Original Article

Autoimmune Haemolytic Anaemia — Approach in a Tertiary Care Centre

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Background : Autoimmune Haemolytic Anaemia (AIHA) is defined as decreased red cell survival or increased destruction, secondary to antibodies directed against own red blood cells. The disease is heterogeneous, with symptoms ranging from fully compensated asymptomatic patients to patients presenting with rapid onset of life-threatening Anaemia.

Aims and Objectives : The main objective of our study was to distinguish Warm antibody-induced AIHA from Cold antibody-induced AIHA on the basis of immunoglobulin class of the antibody. The secondary objectives were to study the type of antibody, result of Direct Antiglobulin Test (DAT), thermal amplitude of involved antibody and to study the association of severity of anaemia with various laboratory parameters and to assess the response of different treatment modalities used to treat AIHA.

Materials and Methods : It was an observational prospective study. Fifty patients diagnosed with AIHA were taken and were categorised on the basis of antibody types, severity of haemolysis and other laboratory parameters.

Results : The parameters, which had the greatest impact on severity of haemolysis were the patients of primary AIHA, patients with multiple auto-antibodies, complement fixation and strength of DAT.

Conclusion : In this study the association of strength of DAT and severity of disease suggest that an algorithm should be designed for testing patients with DAT positive AIHA with a monospecific DAT and IgG subtype analysis. It may allow for identification of this critical subgroup of patients in whom more intense clinical intervention and close follow up might be indicated. Also, the subtyping may help in better treatment as it has been shown in many studies that steroids are more effective first line therapy in warm AIHA while Cold Agglutinin Disease (CAD) show better response to Rituximab.

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Key words **: Autoimmune Haemolytic Anaemia, Warm AIHA, Cold Agglutinin Disease, Direct Antiglobulin Test.**

Autoimmune Haemolytic Anaemia (AIHA) is a clinical condition in which antibodies bind to patient's own red cell surface antigens and initiate red cell destruction. It is classified as either autoimmune, alloimmune or drug induced based on the antigenic stimulus responsible for the immune response[1]. The peak incidence is in between 60 and 70 years of age. The disorder is usually more common in females[2]. It can be idiopathic (50%), secondary to lymphoproliferative diseases (20%), autoimmune (20%), infection and tumours[3].

The disease is heterogeneous with symptoms ranging from fully compensated disease to life-threatening anaemia. It should be suspected in a patient presenting with symptoms attributable to anaemia such as easy fatiguability, dyspnoea, palpitation in the absence of obvious causes like nutritional deficiency, bleeding etc and symptoms of haemolysis eg, jaundice.

Editor's Comment :

- Extended DAT should be done in patients with AIHA as it will help to plan the first line therapy.
- The strength of DAT generally correlates with the severity of AIHA.
- The parameters like LDH may be used as marker to follow up such patients and may help in predicting relapse as specialised tests like DAT may not be available at all centres.

Laboratory parameters helpful for diagnosis are Complete Blood Count (CBC), Direct Antiglobulin Test (DAT), Reticulocyte Count, Serum Lactate Dehydrogenase (LDH) and indirect hyper-bilirubinemia. DAT helps in differentiating immune from non-immune causes.

The autoantibodies agglutinate, sensitize or cause lysis of their own red blood cells. Destruction of red cells causes anaemia and jaundice and without timely intervention, it can be fatal in some patients. The factors that have an impact on the severity of haemolysis include antibody quantity, antibody specificity, thermal amplitude, ability to bind to tissue

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macrophages and ability to fix complement. Multiple immunoglobulins molecules coating red cells are one of the major causes of haemolysis[4]. IgG antibodies are relatively poor activator of classical complement pathways but IgG1 and IgG3 are readily recognised by Fc receptors on reticuloendothelial cells. IgG3 autoantibody is most effective in bringing about red cell destruction. Patients' response to treatment correlated with the type of IgG subclasses[5]. Currently there are no guidelines regarding strength of DAT positivity and the severity of haemolysis.

