapes are referred to as poikilocytosis. (4) A differential diagnosis of diseases in humans and animals can be established by evaluating changes in red blood cell morphology, which offers valuable information. (5), (6), (7), (8) Alterations in erythrocyte morphology can be brought on by illnesses as well as other physiological variables like aging. (9), (10)

β- Thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β-globin chain synthesis. It was the first time defined by Cooley and Lee in 1925. (11) Every year, about 68,000 newborns are diagnosed with different forms of thalassemia. (12) Approximately 80 to 90 million individuals worldwide (or 1.5% of the total population) are carriers of β-thalassemia, indicating its high prevalence. There are two clinically significant forms of beta-thalassemia (β-thalassemia): β- Thalassemia major β-Thalassemia Minor. (13)

AIMS AND OBJECTIVE

The purpose of this study was to determine how frequently patients with β-thalassemia minor had red blood cell abnormalities.

MATERIAL AND METHODS

This study used well-defined criteria, cutting-edge diagnostic methods, and ethical considerations to take a methodical approach to examining the incidence of RBC abnormalities in patients with β-thalassemia minor. RBC abnormalities in the study population are assessed using a methodology that is valid and reliable.

Study Population:

The study population comprises patients diagnosed with β-thalassemia minor. Patients were diagnosed using High-Performance Liquid Chromatography (HPLC) and hemoglobin electrophoresis. Inclusion criteria involve the presence of microcytosis or microcytic anemia

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and hemoglobin A2 values above 3.5%, while exclusion criteria include β-thalassemia major, β-thalassemia intermedia, hemoglobinopathies, and cases of β-thalassemia minor without a stored blood smear slide.

Sample Size:

A total of 66 patients with β-thalassemia minor were included in the study, and their PB smears were analyzed.

Data Collection:

Data regarding demographic and laboratory characteristics of the study population were obtained from medical records. Patients meeting the inclusion criteria were selected for the study.

Anonymization and Coding:

Blood smear slides were anonymized and coded by a designated individual (C.R.) to ensure unbiased examination. The coded slides were examined in a blinded fashion by a specially trained analyst (C.K.) using light microscopy.

Assessment of RBC Abnormalities:

RBC abnormalities were assessed based on the presence or absence (positive or negative) and quantified in case of positivity as cells per 20 high power fields (HPF) at 1000-fold magnification (oil immersion). Standard hematologic literature (14), (5), (15) was used to define the following abnormalities: anisocytosis, poikilocytosis, basophilic stippling, target cells, irregularly contracted cells, dacryocytes, schistocytes, elliptocytes, ovalocytes, pincer cells, stomatocytes, bite cells/degmacytes, erythroblasts, blister cells/prekeratocytes, and Howell-Jolly bodies.

Data Presentation:

The data were presented as n (%) for positive samples and median (range) for the description of abnormal cell counts, providing a comprehensive overview of the frequency and extent of RBC abnormalities in the study population.

RESULT

This section on results provides a thorough summary of the study's findings. It starts with an examination of the participants' gender distribution. The laboratory characteristics of the participants are then explained, providing insight into important parameters like hemoglobin levels, erythrocyte count, and different indices of red blood cell morphology. Furthermore, Table 3 discusses the thorough examination of erythrocyte morphological anomalies seen in

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people with β-thalassemia minor. The talk that follows will give a detailed grasp of the hematological complexities related to β-thalassemia minor and will be helpful in guiding clinical interpretation and future research projects.

Table 1: Gender of respondents

Gender of the respondents		
	Frequency	Percentage
Male	36	54.54%
Female	30	45.45%
Total	66	100%

The above table discusses the Gender of the respondents.

Table 2: Laboratory characteristics of the respondents

Laboratory characteristics	
Erythrocytes $\times 10^6/\mu L$	6.081
Hemoglobin in g/dL	12.53
MCV, fL	65.5
MCH, pg	12.53
MCHC, g/dL	31.5
Reticulocyte count, $\times 10^3/\mu L$	93.45
RDW, %	15.68
HbA_2, %	5.39
HbF, %	1.59

The above table discusses the Laboratory characteristics of the respondents.

