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Original Research Paper

Pathology

WARM ANTIBODY AUTOIMMUNE HEMOLYTIC ANEMIA: A CLINICO-PATHOLOGICAL STUDY OF 10 CASES IN TERTIARY TEACHING INSTITUTES OF BIHAR

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ABSTRACT

Introduction: Autoimmune hemolytic anemia (AIHA) is a rare and heterogeneous disease that affects 1 to 3/100000 patients per year. AIHA is caused by warm Autoantibodies (w-AIHA), i.e., antibodies that react with their antigens on the red blood cell optimally at 37°C, is the most common type, comprising approximately 70% to 80% of all adult cases and approximately 50% of pediatric cases. About half of the w-AIHA cases are called primary because no specific etiology can be found, whereas the rest are secondary to other recognizable underlying disorders.

Materials And Methods: A retrospective study of 10 cases of AIHA done in tertiary teaching medical colleges of Bihar between periods August 2019 to August2020. Detailed clinical features, epidemiological data and laboratory parameters were noted .Peripheral smears and bone marrow studies were also done all patients. Further clinical and special investigations were noted in secondary AIHA in multiple myeloma and chronic lymphocytic leukemia.

Result: Total number of 10 patients was included in this study. It had female preponderance (M: F ratio: 1:2.3), three patients were DAT negative that responded with steroid. All patients had indirect hyperbilirubinemia, raised LDH and most had raised reticulocyte count .PBS revealed normocytic to mildly macrocytic and normochromic blood picture. Bone marrow aspiration revealed normoblastic erythroid hyperplasia. In this series, patients presented with moderate to severe anemia.

Conclusion: w-AIHA is rare disease worldwide, but common in India. It could be fatal in emergency hemolysis crisis, so early diagnosis is essential. Approximately 5% w-AIHA may be DAT- negative however flowcytometric detection of RBC bount IgG antibodies is possible.

KEYWORDS : AIHA, Warm antibodies. DAT, Hemolytic anemia, Polychromasia

INTRODUCTION:

Normal red blood cells (RBCs) have an average life span of 115 days (1). Hemolysis is defined as decreased RBC survival and can be caused because of an inherent abnormality of the cell (intrinsic or intracorpuscular defect), by extrinsic factors, or by a combination of both. When hemolysis occurs at a rate that cannot be compensated by increased RBC production, then the patient presents with hemolytic anemia. The premature RBC destruction can happen intravascularly or extravascularly in the reticuloendothelial system (mainly adjacent to the macrophages of spleen and liver) and can be episodic/acute or chronic. Clinical presentation includes pallor, fatigue, jaundice, dark urine, splenomegaly, and, in chronic cases, gallstones and cholecystitis. Common laboratory findings are anemia, reticulocytosis, elevated unconjugated bilirubin and lactate dehydrogenase and decreased haptoglobin.

Autoimmune hemolytic anemia (AIHA) is caused by increased RBC destruction triggered by autoantibodies reacting against RBC antigens with or without complement activation(1,2).The autoantibodies and/or complement fragments are detected on the RBC surface using the direct antiglobulin test (DAT). DAT, or direct Coombs test, is typically performed in 2 steps. First, the polyspecific reagent containing both anti-immunoglobulin G (IgG) and anticomplement is used to agglutinate antibody-coated cells, and then the monospecific reagents anti-IgG and anti-C3d (anti-C3b, anti-C4b, and anti-C4d reagents also available) are used individually to detect IgG and complement, respectively. Binding of anti-C3d alone often indicates bound IgM(4,5). AIHA is classified into 3 major types based on the optimal temperature in which the autoantibodies bind on the patient's RBCs in vivo: warm antibody AIHA (w-AIHA), cold agglutinin syndrome (CAS), and paroxysmal cold hemoglobinuria (PCH). In some unusual cases, considered as "mixed AIHA," the laboratory data satisfy the serologic criteria of both w-AIHA and CAS(6). The 2 clinical entities of AIHA that are due to cold-reacting autoantibodies are defined by the immunoglobulin isotype against the RBCs: IgM in CAS and IgG in PCH. IgM autoantibodies, typically directed against the I/i system of RBC antigens, are maximally reactive in the cold (4°C), although they may keep a reactivity up to ≥30°C (wide "thermal amplitude"). The IgM pentamers fix complement much more readily than IgG, causing intravascular hemolysis and to a lesser extent extravascular lysis mainly in the liver by macrophages with C3d receptors. Rouleaux formation indicating RBC agglutination is frequently noted on the blood smear. PCH is caused by the Donath-Landsteiner IgG antibodies which are usually directed against the P antigen of RBCs. Donath-Landsteiner antibodies are biphasic hemolysins: they bind to RBCs and fix complement (C1) at cold temperatures, but the complement is then activated at the core temperature of 37°C causing intravascular RBC lysis. PCH is frequently postinfectious and typically has a good prognosis after remission; however, it can be life threatening on presentation due to severe and rapidly progressive anemia(6,7).