The initial treatment aims to stabilise the patient and blood is transfused if patient has severe symptoms. In hemodynamically stable patients consideration of Packed Red Blood Cell (PRBC) transfusion to maintain haemoglobin of 7g/dl. For patients who are experiencing cardio-pulmonary symptoms due to anaemia, PRBC transfusion should not be withheld regardless of haemoglobin level.

The treatment response is varied and not all patients respond to initial treatment. Glucocorticoids are considered the first-line treatment in Warm AIHA. Splenectomy is effective second-line treatment but has never been compared with other treatments. The response rate of splenectomy is 60%-90%. Treatment of Cold agglutinin disease generally depends on etiology. In cases of severe haemolysis immuno-suppression with chlorambucil or cyclophosphamide may be useful[6]. Significant responses are also seen with interferon[7]. Response to steroid is seen in low titre disease. The response to splenectomy is not as effective as in majority of cases haemolysis occurs in liver[8]. Single-agent rituximab is currently considered the first-line systemic therapy for CAD due to its good efûcacy and tolerability.

Aims and Objectives

Aims : In view of the lack of any homogenous data in Indian population, the present study including a series of 50 AIHA cases in Eastern India, aimed at investigating the aetiology, pathogenesis, clinico-haematological profile and efficacy of the treatment.

Objectives : The primary objective of our study was to distinguish Warm antibody-induced AIHA from Cold antibody-induced AIHA on the basis of immuno-globulin class of the antibody.

The secondary objectives were to study the type of antibody, result of Direct Antiglobulin Test (DAT), thermal amplitude of involved antibody and to study the association of severity of anaemia with various laboratory parameters and to assess the response of different treatment modalities used to treat AIHA.

Material and Methods

We studied all patients whether newly diagnosed or on treatment or on post treatment follow-up with initial diagnosis of AIHA. The severity of haemolysis was classified into severe or moderate based on study criteria laid down by Wheeler, *et al*[9].

The laboratory parameters used to categorize severity of haemolysis are-

(1) Haemoglobin <9g/dl
(2) Indirect Bilirubin >2mg/dl
(3) Reticulocytes (>2%)
(4) LDH > 500IU/ml.

The patients were enrolled on the basis of the following categories-

(1) First time diagnosed to have AIHA.
(2) Patients already diagnosed to have AIHA and on treatment at the time of analysis.
(3) Previously diagnosed to have AIHA, currently relapsed after stopping treatment.

Patients were classified as having primary or secondary AIHA based on the history, laboratory and radiological results.

We used Column Agglutination Technique (CAT) to serologically characterize the autoantibodies. Polyspecific Direct Antiglobulin Test (DAT) was performed using Low Ionic Strength Saline (LISS) Coombs ID card to identify the presence of IgG and complement. The Coomb's positive patients were further tested using monospecific DAT LISS Coombs ID to identify the presence of immunoglobulins such as IgG, IgA, IgM and complement C3d, C3c. Results of DAT were graded from 0 to 4 as per manufacturer's recommendations (Fig 1) and strength of DAT was interpreted by two individuals individually.

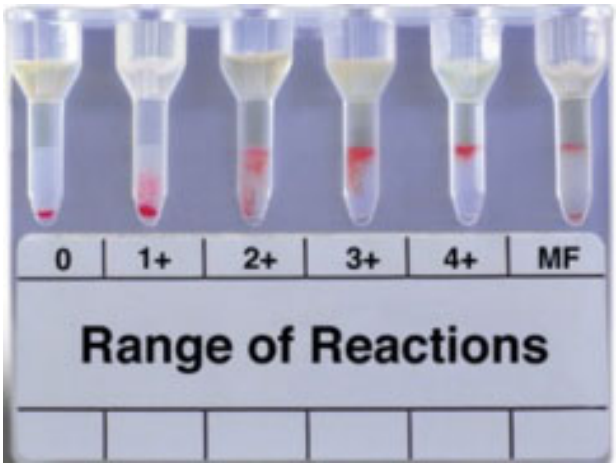

Fig 1 — Grading of DAT reaction (Taken from Card Instruction Manual of Micro Typing System MTS084024 Anti-Human Globulin Anti-IgG MTS Anti-IgG)

Interpretation :

(A) Positive: Agglutinated cells form a red line on the surface of the gel (strong reaction) or the agglutinates tend to disperse in the gel in a weaker reaction.