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Table 3: Abnormalities of erythrocyte morphology in Respondents with β-thalassemia minor

Morphological abnormalities	Prevalence, n (%)	Cells/20 HPF, median (range)
Anisocytosis	66 (100)	n.a.
Poikilocytosis	66 (100)	n.a.
Target cells	66 (100)	42.02 (4–279)
Ovalocytes	64 (96.9)	13.04 (0–85)
Dacryocytes	54 (81.8)	6.05 (0–23)
Stomatocytes	54 (81.8)	10.01 (0–55)
Elliptocytes	50 (75.8)	7.02 (0–46)
Basophilic stippling	48 (72.7)	7.04 (0–101)
Irregularly contracted cells	42 (63.6)	6.01 (0–102)
Schistocytes	10 (15.2)	1 (0–2)
Bite cells/degmacytes	4 (6.06)	2.52 (0–4)
Pincer cells	2 (3.03)	1 (0–1)
Prekeratocytes/blister cells	0	0
Erythroblasts	0	0
Howell-Jolly bodies	0	0

The above table discusses the abnormalities of erythrocyte morphology in Respondents with β-thalassemia minor.

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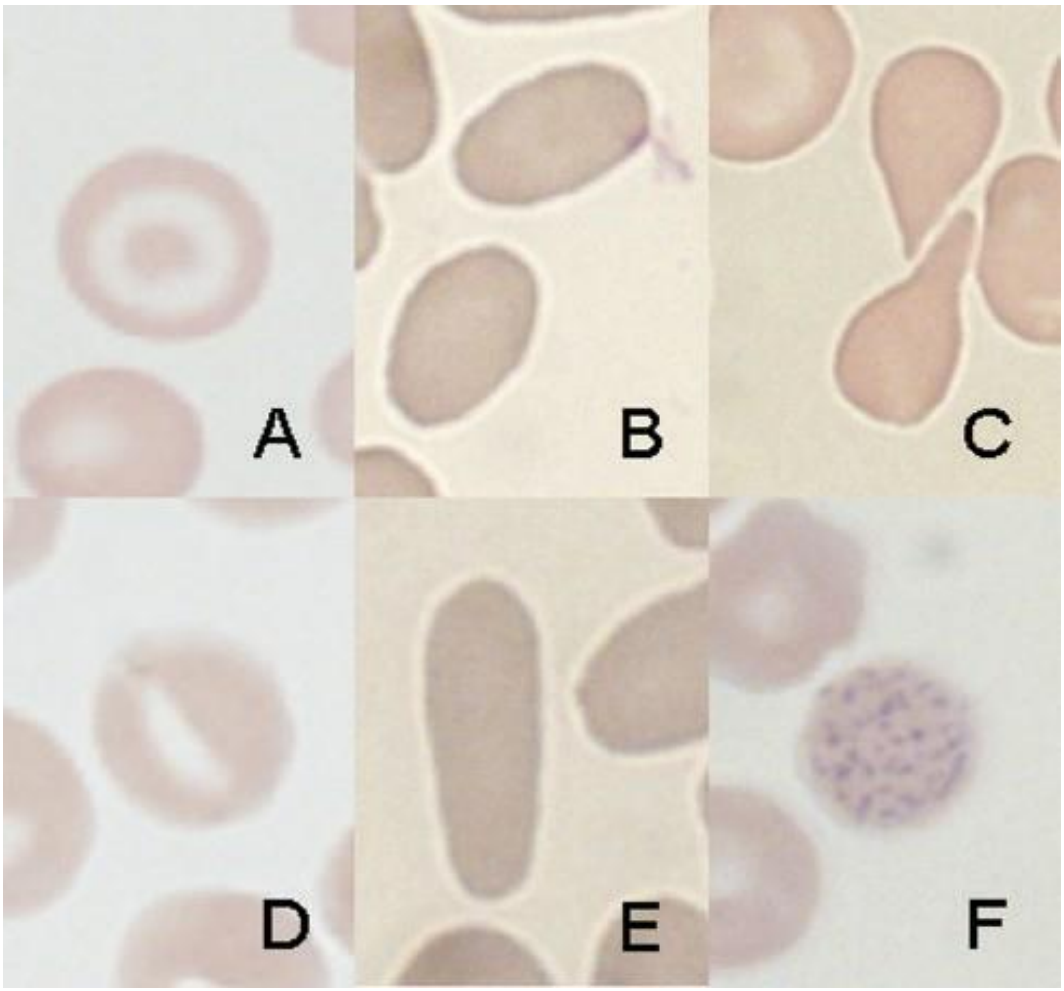
Fig 1: The most frequent red blood cell abnormalities in patients with β-thalassemia minor