AIHA is a rare disease with an incidence of 1 to 3 per 100 000 people per year(8,9).w-AIHA is the most common type of autoimmune hemolytic anemia, comprising ~70% to 80% of all adult cases and ~50% of the pediatric cases(10).About half of the w-AIHA cases are called primary because no specific etiology can be found, whereas the rest are recognized as secondary to lymphoproliferative syndromes; malignant diseases including chronic lymphoblastic leukemia (CLL), non-Hodgkin's lymphoma, and solid tumors; rheumatologic diseases, especially systemic lupus erythematosus; infections (mostly viral); drugs; frequent cephalosporins and piperacillin; or a previous transfusion or transplantation(11,12). In w-AIHA, the autoantibodies react optimally with the RBCs at 37°C. Typically, no autoantibody specificity can be identified; the autoantibodies are polyclonal and react with all RBCs tested

(pan-reactive), even when w-AIHA complicates a clonal B-cell lymphoproliferative disorder like CLL. Microspherocytes are frequently noted on the blood smear (Figure 1). Polychromasia, indicating reticulocytosis, is typical, although the reticulocyte count may not be elevated early in the course, and occasionally relative reticulocytopenia may be continuously observed as a result of autoantibody sensitization and destruction of late erythroid precursors. Complement activation on the RBC surface may lead to the formation of membrane attack complex (C5b9), causing RBC lysis in the circulation, although this is more typical for antibodies that avidly bind complement like the IgM pentamers in CAS. Intravascular complement-mediated cell lysis does not play a significant role in most patients with w-AIHA, implicating that terminal complement inhibitors would be clinically irrelevant(12),except may be for those atypical cases with significant warm antibody-complement–mediated intravascular hemolysis(13). Erythrocytes coated by warm-reacting IgG are bound by spleen macrophages, which carry Fc receptors for the IgG heavy chain, and they are either phagocytosed or have part of their membrane removed, in which case they form microsperocytes subject to further destruction during their next passage through the spleen. Antibody-dependent cell-mediated cytotoxicity (ADCC), mediated by cytotoxic $CD8^+$ T cells and natural killer (NK) cells also with Fc receptors in the spleen, also contributes to RBC destruction/extravascular hemolysis. When either a high concentration of IgG or IgG with high affinity to complement is bound to the erythrocytes, complement (C1q) is bound and gets activated toward C3b. C3b-opsonized RBCs are next phagocytosed by liver macrophages that carry C3b receptors (Figure 2). There are certainly enough data and experience to conclude that w-AIHA can be a fatal disease, either because of the acuity of the presentation or because of being refractory to treatment with acute relapses requiring multiple lines of therapy with frequently life threatening complications. A mortality rate of 11% in adults and 4% in children has been reported(9,10). A recent retrospective study reviewed the course, laboratory data, treatment, and clinical outcome of 308 primary autoimmune hemolytic anemia (AIHA) cases (including 10 pediatric patients) over a follow-up period of 12 to 372 months (median, 33 months)(40). Sixty percent of these patients, had w-AIHA, 27% had CAS, 8% had mixed (DAT positive for IgG and C3d with coexistence of warm autoantibodies and high-titer cold agglutinins), and 5% were atypical (mostly DAT-negative w-AIHA). The mixed and atypical cases presented frequently with severe onset (Hb <6 g/dL) along with reticulocytopenia. The level of Hb at presentation was predictive of the relapse risk, with the more severe cases (including mixed and atypical ones) having >50% cumulative incidence of relapse after 3 years or a threefold increased risk of relapse in comparison with the mild cases (Hb >10 g/dL) and requiring multiple lines of treatment (40, 41).

Pathogenetic Mechanisms In w-AIHA

Autoantibodies, the complement system, phagocytes, cytotoxic $CD8^+$ T cells and NK cells performing ADCC, B and T lymphocytes including the $CD4^+$ T regulatory (Treg) cells, and cytokines are all key players in the pathogenesis of w-AIHA (Figure 2). Several mechanisms leading to breach of normal interactions between these immune system components have been proposed and are being actively investigated as responsible for the breakdown of immunologic tolerance that allows development of w-AIHA(5,6).

