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STUDY OF ACQUIRED IMMUNOHAEMOLYTIC ANAEMIA IN LYMPHOPROLIFERATIVE DISORDERS

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ABSTRACT

The study was done to highlight incidence of immunohaemolytic anemia following lymphoproliferative disorder.
Five cases of immunohaemolytic anaemia were diagnosed, among cases of lymphoproliferative diseases, during last six years. Age ranged from 44 to 68 years, with four males and one female. They presented with lymphadenopathy, splenomegaly and pallor. EDTA blood was collected for complete haemogram. Blood smear examination, reticulocyte count and bilirubin was done. DAT, serum LDH and CD5 antigen were done.
All five patients showed evidence of haemolytic anaemia by presence of normoblasts, polychromatophilia, reticulocytosis, DAT positive, elevated serum LDH & CD5 positive. Four cases were Chronic Lymphatic Leukaemia and one a case of low grade Small Lymphocytic Lymphoma.
Approximately 8 - 25% patients develop immunohaemolytic anaemia as a complication of lymphoproliferative disorders . CD5 positive lymphocytes secrete anti DNA antibodies and rheumatoid factors and play significant role in producing auto-antibodies against red cell leading to predominantly warm antibody type of immunohaemolytic anaemia.

INTRODUCTION :

Auto-immune hemolytic anemia (AIHA) occurs when a patient produces pathologic antibodies that attach to and lead to destruction of their RBCs with consequent anaemia. By definition, both pathologic antibodies and associated RBC consumption must occur. These conditions can be classified according to the characteristic temperature activity of the antibodies into "warm reactive" and "cold reactive" types [3].

Warm active antibodies are found in idiopathic AIHA, lymphoproliferative diseases, autoimmune disorders, immunodeficiency disorders, malignancy and viral infections. Several drugs have also been implicated in the causation of warm active immunohaemolytic anemia [4].

Cold active antibodies are found in cold aggutinin disease, which maybe primary or secondary. Lymphoproliferative diseases, autoimmune disorders and infections are among the common causes of secondary cold agglutinin disease. Paroxysmal cold haemoglobinuria is another condition in which there maybe development of cold active antibodies [4].

Among lymphoproliferative diseases, Chronic Lymphocytic Lymphoma (CLL) is the most common cause of autoimmune haemolytic anemia [2]. AIHA in CLL is mostly associated with a warm type antibody. CLL cells may act as antigen presenting cells for either normal CD5+ or CD5- antibody producing B cells. Alternatively, the CLL cells might be the source of typical warm reactive antibodies, normally seen in autoimmune diseases [2].

Two criteria must be met to diagnose AIHA : laboratory evaluation of haemolysis and serologic evaluation of autoimmune antibody. Diagnosis of warm type AIHA is largely based on Direct Antiglobulin Test (DAT) or Coomb's test [5].

MATERIALS AND METHODS :

Fifty-three patients with lymphoproliferative diseases (42 CLL and 11 Non-Hodgkin Lymphoma, NHL) were evaluated for any haemolytic anaemia. The clinical parameters of these patients were carefully recorded (Table I). These included their age, gender, presence or absence of pallor, icterus, lymphadenopathy and splenomegaly. Chest X-rays of the patients were examined for presence of any hilar lymphadenopathy. Routine examination of urine was also undertaken and presence of protein, in any, was recorded.

EDTA blood samples of these patients were collected and the following laboratory parameters were recorded (Table II):

1. Hemoglobin %
2. Haematocrit
3. Total leucocyte count
4. Platelet count
5. Reticulocyte %
6. Number of norm oblasts/100 WBCs
7. Plasma haemoglobin

Clotted blood samples were also collected for estimation of total and unconjugated bilirubin levels in blood (table II).

Seven patients had evidence of haemolytic anaemia. They were subjected to DAT and five were Coomb's positive. Two of these patients had albumin in urine.

RESULTS :

TABLE I : Clinical parameters of all patients with Lympho-proliferative disorder

AGE	M/F	L.N.	SPL	PALLOR	ICT	CXR	URINE	DIS
57yrs	M	Gen	+	++	Nil	HL	1+	CLL
60yrs	M	Cv	+	++	+	Nil	Nil	SLL
68yrs	M	Gen	+	++	+	Nil	Nil	CLL
44yrs	M	Cv	+	++	+	HL	1+	CLL
65yrs	F	Cv	+	++	+	Nil	Nil	CLL

M : male
F : female
L.N. : lymphadenopathy
Gen : generalised lymphadenopathy
Cv : cervical lymphadenopathy
SPL : splenomegaly
ICT : icterus
CXR : chest X-ray
HL : hilar lymphadenopathy

In table I, we find that the age of the patients ranged from 44 to 68 years. Four of them were male patients and one, a female patient. All of them presented with splenomegaly and pallor. Cervical lymphadenopathy was present in three of them. Generalised lymphadenopathy was detected in two patients. Icterus was detected in all but one. Hilar lymphadenopathy was detected in the chest X-rays of two patients. Protein was detected in the urine of two patients