A, target cell; B, ovalocyte; C, dacryocyte; D, stomatocyte; E, elliptocyte; F, basophilic stippling

DISCUSSION

One common and useful method for diagnosing a variety of RBC disorders is the analysis of PB smears. Previous research on β-thalassemia minor was primarily limited to a small number of quantifiable abnormal morphologic features. (16), (17), (18)

As far as we are aware, no one has yet attempted a quantitative morphologic analysis of a wider range of poikilocyte subtypes. Thus, the purpose of the current study was to quantify the abnormal cells in the event of positivity and to ascertain the prevalence of 15 defined morphological RBC abnormalities in the PB smears of 66 subjects with β-thalassemia minor.

All of the patients in our study population had morphological abnormalities such as target cells, anisocytosis, and poikilocytosis, in addition to numerical abnormalities. With the exception of

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the three categories of anomalies, different frequencies and quantities of all other recognized pathologic erythrocyte forms were noted.

Target cells (n = 66, 100%) and cells with basophilic stippling (n = 48, 72.7%) are common in our study and match patterns found in other studies, indicating that these morphological characteristics are consistently present in β-thalassemia patients. Our results are consistent with a large-scale study carried out in a large Spanish population (n=825) with β-thalassemia trait, where most study participants had changes in red blood cell (RBC) morphology. Remarkably, a startling 96% of cases in this cohort had basophilic stippling (17). The robustness of basophilic stippling as a notable morphological characteristic in the context of β-thalassemia is reinforced by its high prevalence in the Spanish population.

Nonetheless, it is imperative to recognize the presence of contradictory findings in the literature, as demonstrated by an earlier investigation concentrating on thirty instances of β-thalassemia minor. In this specific study, basophilic stippling was found to be much less common—only 17% (5/30) of cases had this morphological characteristic (16). The disparity observed between these results and our research, along with the investigation conducted by Calero et al. (17), underscores the possible fluctuations in basophilic stippling expression across distinct β-thalassemia populations. These variations may be caused by elements like genetic diversity or geographic influences, highlighting the significance of taking into account population-specific subtleties when interpreting morphological findings.

A study by Calero et al. (17) found that target cells were present in 93% (28/30) of subjects with β-thalassemia minor, which is in close agreement with our own findings regarding the prevalence of target cells. Despite possible differences in the prevalence of other morphological features, our study's agreement with Calero et al.'s research further supports the validity of target cells as a consistent morphological marker in β-thalassemia.

In a different investigation into the role that red blood cell (RBC) morphology plays in the diagnosis of β-thalassemia trait, scientists defined a new method by designating the coexistence of both target cells or microcytosis and basophilic stippling as "experimental" morphologic criteria. They also categorized poikilocytosis and microcytosis as "control" criteria at the same time. This novel classification sought to develop a more precise set of criteria for the diagnosis of β-thalassemia trait by utilizing the correlation between particular morphological characteristics.

