Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Research Paper

An Practical Approach Towards Evaluation of Acquired Hemolytic Anemia

Medical Science

KEYWORDS : Acquired hemolytic anemia, immune hemolysis, microangiopathic hemolytic anemia (MHA), spherocytes.

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ABSTRACT

Acquired hemolytic anemia represents a group of disorders in which premature destruction of red cells is triggered by extrinsic factors. In our study, we have evaluated 76 cases of acquired hemolytic anemia where immune hemolysis accounted for 52.6% (n=40) and fragmentation hemolysis in 44.7% (n=34). Autoimmune hemolytic anemia (AIHA) and microangiopathic hemolytic anemia (MHA) were the two most frequent causes accounting for 44.7% (n=34) and 39.5% (n=30) of acquired hemolysis. Peripheral smear examination revealed spherocytes in 94% (n=32) cases of immune hemolytic anemia and schistocytes in all cases of microangiopathic hemolytic anemia (MHA). Also microspherocytes were seen in 40% of MHA. Complete blood counts and morphology of blood smear can gives valuable information regarding the cause of acquired hemolysis.

INTRODUCTION

Acquired hemolytic anemia can be classified as immune hemolytic anemia and non-immune hemolytic anemia (1). It represents a group of disorders in which premature destruction of the red cells is triggered by extrinsic factors unlike congenital hemolytic anemia which results from intrinsic red cell defects. Immune hemolytic anemia (IHA) is a condition where IgG and/ or IgM antibodies bind to RBC surface and initiate RBC destruction via the reticuloendothelial system and complement system. IHA can be classified into autoimmune, alloimmune and drug induced hemolysis.

Non-immune hemolytic anemias result from various causes like mechanical trauma, oxidative damage, infections, drugs, toxins, PNH and others. Mechanical trauma to the RBC's is caused by excessive shearing forces on red cells or it can also result from fibrin strands on the endothelium of small vessels as seen in microangiopathic hemolytic anemia (MHA) (2, 3).

Acquired hemolytic anemia requires an extensive diagnostic work up and careful evaluation of clinical history, biochemical, serological and hematological workup (4,5). In this context, complete haemogram and erythrocyte morphology can yield valuable information and precise identification of spherocytes, schistocytes, microspherocytes, and irregularly contracted cells plays an important role in determining the cause of hemolysis(6,7) .

MATERIALS AND METHODS

This prospective study was conducted in a tertiary care hospital for a period of two years between 2012- 2014 and a total of 80 cases were included after obtaining consent. Four patients lost the clinical follow up. All patients were subjected to following investigations- Complete blood counts (CBC), reticulocyte count, blood smear, lactate dehydrogenase (LDH), liver function tests, renal function tests, serum haptoglobin and Coomb's test (direct and indirect) . Complete clinical details with history of disorders associated with immune hemolytic anemia (IHA), microangiopathic hemolytic anemia (MAH) and drug history were recorded.

Inclusion criteria: Patients having clinical and laboratory evidence of hemolysis.

Exclusion criteria: Patients with G6PD deficiency, Heriditary spherocytosis, Hemoglobinopathies and other congenital causes

Gel cards with polyspecific antihuman globulin and complement were used to screen the patients and the diagnosis of immune hemolytic anemia was based on a positive Direct antiglobulin test (DAT). Presence of schistocytes with/ without microspherocytes on peripheral blood smears was taken as evidence of RBC fragmentation. MAH was diagnosed when fragmentation hemolysis was accompanied by thrombocytopenia ($<1.5x\ 10^9/L$). Fluorescent antinuclear antibody test was used as a screening test for SLE. Prothrombin time, Activated partial thromboplastin time and D-dimer assays were also done to rule out the possibility of Disseminated Intravascular Coagulation (DIC).

RESULTS

Total of 76 cases of acquired hemolytic anemias, Immune hemolysis accounted for 52.6% (n=40) and fragmentation hemolysis 44.7% (n=34) (table-1). The two most frequent causes of acquired hemolytic anemia were AIHA (n=34) and MHA (n=30). Oxidative hemolysis (n=2) was uncommon cause of acquired hemolysis (table 2).

Of the 40 cases of IHA, 6 cases were due to alloimmunization (4 cases of hemolytic disease in newborns and 2 cases of delayed transfusion reaction). The remaining 34 cases were grouped as autoimmune hemolytic anemia (AIHA). Of the 34 cases diagnosed as AIHA, 58.8% (n=20) had serological of underlying disorders and were classified as secondary AIHA. This group included SLE (n=10), Rheumatoid arthritis (n=2), pulmonary tuberculosis (n=2), HIV (n=2), Mycoplasma infection (n=2), Small lymphocytic lymphoma (n=2). The remaining 14 cases (41.2%) which did not have any underlying causes were regarded as primary AIHA (table-1).