Molecular mimicry of foreign antigens from viruses or other exogenous infectious and noninfectious agents, eg, drugs, that cross-react with RBC self-antigens, most frequently Rh proteins, and occasionally glycophorins and band 3, may overcome self-tolerance and trigger AIHA. Additionally, polyclonal activation of B lymphocytes by viruses may trigger the emergence of "forbidden clones," occasionally along with a congenital or acquired lymphopro life rative disorder.

The $CD4^+$ T-helper cell subsets, Th1, Th2, Th17, and the Tregs control the humoral immune system and have a critical role in maintaining or losing self-tolerance. Elevated frequency of Th17 cells producing increased interleukin-17 (IL-17) were found to correlate with disease activity in patients with AIHA(18). $CD4^+CD25^+$ Treg cells express high levels of the IL-2 receptor CD25 and secrete transforming growth factor-β and IL-10. Naturally occurring Tregs contribute to immunologic self-tolerance by suppressing potentially autoreactive T cells. Treg cells were indeed found reduced in patients with AIHA (20).Imbalance between Th1 and Th2 cells and the cytokines they secrete may also play a role. Th1 secrete IL-2, IL-12, interferon-γ, and tumor necrosis factor-β and promote cell-mediated immunity, whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 and promote humoral responses. Although the data on increased and decreased cytokine levels in patients with AIHA may be somewhat conflicting, they favor that a reduced Th1 and a prominent Th2 profile promote the pathogenesis of AIHA.

Regarding unique mechanisms in secondary w-AIHA, a decrease of the CR1 complement receptor (CD35) and CD55 (decay-accelerating factor) or CD59 was found on RBCs and $CD3^+$ lymphocytes in systemic lupus erythematosus–associated AIHA, whereas after bone marrow transplantation, an activation of host self-reactive B cells by allo-reactive donor T cells was seen in chronic graft-versus-host disease.

DAT-negative AIHA

In 1957, Evans and Weiser reported on the serology of immune hemolytic disease, describing 41 cases of autoimmune hemolytic anemia, 4 of which had negative direct and indirect Coombs(19). In 1 case, RBC agglutination in the DAT was achieved only after preparing the antiglobulin serum by injecting the patient's own serum into rabbits. In the other 3 cases, the 2 associated with infectious mononucleosis, autoantibodies or isoantibodies, could not be demonstrated but transfused RBCs in these patients had a severely decreased survival, indicating that a factor extrinsic to the patients' red cells was responsible for their destruction. Since then, numerous case series have studied anemia with clinical characteristics of w-AIHA but with negative DAT. Based on such publications, the incidence of DAT-negative w-AIHA has been estimated at 3% to 11% of all cases, depending, at least in part, on the potency of the direct antiglobulin reagent used for the DAT(25).

There are several inherent characteristics of the RBC autoantibodies causing DAT-negative w-AIHA, and thankfully there are now alternative methods to prove their existence, which are easier and faster than immunizing a rabbit with the patient's serum.

Anti-RBC IgG may bind to the erythrocytes at relatively low levels causing hemolysis but being below the threshold of detection for the commonly used DAT reagents. At the same time, the commercial DAT reagent and assay could not identify positivity below ~500 molecules of IgG/cell(25). Flow cytometry assays have been now developed and used by reference laboratories; they are calibrated so that the fluorescently labeled anti-human IgG on red cells is detected at a sensitivity greater than that of the commercial DAT reagent, decreasing the frequency of a false-negative antiglobulin test(32).

RBC autoantibodies may have low affinity and therefore are removed easily from the red cell surface during preparative washings of the cells at room temperature, to perform direct Coombs. Cold washing with isotonic or with low ionic strength

saline at 4°C using refrigerated centrifuges can prevent the antibody loss from the RBC surface, improving the sensitivity of the commercial DAT assay(33,34).

w-AIHA may be rarely due to sensitization of RBCs with IgAor a warm-reacting, monomeric IgM alone instead of IgG, without complement fixation,[27] in which cases most commercial DAT reagents, containing only anti-IgG against the heavy chain portion of the IgG molecule and anti-C3, are not detecting the antibodies(36,37).

