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Pathology

Spherocytosis: Acquired Causes and Morphological Distinctions: An Observational Study Done in a Tertiary Care Setting

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Abstract **Original Research Article**

This study is conducted in Pathology department of Bombay Hospital in duration of one year (Feb 2018 - 19). Fifty patients that presented to Bombay Hospital pathology department with features of hemolysis were chosen for this study. Hereditary spherocytosis (HS) is a genetic aberration in the red blood cells which predisposes the person affected with this disorder to chronic hemolysis. Development of accurate, sensitive, and specific diagnostic laboratory tests for hereditary spherocytosis.

Keywords: Spherocytosis & Morphological.
Study Design: Observational Study.

INTRODUCTION

Spherocytosis is a common morphological finding seen in peripheral smears of patients in tertiary care settings. Spherocytosis are RBC's with smaller surface area and increased volume. In patients of hemolytic anaemias; hereditary as well as acquired, spherocytes can be seen as secondary finding and at times masquerade the primary diagnosis. This study demonstrates cases of hemolytic anaemias wherein spherocytes were seen n discusses differential diagnosis based on other morphological findings.

MATERIALS AND METHODS

This study is conducted in Pathology department of Bombay Hospital in duration of one year (Feb 2018 - 19). Fifty patients that presented to Bombay Hospital pathology department with features of hemolysis were chosen for this study. There peripheral smears were stained by Leishman and Field stain. Peripheral smear were examined for each patient in the light of clinical findings. A reticulocyte stain was done to assess marrow activity. Furthermore based on the findings of PS they were subjected to other tests like Hb electrophoresis, G6PD test and DCT. Relevant Biochemical analysis was done to further support the findings.

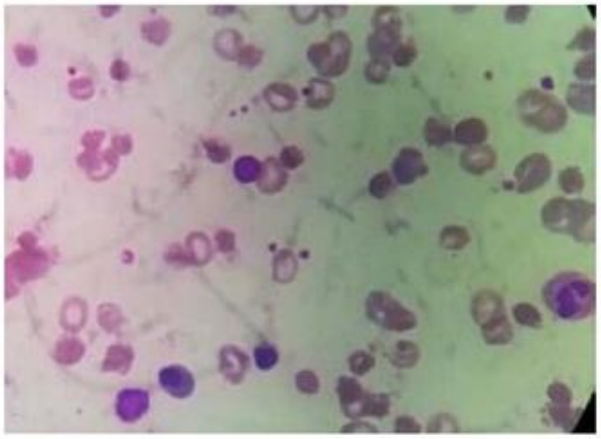

Fig-01: Microphotograph showing sickle cell in AIHA crisis with several spherocytes and nRBC

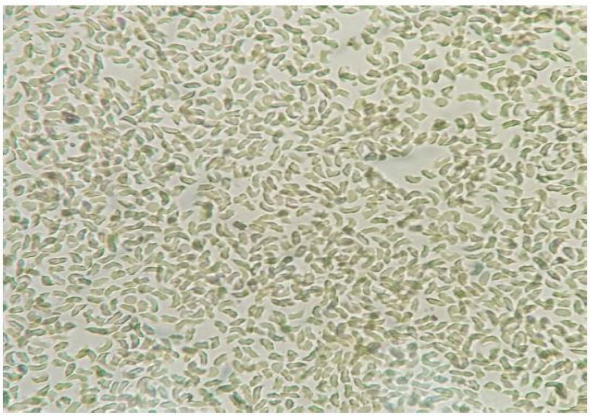

Fig-02: Microphotograph showing positive sickling test

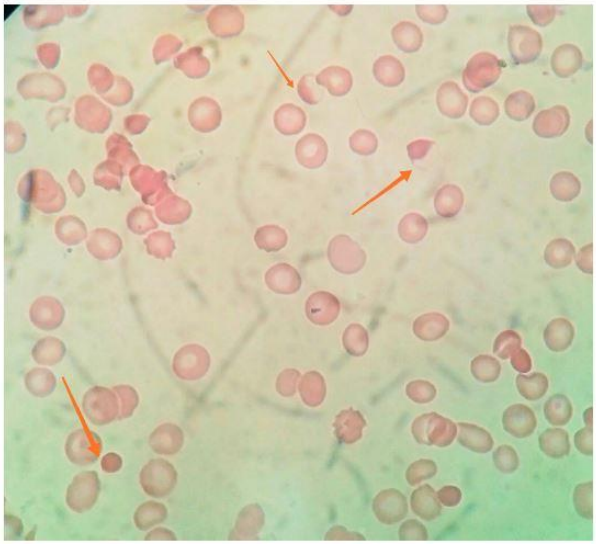

Fig-03 Microphotograph showing spherocytes and blister cell in a patient of G6PD deficiency

