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HEMATOLOGICAL AND CLINICAL FEATURES OF HEMOGLOBIN H DISEASE

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Abstract:

Purpose: *To examine the clinical and hematological features of hemoglobin H disease.*

Study design: *A descriptive cross-sectional study.*

Place and duration: *In the Medicine Unit II and hematology department of Nishter Hospital Multan for one year duration from March 2019 to March 2020.*

Methods: *We evaluated the clinical features and hematological values of patients for hemoglobin studies using non probability consecutive sampling. Complete blood count was performed on Sysmex KX 21 automated hematology analyzer. Hemoglobin H inclusions were visualized with supra vital staining with New methylene blue. Hemoglobin electrophoresis of the sample was carried out on cellulose acetate membrane in Tris EDTA borate buffer at 8.9pH.*

Results: *Compared with 557 patients diagnosed with high / indirect thalassemia, 10 patients were diagnosed with hemoglobin H during the study period. Of the ten patients, 4 (40%) were male and 6 (60%) were female. Patient's ages ranged from 7 to 32 years old. The clinical picture of patients included anemia 100% and jaundice 10%. 90% of patients had a transfusion history. During the study, pallor was 100% present, the liver was palpable in 20% and the spleen in 80% of cases. The average hematological parameters were TRBC 4.5 (± 1.6), Hb 8.8 (± 2.2), MCV 71.9 (± 11.8), MCH 20.4 (± 2.9), and MCHC 25.7 (± 8.7). The mean reticulocyte count was 15.8% (± 9.4). Hemoglobin H inclusions were observed in 100% of cases.*

Conclusion: *Hemoglobin H disease is an uncommon disorder and out of 557 Thalassemia major/Intermedia patients diagnosed during this period 10(1.7%) patients were diagnosed as having Hemoglobin H disease.*

Keywords: *Hemoglobin H disease, α thalassemia, unstable hemoglobin.*

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INTRODUCTION:

α- thalassemia is the most common hereditary disorder found in Southeast Asia and China. Hemoglobin synthesis disorder caused by deletion or mutations (or both) of α-globin genes in the short arm of chromosome 16². The normal genome has four α-globin genes (two per chromosome), and the clinical presentation of genetic abnormalities varies from a silent carrier state to a fetal Hydrops with a single deletion of the α-globin gene; All four a-globin genes are deleted[1-2]. Hemoglobin H disease is a moderate thalassemia in which three of the four a-globin genes are affected, and in most cases it is characterized by the presence of an abnormal hemoglobin, hemoglobin H[3-4]. Results from compound heterozygousity for a-thalassemia α-thalassemia (- - / - a genotype). However, it may be the result of interactions between a-thalassemia and indelible mutations (usually a point mutation) containing the a-globin gene (αTa or a-). The hemoglobin molecule is a tetramer consisting mainly of two a-globin genes and two globin genes that make up the "hemoglobin A" component of adult hemoglobin[5-6]. Balanced production of these globin chains is important for normal hemoglobin formation and integrity of erythrocytes. Hemoglobin H disease is characterized by an excess of the excess-globin chain in erythroblasts and erythrocytes, and decreased synthesis of a-globin chains[7-8]. This leads to abnormal hemoglobin formation with β-globin chain tetramers known as hemoglobin H. These tetramers are unstable and precipitate erythroblasts / erythrocytes that cause lysis of these cells. Hemoglobin H patients may occur clinically with symptoms of anemia and hepato-splenomegaly, or, by chance, hypochromic microcytic anemia[9]. Most patients are classified as "Intermediate Thalassemia" with an initial hemoglobin level compensated hemolytic anemia of 8 to 10 g / dl. These patients have mild or no hepato-splenomegaly and do not require regular blood transfusions. A-thalassemia is a common genetic disorder in Pakistan. However, full data on hemoglobin H disease is not available for our population[10]. In this study, we presented the clinical and hematological features of hemoglobin H disease in Pakistani patients.

MATERIALS AND METHODS:

This descriptive cross-sectional study was conducted in the Medicine Unit II and hematology department of Nishter Hospital Multan for one year duration from March 2019 to March 2020. The study was carried out over a period of one year. All individuals who were part of the study were selected by sequential probability sampling. The patients were evaluated in terms of clinical features. The following factors were identified: age at diagnosis, symptoms at presentation, and history of transfusion. Physical examination was performed to determine whether there was pallor and jaundice in each patient, and the size of the spleen and liver was determined. Five ml of venous blood was withdrawn from the ante-cubital vein using an aseptic technique and collected in EDTA. In the Sysmex KX 21 Automated Hematology Analyzer, complete blood count was performed within 1-3 hours after blood collection. The hematological parameters evaluated were TRBC, Hb, MCV, MCH and MCHC. Hemoglobin H inclusions were microscopically visualized by staining red blood cells vitally with new methylene blue for 1-2 hours at 37 ° C and making blood films. Hemolysate was prepared from washed red blood cells and for example hemoglobin electrophoresis was performed on cellulose acetate membrane in Tris EDTA borate buffer at pH 8.9. Visual evaluation of the hemoglobin H bands of the patients was made by comparison with the normal control.