(B) Negative: Unagglutinated RBCs form a well-defined button at the bottom of the microtube.

The results of the test are graded as shown in Fig 1. The reaction on the card was read from both the sides and interpreted by two individuals independently to avoid interpretation error.

1+Reaction — RBC agglutinates are observed predominantly in the lower half of the gel microtube. Unagglutinated RBCs form a button in the bottom of the microtube

2+Reaction — RBC agglutinates are dispersed throughout the length of the gel microtube. Few unagglutinated RBCs may be observed in the bottom of the microtube.

3+ Reaction — The majority of RBC agglutinates are trapped in the upper half of the gel microtube.

4+ Reaction — A solid band of RBC agglutinates on top of the gel. A few agglutinates may filter into the gel but remain near the predominant band.

Weak Reaction — Granular suspension

MF Reaction — Mixed field agglutination / double population- RBC agglutinates at the top of the gel or is dispersed throughout the gel microtube accompanied by a button of negative red blood cells in the bottom of the microtube

Clinical and laboratory data relevant to the study were collected. These were age, gender, presence or absence of organomegaly, causes of secondary AIHA, new onset of AIHA or already diagnosed patients on treatment or relapsed AIHA.

Observations and Results

Fifty patients of any age/sex whether newly diagnosed / on treatment / on post treatment follow up with initial diagnosis of AIHA were included in the study. The medical records were analyzed for the demographic data (age and sex), clinical features, co-morbid conditions, investigations, mode and results of the treatment.

In our study, 66.00% of patients were Females and 34.00% of patients were Males. Mean age (years) was 45.88 ± 20.2 with median. In our study, majority (60.00%) of patients had primary AIHA. Only 20 out of 50 patients (40.00%) had secondary AIHA. The majority (60.00%) of patients were diagnosed as Warm AIHA followed by Cold AIHA (30.00%). Diagnosis was mixed AIHA in only 5 out of 50 patients (10.00%) (Table 1).

Table 1 — *Distribution of diagnosis of study subjects*

Diagnosis	Frequency	Percentage
Warm AIHA	30	60.00%
Cold AIHA	15	30.00%
Mixed AIHA	5	10.00%
Total	50	100.00%

In majority 39 (78.00%) of patients there was no history of blood transfusion. The majority (76.00%) of patients had moderate anaemia. In 12 out of 50 patients (24.00%) had severe anaemia. Mean value of haemoglobin (g/dL) of study subjects was 5.86 ± 1.02 with median (25th-75th percentile) of 5.9 (5.225-6.2). The mean value of haematocrit (%) was 17.63 ± 3.48, retic count (%) was 6.34 ± 2.03, total bilirubin (mg/dL) was 3.06 ± 1.58, indirect bilirubin (mg/dL) was 2.43 ± 1.41, LDH (U/L) was 889.1 ± 476.86, total leucocyte count (cells/mm^3) was 11809.68 ± 20660.9 and platelet count (cells/mm^3) was 2.45 ± 1.35. The median haematocrit was 17.7 (15.225-18.6), reticulocyte count was 6.15 (4.825-7.2), total bilirubin was 2.8 (1.925-3.975), indirect bilirubin was 2.05 (1.425-3.2), LDH was 760 (562.25-1045), Total Leucocyte Count was 7596.5 (5992.5-8701.75) and Platelet Count was 2.46 (1.7-3.098) respectively.