Approach To The Diagnosis Of Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) is a decompensated acquired hemolysis caused by the host's immune system acting against its own red cell antigens. Consequent complement activation can impact the clinical picture and is an emerging target for therapeutic approaches.

When a patient presents with anemia, a stepwise approach should be followed. Initial simple investigations will first alert the physician to the suggestion of hemolysis as the cause of the anemia. These include a normo-/macrocytic anemia, raised reticulocyte count, raised unconjugated bilirubin, reduced haptoglobin, and blood smear with polychromasia or more specific features, such as spherocytes or agglutination (Figure 1). Although the typical pattern is presented, none of these tests are fully sensitive or specific for hemolysis. For example, liver disease can increase lactate dehydrogenase (LDH) and reduce haptoglobin. The bilirubin may be normal with milder hemolysis, and spherocytes are not always visible. Reticulocytopenia can occur in AIHA, secondary to bone marrow infiltration by a lymphoproliferative disorder or to parvovirus B19 infection. However, reticulocytopenia is also observed in a significant minority of patients at presentation, despite erythroid hyperplasia in the marrow. This may be caused by immune attack on late-stage erythroid precursors or reflect a lag in marrow responsiveness, but it can sometimes persist and predict a more severe clinical course.[2] A significantly raised LDH, red cell fragments on smear, or the presence of urinary hemosiderin suggests a predominant intravascular component to the hemolytic process. There are fewer causes of intravascular hemolysis; therefore, these can be very useful for subsequent investigations (Table 2). Once hemolysis is confirmed, further investigation is needed to establish whether that hemolysis is immune, principally by the direct anti-globulin test (DAT). The standard DAT demonstrates that immunoglobulin G (IgG) and/or complement (usually C3d) is bound to the red cell membrane. Autoantibodies can also be of IgM and IgA classes, and, in some circumstances, an extended DAT panel can be used to detect these.

METHODS AND MATERIALS:

A retrospective study of 10 cases of AIHA done in tertiary teaching medical colleges of Bihar between periods Agust2019 to agust2020. Detailed clinical features, epidemiological data and laboratory parameters were noted .Peripheral smears and bone marrow studies were also done all patients. Further clinical and special investigations were noted in secondary AIHA in multiple myeloma and chronic lymphocytic leukemia.

RESULT:

Total number of 10 patients was included in this study. It had female preponderance (Male: Female ratio: 1:2.3), three patients were DAT negative that also responded with steroid. All patients had indirect hyperbilirubinemia, raised LDH and raised reticulocyte count except one (Table 1 & 2).PBS revealed normocytic to mildly macrocytic and normochromic blood picture. Bone marrow aspiration revealed normoblastic erythroid hyperplasia. In this series, patients presented with moderate to severe anemia (Table 1).One patient on steroid presented with bilateral femoral head fracture due to avascular necrosis.

Table 1. Hematological Parameters Hemolytic In W-AIHA

CASE NO	AGE	SEX	RBC	HB	MCV	MCH	PLT	RETIC COUNT	DAT	REMARK
1	55	F	2.5	6.5	100	35	170	3.5	NEGATIVE	
2	18	F	2.8	7.0	78	32	190	4.8	POSITIVE	
3	64	F	2.0	6.0	90	33	176	5.0	POSITIVE	
4	13	F	1.8	5.8	105	34	199	8.8	POSITIVE	Auto agglutination
5	68	F	2.6	6.9	88	33	155	1.2	Positive	Multiple myeloma
6	45	M	2.8	7.8	99	32	180	2.8	NEGATIVE	
7	40	F	1.6	3.8	100	35	198	4.8	POSITIVE	STEROID INDUCED B/L FEMORAL FRACTURE
8	29	F	2.0	5.8	86	31	299	6.8	POSITIVE	MALIGNANT HTN
9	35	M	2.5	6.2	84	30	200	3.9	POSITIVE	
10	85	M	2.9	6.5	101	33	250	4.0	NEGATIVE	

Table 2. Biochemecal Change In W-AIHA

CASE NO	AGE	SEX	T.BILIRUBIN	DIRECT	INDIRECT	LDH	ANA	HEPTOGLOBIN	REMARK
1	55	F	3.5	0.7	2.8	300	-	REDUCED	
2	18	F	3.8	1.5	2.3	350	-	REDUCED	
3	64	F	2.9	0.9	2.0	465	-	REDUCED	
4	13	F	5.5	1.5	4.0	288	-	REDUCED	
5	68	F	3.9	1.9	2.0	290	-	REDUCED	
6	45	M	15	5.0	10	310	+	REDUCED	
7	40	F	6.8	2.8	4.0	388	-	REDUCED	
8	29	F	7.0	2.5	4.5	400	-	REDUCED	
9	35	F	4.8	1.3	3.5	299	-	REDUCED	
10	85	M	5.9	1.9	4.0	355	-	REDUCED	