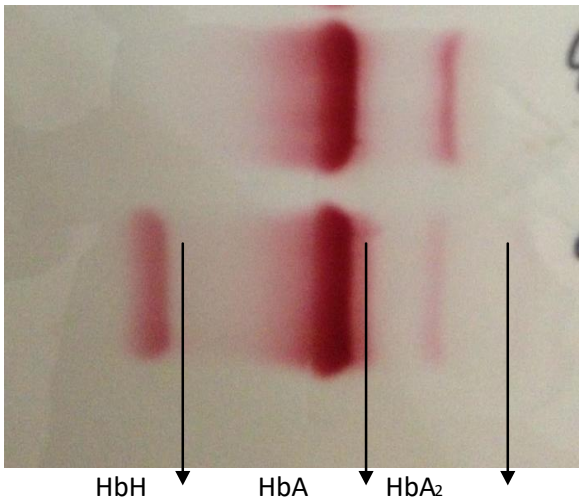
RESULTS:

During this period, a total of 557 patients were diagnosed with beta thalassemia major / mid-level. Of these 557 patients, 10 patients were diagnosed with hemoglobin H disease, 4 (40%) male and 6 (60%) female. The ages of the patients vary between 7 and 32, and the median age is 17 ± 08 years. The clinical presentation of the patients was 100% anemia and jaundice was 10%. The history of transfusion was present in 90% of patients, but was not due to transfusion. On examination, pallor in 100%, liver in 20% can be palpated and 80% of patients had palpable spleen. The average hematological parameters obtained by the analyzer are presented in Table 1.

Table 1: Red blood cell parameters

RBC parameters	Mean values	St. deviation
TRBC (x 10^{12}/L)	4.5	± 1.6
Hb (g/dl)	8.8	± 2.2
MCV (fl)	71.9	± 11.8
MCH (pg)	20.4	± 2.9
MCHC (g/dl)	25.7	± 8.7

The mean reticulocyte count was 15.8% (± 9.4). Hemoglobin H inclusions were observed in 100% of cases by supra vital staining with new methylene blue (Figure 1).



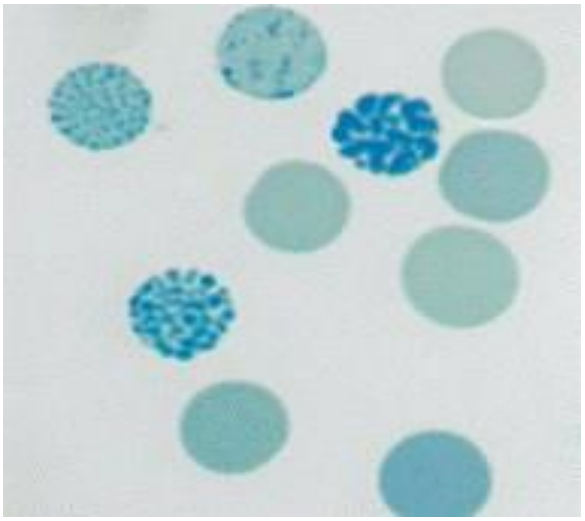


Fig. 1: Haemoglobin H inclusions **Fig. 2: Haemoglobin Electrophoresis on Cellulose acetate membrane at pH 7.9 shows fast moving band of Hb H.**

The rapidly moving hemoglobin H band was observed on the cellulose acetate membrane in all patients (Figure 2).

DISCUSSION:

α -thalassemia is one of the most common genetic disorders that affect 5% of the world's population. While the prevalence of α + -thalassemia in Pakistan is 15-20%, the prevalence of a-thalassemia has not been documented[11-12]. High prevalence of α + thalassemia trait in Pakistan is the most likely cause of low frequency of hemoglobin H disease in this region. Hemoglobin H disease is of intermediate clinical severity as a result of three deletions and / or mutations of the α-globin gene and is clinically presented as 'thalassemia intermedia[13]. This disease has a significant phenotypic variability, some patients have recurrent blood transfusions due to anemia, and some patients have variable hepato-splenomegaly. These patients also develop iron overload as they age or due to repeated blood transfusions. Eight of the 10 patients in our study had splenomegaly and 02 had hepatomegaly. Nine of them were transfected once or twice, but none were due to transfusion. Laboratory diagnosis of hemoglobin H disease begins with a complete blood image showing hypochromic microcytic anemia in the width of red blood cells due to aniscoikilocytosis seen in the peripheral film10. Red blood cell inclusion bodies are observed in most red blood cells after staining with supravital dyes such as methylene blue or bright cresyl blue. Hemoglobin H is detected as a fast-moving band by the electrophoresis of a freshly prepared hemolysate at alkaline pH and typically represents 3 to 30% of total hemoglobin[14]. The diagnosis can be confirmed by DNA analysis by polymerase chain reaction. In our study, hypochromic microcytic anemia of 10 patients; with average Hb 8.8 g / dl, average MCV 71.9 fl and MCH 20.4pg average. In 100% of the cases, hemoglobin H inclusions were observed by supra vital staining with new methylene blue, and a rapidly moving hemoglobin H band was visualized by electrophoresis in all patients. The phenotypic variability of hemoglobin H disease depends on the patient's mutation status, because non-selective hemoglobin H disease is generally known to be more severe than the delusional form of the disease. These mutations can be diagnosed in Pakistan by limited molecular methods[15].

CONCLUSION:

Hemoglobin H disease is an uncommon disorder. Against the 557 Beta Thalassemia major/Intermedia patients, only 10(1.7%) patients were diagnosed as having Hemoglobin H disease. These patients present as chronic anemia, occasional jaundice and splenic enlargement. Diagnosis is confirmed by demonstrating Hb-H inclusions and Hemoglobin electrophoresis.

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