On clinical examination, fatigue was the most common symptom followed by pallor. Fatigue was present in 58.00% of patients, pallor in 56.00%, dyspnoeal and splenomegaly in 38.00%, jaundice in 12.00%, haematuria in 6.00%, fever in 10.00%, weakness in 8.00%, lymphadenopathy in 4.00%, lumbar region pain in 4.00% and anaemia in 4.00%. Clinical features were nose bleeding, hepato-splenomegaly, ascites, shortness of breath on exertion, pericardial effusion, joint pain and abdominal pain in only 1 out of 50 patients (2.00%) each.

The most common Blood Group was 'O' positive (42.00%) followed by 'A' Positive (32.00%) and 'B' Positive (24.00%). Blood Group was 'AB' Positive of only 1 out of 50 patients (2.00)

Distribution of DAT grade of study subjects — In present study, in majority (46.00%) of patients DAT grade was 3+, followed by 4+(38.00%) and 2+ (12.00%). DAT grade was 1+ in only 2 out of 50 patients (4.00%) (Table 2).

Table 2 — *Distribution of DAT grade of study subjects*

DAT grade	Frequency	Percentage
1+	2	4.00%
2+	6	12.00%
3+	23	46.00%
4+	19	38.00%
Total	50	100.00%

Distribution of extended DAT of study subjects **—** In present study, in majority (42.00%) of patients, extended DAT was only IgG followed by IgG + Others (34.00%). Extended DAT was only C3D in only 12 out of 50 patients (24.00%) (Table 3).

In the present study, in majority (84.00%) of patients, underlying alloantibody were not detected. Underlying alloantibody was detected in only 8 out of 50 patients (16.00%). The majority (60.00%) of patients were idiopathic. Amongst the remaining patients (40%) the common causes were Chronic liver disease (6.00%), Systemic lupus erythematosus (6.00%), Lymphoma (6.00%) and ANA positivity (4.00%). 1 out of 50 patients (2.00%) was diagnosed with paroxysmal cold haemoglobinuria.

In majority (68.00%) of patients, first line therapy given was steroids followed by Rituximab (20.00%). The majority (64.00%) of patients had complete response after first line therapy and partial response was seen in (36.00%). In majority 31 out of 50 pateints (62.00%) second line therapy was not required. In patients with partial response, 13 out of 18 (72.22%) had complete response after second line therapy. In 1 out of 50 patients (2.00%) died because of disease relapse.

Distribution of diagnosis was comparable between severity of anaemia (Severe *versus* Moderate). (Cold AIHA:- 25% *versus* 31.58% respectively, Mixed AIHA:- 8.33% *versus* 10.53% respectively, Warm AIHA:- 66.67% *versus* 57.89% respectively) (p-value=0.886).

Distribution of history of blood transfusion was comparable between severity of anaemia (Severe *versus* Moderate). (8.33% *versus* 26.32% respectively) (p value=0.257).

Significant association was seen in LDH (U/L) with severity of anemia (p-value <0.05). Mean ± SD of LDH (U/L) in severe anemia was 1213.83 ± 427.73, which was significantly higher as compared to moderate anemia (786.55 ± 449.17) (p value=0.006). No significant association was seen in reticulocyte count (%) (p value=0.91), total bilirubin (mg/dL) (p value=0.841), indirect bilirubin (mg/dL) (p value=0.952) with severity of anemia. Mean±SD of reticulocyte count (%), total bilirubin (mg/dL), indirect bilirubin (mg/dL) in severe anemia was 6.28 ± 1.99, 2.98 ± 1.48, 2.41 ± 1.41 respectively and in moderate anemia was 6.36 ± 2.07, 3.08 ± 1.62, 2.44 ± 1.43 respectively with no significant association between them.