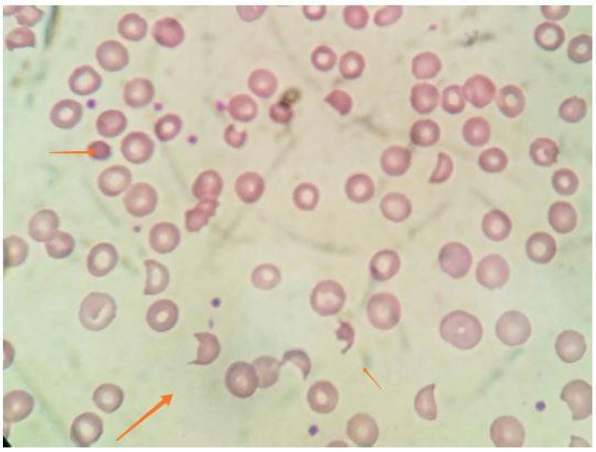

Fig-04: Microphotograph depicting features of intravascular hemolysis in HUS, helmet cells And spherocyte (arrow mark)

REVIEW OF LITERATURE

Normal red blood cells or erythrocytes are biconcave shaped and can pass through small blood vessels. In HS the erythrocytes become deformed (actually, spherical) hence the term spherocytes. Spherocytes lack flexibility, are fragile and tend to fragment when forced to pass through a small vessel, releasing hemoglobin, an essential component of iron. The process is called hemolysis and the result, hemolytic anemia and/ or jaundice. Reticulocytosis is seen in response to hemolysis. l. HS

is also manifested by the passage of "coke-colored" urine and formation of gallstones [2].

In all patients with suspected MAHA, a complete blood count (CBC) should be performed, which will reveal anemia, thrombocytopenia, and a markedly increased red cell distribution width (RDW). With these findings, a peripheral blood smear examination should be performed, which will reveal the presence of schistocytes [3].

Additional laboratory tests should include bilirubin, haptoglobin, fibrinogen, lactate dehydrogenase, prothrombin time (PT), serum electrolytes, creatinine, blood urea nitrogen (BUN), urinalysis, and stool examination. Fibrinogen, D-dimer, and increased PT are seen in states of increased coagulation, such as DIC, while kidney function parameters may be deranged as well. Urinalysis may include hemoglobinuria, while stool examination should include cultures for Enterobacteriaceae, especially in those that present with features of HUS. LDH is an indicator of cellular breakdown, and its values may rise together with liver transaminases. In all patients, direct Coombs' test should be performed to exclude autoimmune causes of anemia, and MAHA is distinguished from other forms of hemolytic anemia with a negative Coombs' test. Additionally, antiphospholipid antibodies should be measured in all patients, since a significant correlation between MAHA and these antibody subsets have been established [4]

Autoimmune hemolytic anemia (AIHA) is a rare disorder in which the body' s immune system destroys its own red blood cells by releasing proteins that are meant to protect it from "outsiders" or so-called "non-self", such as infectious disease agents. The proteins are antibodies or immunogloblins G and M produced by the bone marrow and thymus gland, and complement, from the liver. Autoantibody and complement-coated red cells rupture or lyze and are eliminated by phagocytic while blood cells (phagocytes) in the liver and spleen. Red blood cells (erythrocytes) are killed prematurely, thus, are unable to attain their normal life span of 120 days, resulting in anemia (loss of hemoglobin) and symptoms like pallor and general debility [5].

The disorder affects women more than men. The exact cause of AIHA is not known, hence, it is called primary idiopathic AIHA. Secondary AIHA is usually associated with some underlying condition (infection, other autoimmune disease, malignancy, drugs) that need to be diagnosed and treated together with AIHA[6].