DISCUSSION:

There are certainly enough data and experience to conclude that w-AIHA can be a fatal disease, either because of the acuity of the presentation or because of being refractory to treatment with acute relapses requiring multiple lines of therapy with frequently life threatening complications. A mortality rate of 11% in adults and 4% in children has been reported (15, 35). A recent retrospective study reviewed the course, laboratory data, treatment, and clinical outcome of 308 primary autoimmune hemolytic anemia (AIHA) cases (including 10 pediatric patients) over a follow-up period of 12 to 372 months (median, 33 months), sixty percent of these patients had w-AIHA, 27% had CAS, 8% had mixed (DAT positive for IgG and C3d with coexistence of warm

autoantibodies and high-titer cold agglutinins), and 5% were atypical (mostly DAT-negative w-AIHA) (40,41,42). The mixed and atypical cases present frequently with severe onset (Hb <6 g/dL) along with reticulocytopenia. The level of hemoglobin(Hb) at presentation was predictive of the relapse risk, with the more severe cases (including mixed and atypical ones) having >50% cumulative incidence of relapse after 3 years or a threefold increased risk of relapse in comparison with the mild cases (Hb >10 g/dL) and requiring multiple lines of treatment(40). In 1957, Evans and Weiser reported on the serology of immune hemolytic disease, describing 41 cases of autoimmune hemolytic anemia, 4 of which had negative direct and indirect Coombs (20). In 1 case, RBC agglutination in the DAT was achieved only after preparing the antiglobulin serum by injecting the patient's own serum into rabbits. In the other 3 cases, the 2 associated with infectious mononucleosis, autoantibodies or isoantibodies, could not be demonstrated but transfused RBCs in these patients had a severely decreased survival, indicating that a factor extrinsic to the patients' red cells was responsible for their destruction. Since then, numerous case series have studied anemia with clinical characteristics of w-AIHA but with negative DAT. Based on such publications, the incidence of DAT-negative w-AIHA has been estimated at 3% to 11% of all cases, depending, at least in part, on the potency of the direct antiglobulin reagent used for the DAT (22)

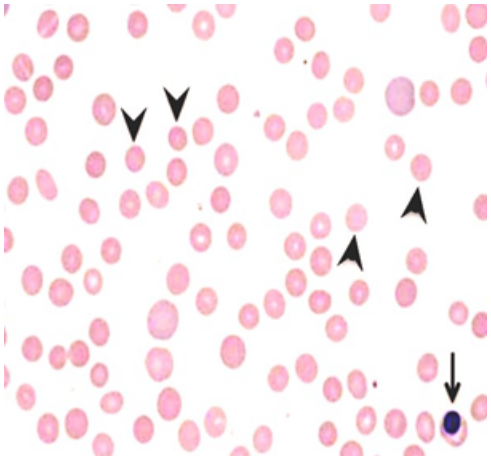

Figure1.polychromatic, Microspherocytes And nRBC.

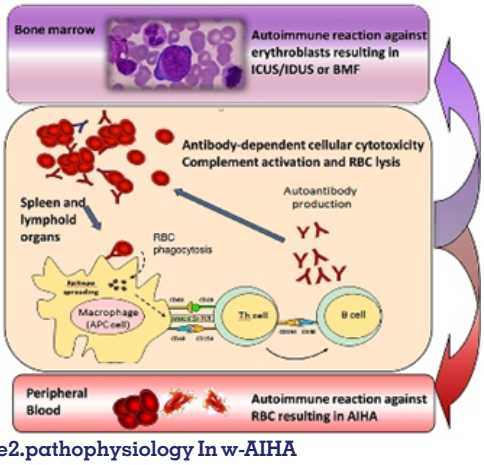

Figure2.pathophysiology In w-AIHA

CONCLUSION:

w-AIHA is rare disease worldwide, but common in India. It could be fatal in emergency hemolysis crisis, so early diagnosis is paramount importance. Approximately 5% w-AIHA may be DAT- negative, however flowcytometric detection of RBC bount IgG antibodies is possible .Treatment on basis of clinical, biochemical and hematological parameters must be started to save the life of patients in w-AIHA DAT- negative.

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