Association of blood group with severity of anaemia **—** Distribution of blood group was comparable between severity of anaemia (Severe *versus* Moderate). (A Positive:- 33.33% *versus* 31.58% respectively, AB Positive:- 0% *versus* 2.63% respectively, B Positive:- 33.33% *versus* 21.05% respectively, O Positive:- 33.33% *versus* 44.74% respectively) (p value=0.682).

Table 3 — *Distribution of extended DAT of study subjects*

Extended DAT	Frequency	Percentage
Only IgG	21	42.00%
Only C3D	12	24.00%
IgG + Others	17	34.00%
Total	50	100.00%

Association of DAT grade with severity of anaemia **—** Distribution of DAT grade was comparable between severity of anaemia (Severe *versus* Moderate). (1+:- 0% *versus* 5.26% respectively, 2+:- 8.33% *versus* 13.16% respectively, 3+:-41.67% *versus* 47.37% respectively, 4+:- 50% *versus* 34.21% respectively) (p-value=0.798) (Table 4).

Association of extended DAT with severity of Anaemia **—** Distribution of extended DAT was comparable between severity of Anaemia (Severe *versus* Moderate). (Only IgG:- 33.33% *versus* 44.74% respectively, Only C3D:- 16.67% *versus* 26.32% respectively, IgG + Others:- 50% *versus* 28.95% respectively) (p value=0.482) (Table 5).

Association of complete/partial response after first line therapy **—** Distribution of complete/partial response after first line therapy was comparable between first line therapy (Rituximab, steroids, chemotherapy, others). (Complete response:- 90% *versus* 55.88% *versus* 50% *versus* 75% respectively, Partial response:- 10% *versus* 44.12% *versus* 50% *versus* 25% respectively) (p-value=0.174).

Discussion

Autoimmune Haemolytic Anaemia (AIHA) is a collective term for several diseases characterized by

Table 4 — *Association of DAT grade with severity of Anaemia*

DAT grade	Moderate (n=38)	Severe (n=12)	Total	P value
1+	2 (5.26%)	0 (0%)	2 (4%)	0.798
2+	5 (13.16%)	1 (8.33%)	6 (12%)	
3+	18 (47.37%)	5 (41.67%)	23 (46%)	
4+	13 (34.21%)	6 (50%)	19 (38%)	
Total	38 (100%)	12 (100%)	50 (100%)	

Table 5 — *Association of extended DAT with severity of Anaemia*

Extended DAT	Moderate (n=38)	Severe (n=12)	Total	P value
Only IgG	17 (44.74%)	4 (33.33%)	21 (42%)	0.482
Only C3D	10 (26.32%)	2 (16.67%)	12 (24%)	
IgG + Others	11 (28.95%)	6 (50%)	17 (34%)	
Total	38 (100%)	12 (100%)	50 (100%)	

autoantibody-initiated destruction of red blood cells[10]. It is greatly heterogeneous, with symptoms ranging from fully compensated to patients presenting with fulminant, rapid onset of life-threatening Anaemia[10]. Immunoglobulin class, subclass, titre, ability to activate complement, thermal amplitude and strength of Direct Antiglobulin Test (DAT) have been implicated as factors affecting the severity of the disease[11,12]. In view of this, it becomes very important to identify patients who are at risk of severe haemolysis, so that treatment and management is initiated under close supervision. With this background this study was undertaken, to serologically characterize the auto antibodies resulting in AIHA in the Indian population and to analyse the correlation between severity and the various factors implicated in AIHA along with assessment of treatment outcomes with various therapeutic strategies.

The incidence of AIHA is 0.8 to 1/80,000 to 1,00,000/year in western population[6,7] and the reported prevalence is 17/100,000[13]. In literature there are no population-based studies for incidence and prevalence of AIHA from India. It is known from various studies that frequency of AIHA is usually greater in females than in Males[6,14]. Similar trend was seen in our study with a Male : Female ratio of 1:1.9. This finding was in accordance with an Indian study by V Alwar, *et al* where it was noted that male: female ration was 1:2.2[14].