Glucose-6-phosphate dehydrogenase deficiency, the most common enzyme deficiency worldwide, causes a spectrum of disease including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis [7] Persons with this condition also may be asymptomatic. This X-linked inherited disorder most commonly affects persons of African, Asian, Mediterranean, or Middle-Eastern descent. Approximately 400 million people are affected worldwide. Homozygotes and heterozygotes can be symptomatic, although the disease typically is more severe in persons who are homozygous for the deficiency. The conversion of nicotinamide adenine dinucleotide phosphate to its reduced form in erythrocytes is the basis of diagnostic testing for the deficiency [8].

OBSERVATIONS AND RESULTS

Of the fifty patients sampled, there were patients of Sickle cell anaemia(10), G6PD deficiency(5) Hemolytic Uraemic Syndrome(5), AIHA(10) transfusion associated hemolysis (7), and hereditary Spherocytosis (2). There were 11 patients that presented with spherocytosis with no supportive clinical or serological evidence of hemolysis.

In patients of Sickle cell disease hemoglobin was low, Rbc indices revealed low to normal values and variable number of n RBC. Peripheral smear showed sickle cells, spherocytes, fragmented cells, polychromatophils and nRBC. In 4 of 10 cases only spherocytes and polychromatophils were seen and no sickle cells were observed. Hb electrophoresis alongwith sickling test confirmed the diagnosis. DCT was positive in 8 of 10 cases (Fig 1,2,3).

In patients of G6PD deficiency Coulter showed a low Hb and normal RBC indices, peripheral smear revealed normocytic normochromic blood picture with presence of spherocytes and blister cells. Urine was cola coloured and positive for benzidine test. Methemoglobin reduction test revealed G6PD deficiency. Clinical history was suggestive of intravascular hemolysis (fig 4,5).

Patients of HUS/TTP presented with deteriorating Hemoglobin and platelet count in known renal patients. On peripheral smear examination microcytic hypochromic anaemia was seen alongwith large number of shistocytes, helmet cells, bites cells and spherocytes. Thrombocytopenia was noted. Biochemical analysis revealed a raised Serum LDH and Creatinine (Fig 6,7). AIHA patients and patients with transfusion associated hemolysis revealed a low Hb, a normocytic normochromic anaemia alongwith spherocytic blood picture. DCT was positive in all patients (Fig 8).

In patients of hereditary spherocytosis uniform population of Spherocytosis were seen. RBC indices revealed a normal MCV, and a high MCHC (.> 34). Splenomegaly was noted in one patient while another patient was a neonate with rapidly decreasing hemoglobin levels and a positive family history (Fig 9).

In patients with transfusion associated spherocytosis; spherocytosis were seen admixed with normocytic normochromic transfused cells. History of transfusion with a positive Coombs test could clinche the diagnosis (Fig 10). In a subset of patients no obvious cause of spherocytosis was seen. All biochemical parameters and supportive evidence were lacking.

DISCUSSION

Spherocytosis is a common finding seen in peripheral smear of hemolytic anaemia patients. Apart from being secondary accompaniment it also points towards active hemolysis going on in the patient with chronic disorder. This study emphasise on observing not only spherocytes but also the accompanying features that could point towards the primary diagnosis, whether acquired or hereditary.

Although primary investigation to be offered in the clinically suspected patients of hemolysis is peripheral smear examination, it should always be supported by biochemical panel. Biochemical parameters that contributed to our study are Se LDH, Se Creatinine, Se Bilirubin and fractions and direct Coombs test.

Whenever required patients were subjected to relevant investigation like Hb electrophoresis, MRT and OFT for confirmation. Patients of hereditary Spherocytosis have a typical morphological finding of uniform population of spherocytes alongwith splenomegaly and a positive family history. MCHC is highly indicative with value more than 34 suggestive of hereditary Spherocytosis In aquired spherocytosis the distribution is not uniform.

Patients with G6PD deficiency typically have blister cells on their smear with spherocytes. They might present with cola coloured urine as was seen in one of our patients. MRT test was p. Based on our study a flow chart was made for work up of patient with spherocytes on their peripheral smear Although G6PD on peripheral smear shows blister cells in our two cases it classically presented with spherocytic blood picture. In a study by Karen A. Brown it is mentioned that G6 PD can present with spherocytes on smears.

CONCLUSION

Hereditary spherocytosis (HS) is a genetic aberration in the red blood cells which predisposes the person affected with this disorder to chronic hemolysis. Development of accurate, sensitive, and specific diagnostic laboratory tests for hereditary spherocytosis.

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