Fragmentation hemolysis was noticed in 34 cases, four of these cases was identified as has having severe aortic stenosis and prosthetic heart valve and were classified among cardiac hemolysis. They had normal platelet counts. Rest 30 cases showed varying degrees of thrombocytopenia in addition to fragmentation and were regarded as microangiopathic hemolytic anemia (MHA). Platelet count in these cases ranged from 0.05- 1.2 $x10^9/L$ and LDH level was raised in 28 cases (94%).

A thorough clinical and laboratory workup was performed in all the 30 cases of MAH to identify the underlying cause. Preeclampsia accounted for 20% (n=6) of MHA, SLE (n=2), DIC (n=4), HIV infection (n=2), vacuities (n=2) and disseminated malignancy noted in four cases (n=4). In remaining 10 cases, a diagnosis of thrombocytopenic purpura (TTP) was made in 6 cases and Hemolytic uremic syndrome (HUS) in 4 cases.

Erythrocyte morphology was carefully evaluated in all cases on peripheral blood smears. Spherocytes were noted in 90% (n=36) cases of IHA (fig 2). Red cell agglutinates in 2 cases of cold agglutinin AIHA, which was secondary to mycoplasma infection. Two cases of chronic AIHA without spherocytes were encountered and these patients had thrombocytopenia and secondary folic acid deficiency. Their platelet counts improved after correction of nutritional deficiency. Thrombocytopenia was attributed to folic acid deficiency. Thrombocytopenia

was also seen in 4 cases of AIHA secondary to SLE and these were thought to represent Evan's syndrome (7). Schistocytes were seen in 30 cases of MHA, being numerous in (80%) 24 cases (fig 1). 12 cases of MHA (40%) displayed microspherocytes along with schistocytes. Spherocytes were also seen in 4 cases (13.3%) of MHA, but were accompanied by schistocytes. Blister cells and irregularly contracted cells were seen in the cases of oxidative hemolysis.

DISCUSSION

Many studies in India have reported the frequency of secondary AIHA as 77% and 29% (8,9). Baek et al. noted that majority of patients with primary AIHA were found to have SLE during a follow up of two years (10). The present study demonstrated that AIHA and MHA were the most frequent causes of acquired hemolytic anemia and 58.8% of AIHA were due to underlying diseases where SLE being the most frequent.

We noted fragmentation hemolysis represented the most frequent form of non-immune acquired hemolytic anemia in which MHA accounted for 88% (n= 30) of fragmentation hemolysis, Cardiac hemolysis was rare, observed in only 4 cases, and caused by damaged heart valves and prosthetic valves have often been reported (11, 12).

Microangiopathic hemolytic anemia is a Coombs-negative intravascular hemolytic anemia, with schistocytes (13, 14). The term thrombotic microangiopathy (TMA) has been used to describe multiple disease entities characterized by MHA, thrombocytopenia, and organ injury that result from microthrombi in capillaries and arterioles (14, 15) and includes thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). The primary differentiating factor between HUS and TTP is the presence of significant renal impairment in HUS. A TMA syndrome can also be associated with pregnancy, disseminated cancer and chemotherapy, human immunodeficiency (HIV) infection, malignant hypertension, autoimmune disorders, Disseminated intravascular coagulation (DIC), vasculitis and following hematopoietic stem cell transplantation (6, 14, 15). In the setting of MHA, a thorough clinical and laboratory workup is warranted to arrive at a definite diagnosis. We observed, MHA resulted from a variety of causes which included Preeclampsia, TTP and HUS. It is important to distinguish preeclampsia from TTP/HUS and HELLP since the treatment modalities are different in each case (16). We also encountered 2 cases of HIV infection. The association of HIV and TTP has been reported (6, 17).

Since IHA and MHA surfaced as the most frequent causes of acquired hemolysis, their hematological and biochemical features were compared (table 2), both showed female predilection. The mean platelet count in MAH was 0.46 /L, which was much lower than 2.3/L in IHA. Spherocytes were observed in 88% of immune hemolysis and only 13% of non-immune hemolytic process. Spherocytes most often indicate either hereditary spherocytosis or autoimmune haemolytic anemia and has been established that spherocytes in the blood smear of IHA are erythrocytes that are coated with IgG and/ or complement that have been damaged by phagocytic cells (18). Presence of spherocytes in the blood smear warrants a DAT (5).

In our study, schistocytes were noted in 86.7% of MHA and only 11.7% of immune hemolysis. Schistocytes are circulating fragments of erythrocytes from which fragments have been lost and result from mechanical fragmentation of erythrocytes caused by fibrin strands on the endothelial surface and/or excess of turbulence of blood. They are usually absent in blood films of healthy individuals and should be identified by specific morphological criteria. They are always smaller than intact red cells and can have the shape of fragments with sharp angles and straight borders, small crescents, helmet cells, keratocytes (19).