Naithani, *et al* (2006) studied the clinico-haematological profile and treatment outcome of patients with Autoimmune Haemolytic Anaemia (AIHA)[15]. There were 79 (52 primary; 27 secondary) consecutive patients identiûed with a median age of 30.5 years. The main presenting complaints were pallor (94%), jaundice (51%), and splenomegaly (68%). Jaundice was much more common in primary (63%) as opposed to secondary (26%) AIHA. The direct antiglobulin test was negative in six patients which shows us the importance of clinical examination and aniciliary tests. Oral prednisolone produced remission in 87.5% patients. Six patients (three children, three adults) relapsed after a median period of 2 months after response. All of these responded to a second course of steroids, in a median period of 14 days. No correlation was found between response and the parameters like age, sex, jaundice, low pretreatment haemoglobin, reticulocyte count, Total Leucocyte Count (TLC), Platelet Count, subtype of AIHA and hepatosplenomegaly. The only factor which predicted relapse was increased duration between the onset of symptoms and start of treatment ($r = 0.996$; $p=0.0001$)[15].

Primary AIHA affects all age groups with the peak incidence of disease in the fourth and fifth decades. Secondary AIHA reflects the age distribution of the underlying disease. For example, in the case of SLE, AIHA tends to present at much earlier age group and if AIHA is secondary to lymphoproliferative disorder, it tends to present in a relatively older age group. In our study, the median age group of AIHA patient was 47.5 years (Range 3-78 years). The most common secondary cause of AIHA was autoimmune disorders (10%).

Haemolysis was classified into severe and moderate based on study criteria laid down by Das SS, *et al*[16]. Of the total patients, 24% were categorised to have severe haemolysis. The severity of haemolysis was higher in patients with primary AIHA (68.2%) as compared to patients with secondary AIHA (31.8%). The association of primary AIHA with severe haemolysis was statistically significant with $p<0.001$.

Das SS, *et al* (2018) studied patients of “mixed” AIHA (M-AIHA), characterizing M-AIHA both clinically and serologically[13]. Out of 134 AIHA patients, 13 diagnosed as M-AIHA were subjected to detailed immune-hematological characterization. Most patients were severely anaemic and required urgent transfusions. The median age of patient was 37 years with a female preponderance and secondary M-AIHA was observed in 8 (61.5%) patients[13].

In present study, in majority (84.00%) of patients, underlying alloantibody was not detected. Adsorption study and underlying alloantibody were detected in only 8 out of 50 patients (16.00%). The majority of patients were idiopathic followed by secondary causes like Chronic Liver Disease (6.00%), Systemic Lupus Erythematosus (6.00%), Lymphoma (6.00%) and ANA positivity (4.00%). Associated disorder was paroxysmal cold haemoglobinuria in only 1 out of 50 patients (2.00%).

Berensten, *et al* (2004) studied treatment with the anti-CD20 antibody rituximab for chronic Cold Agglutinin Disease (CAD)[17]. Thirty-seven courses of rituximab were administered prospectively to 27 patients. 14 of 27 patients responded to their first course of rituximab, and 6 of 10 responded to re-treatment. In both groups combined, responses were achieved after 20 of 37 courses, giving an overall response rate of 54%. They observed 1 complete and 19 partial responses. Two non-responders and three patients who experienced relapse received second-line therapy with interferon-α combined with a new course of rituximab and one non-responder and two patients who experienced relapse achieved partial responses[17].

In present study, majority (64%) of patients had complete response after first line therapy. Partial response was seen in only 18 out of 50 patients (36%). First line therapy was failed in only 17 out of 50 patients (34%). In majority (62.00%) of patients, second line therapy was not required. Second line therapy was required in only 19 out of 50 patients (38%). Majority (72.22%) of patients had complete response after second line therapy. Partial response was seen in only 5 out of 18 patients (27.78%). One out of 50 patients died of AIHA.