TABLE II : Laboratory parameters of patients with Hemolytic anemia

Hb	HCT	Plt	WBC	Retic	NB	DAT	Pl Hb	Bi	CD 5	CD 23
6.8	20	263	14.5	6%	5/100	++	<20	3	+	+
5.8	10	178	27.6	9%	17/100	++	<40	5	+	+
3.7	16	89	40.7	20%	9/100	++	>600	15	+	+
5.6	18	104	35.5	14%	13/100	++	>40	7	+	+
4.5	15	219	28.6	10%	6/100	++	>100	9	+	+

Hb : hemoglobin in g/dl
HCT : haematocrit
Plt : platelet count 1000
WBC : 1000
Retic : reticulocyte %
NB : normoblasts/100 WBCs
Pl Hb : plasma hemoglobin in mg/l
Bi : unconjugated bilirubin in mg/dl

All five patients, according to table II, showed evidence of immunehaemolytic anaemia by the presence of normoblasts, polychromatophilia, reticulocytosis, DAT positivity and elevated unconjugated bilirubin levels. CD5 and CD23 positivity were noted in all of them. Four cases were that of CLL and one case of low grade Small Lymphocytic Lymphoma (SLL).

In this study an incidence of approximately 9.4% of immunohaemolytic anaemia was found among patients with lymphoproliferative diseases.

DISCUSSION :

Although CLL patients are immune deficient, they have an increased incidence of autoimmune diseases. Overall 4 to 25% of CLL patients develop AIHA and this is usually associated with a warm type antibody. The incidence is higher in men, those with lymphocyte counts >60,000/cu.mm and those >60 years of age [2].

Apoptosis through the TNF receptors plays an important role in controlling lymphoid cell populations, and defects either in Fas ligand or the receptor result in the autoimmune lymphoproliferative syndrome (ALPS), with lymphaden opathy, splenomegaly, and an increase in the risk of subsequent autoimmune diseases and lymphomas. Fas is normally upregulated in activated lymphocytes, and CLL cells are not sensitive to Fas ligand [2].

Normal CD5+ B cells can produce autoantibodies to IgG and single and double stranded DNA, as well as, other autoantigens and the number of these cells in increased in autoimmune disorders. CLL cells can be induced to secrete IgM molecules that react with a comparable spectrum of antigens. However, several observations suggest that clinically significant autoantibodies are not produced by the leukaemic clone. First, the antibodies are polyclonal and are usually IgG. Second, the autoimmune disorders can occur while the patient's disease is responding to therapy. It has been suggested that the CLL cells act as antigen presenting cells for either normal CD5+ or CD5- antibody producing B cells and that antibody production is increased after inhibition of T cells, either with advancement of disease or because of therapy. Alternatively, the CLL cells might be the source of antibody, as it has been demonstrated that IgM+ CLL cells can undergo isotype class switching to IgG+ cells, which could be the source of the typical "warm reactive" antibodies normally seen in autoimmune disease. Additional support for CLL cells being the source of autoantibody is provided by the association between AIHA in CLL and the expression by the leukemia cells of specific Ig variable region genes [2,6].

Immune thrombocytopenia occurs in 2% of patients [7]. One third of these patients with ITP also have a positive Coomb's test. Nephrotic syndrome, acquired angioedema and autoimmune blistering skin diseases also occur in CLL [8]. Paraneoplastic pemphigus in CLL is diagnosed by distinct histologic changes in skin and the presence of autoantibodies in blood, directed against cutaneous epitopes [9].

Mauro et al [10] undertook a study in which 52 cases of AIHA were observed within a series of 1203 patients (4.3%) with CLL. The antierythrocyte antibody was IgG in 87% cases. Patients previously treated with chlorambucil and with fludarabine showed a similar rate of AIHA.

Sikora et al [11] concluded in their study that the autoantibody in patients of CLL with AIHA represented a reactive response and was not the product of malignant clone of B cells. The antibody responsible for the autoimmune manifestations is secreted by normal autoreactive B lymphocytes.

CONCLUSION :

Lymphoproliferative disorders are one of the important cause of secondary autoimmune hemolytic anemia [4]. However, many cases are transient and self limiting. Management of primary disease is important to ameliorate the disease. Most of the antibodies are warm antibody and IgG in type, although cold antibody and mixed warm and cold antibody have also been observed in CLL patients [3]. However, a positive DAT does not always mean decreased RBC survival or clinical AIHA. Study on normal blood donors showed DAT positive in 1:10,000 population. Many more AIHA are transient and self limiting. DAT interpretation must consider the context of clinical history and other laboratory findings. AIHA seems more frequent in patients with low grade lymphoma and CLL treated with fludarabine [12] or cladribine [13]. Most antibodies are IgG with preponderance of IgG_1 and to a lesser extent IgG_3. The subtypes vary in their efficiency at causing haemolysis because of higher affinity of macrophage Fc receptors for IgG_1 and IgG_3 subclasses. Treatment of primary disease usually ameliorates the condition. Rituximab, a monoclonal antibody specific for CD20 antigen expressed on B lymphocytes has been used with success in patients, particularly AIHA associated with CLL and B cell lymphomas, with or without association with fludarabine therapy [14].

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