Schistocytes, if accompanied by thrombocytopenia suggest TMA and in such cases, HUS/ TTP should always be considered because of the urgent need for institution plasma exchange therapy (13). However, schistocytes are not pathognomonic of TMA as they are also seen in other conditions like structural abnormalities of the heart and great vessels or a malfunctioning prosthetic valve (11, 12). Schistocytes can occur in a backdrop of severe anisopoikilocytosis and do not essentially reflect erythrocyte fragmentation. However, isolated schistocytes is a strong indicator of MHA (19, 20). The presence of an high LDH in the presence of microangiopathic hemolysis and thrombocytopenia should prompt a diagnosis of TMA (6).

Microspherocytes are small spherocytes, which have a smooth outline and lack central pallor and they represent erythrocyte fragments which have re-spherized in circulation indicating that red cell fragmentation has occurred. In the context of MHA, microspherocytes are invariably accompanied by schistocytes (4). In the present study, microspherocytes were observed in 40% of MAH and all were accompanied by spherocytes.

Microspherocytes should be distinguished from irregularly contracted cells which also appear hyperchromatic in nature but have an irregular outline. They result from oxidative damage and are usually accompanied by blister cells and bite cells (4).

CONCLUSIONS

AIHA and MHA are the most common forms of acquired hemolytic anemia. A simple morphological peripheral blood smear evaluation offers extremely valuable information that no advanced equipment can offer. Precise identification of spherocytes, microspherocytes and schistocytes is very important in discerning the cause of hemolysis.

Tables and figures-

ACQUIRED HEMOLYTIC ANEMIA (n=76)	
Cause of Acquired Hemolysis	n
1. Immune hemolysis (52.6%)	40
i. Autoimmune	34
• Primary (41.2%)	14
• Secondary (58.8%)	20
ii. Alloimmune	06
• Hemolytic disease of newborn	04
• Transfusion reaction	02
2. Fragmentation hemolysis (44.7%)	34
• Microangiopathic hemolysis	30
• Cardiac hemolysis	04
3. Oxidative hemolysis (2.6 %)	02
• Drug induced	02

Table 1 - showing the various types of acquired hemolytic anemia encountered in our study

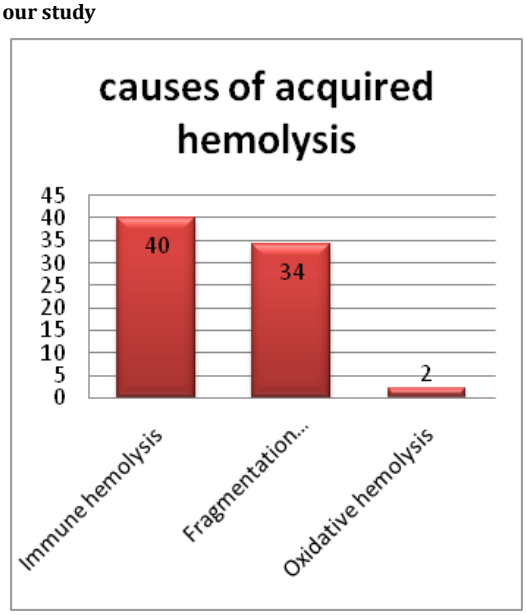

Table 2- Graphical representation showing immune hemolytic anemia as a major cause of acquired hemolytic anemia

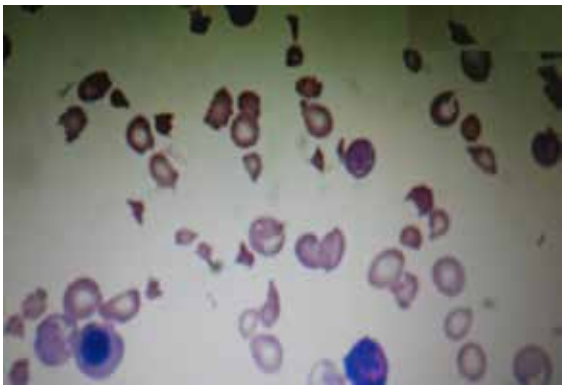

Figure 1 - peripheral blood smear from a patient diagnosed as TTP showing numerous schistocytes.