Hantaweepant, *et al* (2019) investigated among the Thai patients the efficacy and safety of second-line treatment in primary warm-type AIHA that failed corticosteroid treatment[18]. This retrospective descriptive study included patients aged >14 years who were diagnosed with and treated for primary warm-type AIHA at the Haematology division of the Medicine Department, Siriraj Hospital, Mahidol University, Bangkok, Thailand. All 54 included patients failed first-line corticosteroid treatment after which second-line treatment was prescribed. Majority (83.3%) of the included patients were female and had a mean age at onset of 55.8 years (14.5-87.4). Most patients (63%) were refractory to steroids, and the rest of them relapsed while on steroids. The second-line medications were azathioprine (61.1%), cyclophosphamide (31.5%), danazol (3.7%), rituximab (1.9%) and chlorambucil (1.9%) and with respective response rates of 78.8%, 58.8%, 2/2 patient, 0/1 patients and 1/1 patient. Strong positive direct Coombs' test (3+ to 4+) was the only predictive factor of treatment response (p = 0.008). Males had better relapse-free survival than females (not reached *versus* 20.6 months) (p = 0.023).

Distribution of complete/partial response after first line therapy was comparable between first line therapy (Rituximab, steroids, chemotherapy, others). (Complete response:- 90% *versus* 55.88% *versus* 50% *versus* 75% respectively, Partial response:- 10% *versus* 44.12% *versus* 50% *versus* 25% respectively) (p-value=0.174).

Fan, *et al* (2016) analyzed 68 children diagnosed as AIHA and showed that 39.7% of all patients had primary AIHA whereas 60.3% had secondary AIHA[19]. Average haemoglobin was lower in the ≤1-year age group than > 1-year age group, IgM containing group than non-IgM containing group and combined-antibody group than single-antibody group (p < 0.05 for all). Twenty nine cases (29/45, 64.4%) remained in continuous remission during the follow-up period. After first complete remission, total 35.6% patients relapsed but after relapse still 56.3% of them responded to glucocorticoid[19].

In present study majority (60.00%) of patients had primary AIHA while 40.00% had secondary AIHA. In the present study, in majority (60.00%) of patients, diagnosis was warm AIHA followed by cold AIHA (30.00%). Diagnosis was mixed AIHA in only 5 out of 50 patients (10.00%).

Distribution of gender was comparable between severity of anaemia. Females had more severe anaemia as compared to males. Distribution of primary/ secondary AIHA was comparable between severity of anaemia. Cold and mixed AIHA had more severe disease. The higher LDH levels were associated with more severe disease. No significant association with severity was seen with reticulocyte count and bilirubin. Distribution of DAT grade was comparable between severity of anaemia, higher DAT positivity was associated with higher severity. Distribution of extended DAT was comparable between severity of anaemia (Severe and Moderate). (Only IgG:- 33.33% *versus* 44.74% respectively, Only C3d:- 16.67% *versus* 26.32% respectively, IgG + Others:- 50% *versus* 28.95% respectively) (p-value=0.482). Distribution of complete/partial response after first line therapy was comparable between first line therapy.

Conclusion

The auto-antibodies profile identified in patients with AIHA was predominantly IgG along with complement followed by a few cases of complement alone and occasional cases with IgM and IgA. The parameters, which had the greatest impact on severity of haemolysis are primary AIHA, presence of multiple auto-antibodies, complement fixation and strength of DAT. Those with subtypes had significantly greater risk of severe haemolysis when compared with patients having negative for the same. The association in this study of DAT strength and complement fixation on severity of haemolysis suggest that an algorithm of following up DAT positivity in patients with AIHA with a monospecific DAT and IgG subtype analysis will allow for identification of this critical subgroup of patients in whom more intense clinical intervention and close follow-up might be indicated. Steroids are effective as first line therapy in Warm AIHA while CAD show better response to Rituximab.

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