Using this framework, 59 patients with β-thalassemia trait, 60 individuals with microcytosis from non-β-thalassemia-related causes, and 64 nonmicrocytic subjects were all thoroughly

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evaluated in the study. Crucially, the evaluations were carried out in a blinded manner to guarantee an objective examination of RBC morphology.

The results showed a reasonable ability to correctly identify individuals with β-thalassemia trait using the experimental morphologic criteria, with a moderate sensitivity of 73% for both sets of criteria. Nevertheless, the true power of these standards was revealed by their remarkable 99% specificity. With a low chance of false positives, this high specificity highlights the validity of the experimental criteria in distinguishing the β-thalassemia trait from other illnesses.

Additionally, the experimental criteria's predictive value reached a remarkable 98%, highlighting how accurately it could predict the presence of the β-thalassemia trait. This high predictive value indicates that there is a high probability that an individual genuinely possesses the β-thalassemia trait when basophilic stippling and either microcytosis or target cells are seen. These results highlight the potential clinical utility of combining particular characteristics of RBC morphology as β-thalassemia trait diagnostic criteria. The experimental criteria exhibit strong specificity and predictive value, indicating that this method may prove to be a useful tool in clinical practice, supporting medical practitioners in rendering dependable and precise diagnoses. In the end, better patient care and management may result from increased β-thalassemia trait diagnosis accuracy and efficiency, which could be improved with more investigation into such improved diagnostic criteria. (18)

Anisocytosis and poikilocytosis, two common morphological features found on all of the slides we looked at, have been repeatedly reported as being present in people with β-thalassemia (5), (14), (19). These variations in red blood cell (RBC) size and shape are useful markers in the diagnostic landscape of carriers of β-thalassemia, adding to our understanding of the full range of morphological changes linked to this hereditary disorder.

Anisocytosis and poikilocytosis are well-known characteristics; however, there has been little research on the occurrence of other abnormalities beyond these traditional traits, with inconsistent findings when compared to our study. In 11 out of 30 subjects (37%) in a previous investigation, prekeratocytes—a unique RBC morphology—were reported (16). Interestingly, our study's patients did not show any prekeratocytes, highlighting the variation in how particular morphological abnormalities manifest among various cohorts of β-thalassemia carriers.

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Comparably, our research showed that 75.8% of participants had pencil cells, or elliptically shaped red blood cells. This is significantly higher than the results of the previously mentioned study, which showed that only 30% of participants had this morphology (16). Elliptocytes are generally not thought of as a typical feature of β-thalassemia minor, although they can occasionally appear in very rare cases (14). Even within the spectrum of β-thalassemia minor, there could be variability in RBC morphology, and these questions are intriguingly raised by the higher incidence observed in our study population.

Unlike the widely held belief that irregularly contracted cells are rare in β-thalassemia minor patients (14), our study found a higher frequency of these cells, with a prevalence of 63.6%. This disparity underlines the complexity and diversity of the morphological spectrum associated with carriers of β-thalassemia and raises the possibility that some abnormalities are more common in particular populations or subgroups.

CONCLUSION

The study shows that peripheral blood smears from patients with β-thalassemia minor frequently show erythrocyte morphological abnormalities. Findings of the study, in particular, highlight how target cells, poikilocytosis, and anisocytosis are consistently present in all examined samples. On most of the slides that were analyzed, there were also other distinguishing characteristics like elliptocytes, dacryocytes, stomatocytes, irregularly contracted cells, basophilic stippling, ovalocytes, and stomatocytes. When these various morphological abnormalities are recognized, important information is revealed that can greatly improve the accuracy of the differential diagnosis of anemia in standard clinical laboratory settings. Healthcare professionals who possess knowledge of the particular erythrocyte abnormalities linked to β-thalassemia minor are better able to differentiate this condition from other types of anemia quickly and accurately. Moreover, the incorporation of these discoveries into conventional laboratory protocols may enhance the diagnostic proficiency of medical practitioners, resulting in enhanced patient monitoring.

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