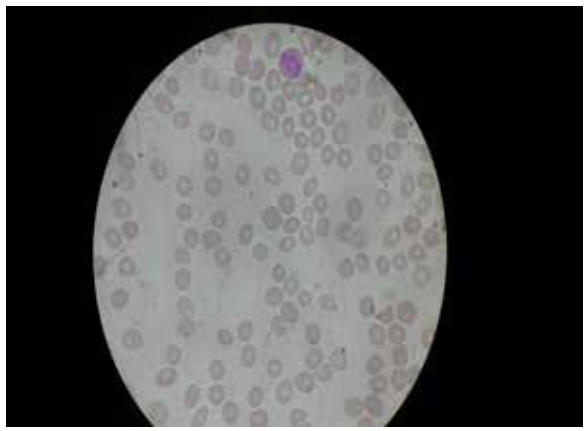

Figure 2 – Blood smear from a case of AIHA showing numerous spherocytes.

REFERENCE

Kelton JG, Chan HH, Heddle NM, Whittaker S. Acquired hemolytic anemia. In: Wickramasinghe SN and McCullough J (eds). In: Blood and Bone Marrow Pathology, Edinburgh, Scotland: Churchill Livingston, Elsevier Science Limited, 2003:185-202. | | 2 Han-Mou Tsai. Thrombotic thrombocytopenic purpura, Hemolytic Uremic syndrome and related disorders (1314-1324) in Wintrobes Clinical Hematology-Volume 2. 12 th edition, Published in 2009 at Philadelphia USA, by Lippincotts Williams and Wilkins. | 3 Zeerleder S. Autoimmune haemolytic anaemia | – a practical guide to cope with a diagnostic and therapeutic challenge. The Netherlands Journal of Medicine. 2011;69(4):177-184 | | 4 Barbara J. Bain. Spherocytes and irregularly contracted cells. Basic Haematology. Haematology Watch, Vol 3, Issue 1. | 5 Bain BJ. Morphology in the diagnosis of red cell disorders. Hematology 2005; 10(1): 178 /181 | 6 Thompson CE, Lloyd E, Damon LE, Ries CA, Linker CA. | Thrombotic Microangiopathies in the 1980s: Clinical Features, Response to Treatment, and the Impact of the Human Immunodeficiency Virus Epidemic. Blood 1992; l80 (8):1890-1895 | | | 7 Michel M, Chanet V, Dechartres A et al. The spectrum of Evans syndrome in adults: new insight into the disease based on the analysis of 68 cases. Blood. 2009; 114 (15): 3167-3172 | | 8 Alwar V, Devi AM Shanthala, S Sitalakshmi, Karuna R K. Clinical Patterns and Hematological Spectrum in Autoimmune Hemolytic Anemia. J Lab Physicians. 2010 ; 2(1): 17–20. | | 9 Choudhry VP, Passi GR, Pati HP. Clinico-hematological spectrum of auto-immune hemolytic anemia: an Indian experience. J Assoc Physicians India. 1996 ;44(2):112-4. | | 10 SW Baek, MW lee, HW Ryu et al. Clinical fearures and outcomes of autoimmune hemolytic anemia: a retrospective analysis of 32 cases. Korean J of Hematology. 2011;46(2):111- 117 | | 11 Tsuji A, Tanabe M, Onishi K, Kitamura T, Okinaka T, Ito M, Isaka N, Nakano T. Intravascular hemolysis in aortic stenosis. Intern Med. 2004;43(10):935-8. | 12 Shapira Y, Vaturi M, Sagie A. Hemolysis associated with prosthetic heart valves: a review. Cardiol Rev. 2009;17(3):121-4. | | 13 Tefferi A, Elliott MA. Schistocytes on the Peripheral Blood Smear. Mayo Clin Proc. 2004;79(6):809. | | 14 Halevy D, Radhakrishnan J, Markowitz et al. Thrombotic microangiopathies. Crit Care Clin. 2002;18:309-320. | | 15 Coppo P, Veyradier A. Thrombotic Microangiopathies: Towards a Pathophysiology-Based Classification. Cardiovascular & Haematological Disorders-Drug Targets, 2009;9:36-50 | | | 16 Stella CL, Dacus J, Guzman E, et al. The diagnostic dilemma of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome in the | obstetric triage and emergency department: lessons from 4 tertiary hospitals. Am J Obstet Gynecol 2009;200:381.e1-381.e6. | 17 Ahmed S, R K Siddiqui, A K Siddiqui, S A Zaidi, J Cervia. HIV associated thrombotic microangiopathy. Postgrad Med J 2002;78:520–525 | | | 18 Sir John Dacie. The immune hemolytic anemias- a century of exciting progress in understanding. British Journal of Haematology, 2001, 114, 770-785 | | | 19 Zini G, D'Onofrio G, Briggs C, Erber W et al. ICSH recommendations for identification, diagnostic value, | and quantitation of schistocytes. International Journal of Laboratory Hematology 2012; 34(2):107-116 | 20 J.-f Lesesve, Salignac S, Lecompte T. Diagnosis of mechanical hemolytic anemias: contribution of complete blood count. Annals of clinical biology. 2001;59(5): 551-8 |