A clinico hematological correlation of autoimmune hemolytic anemia and its clinical implication

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Abstract

Introduction: Autoimmune hemolytic anemia characterized by production of antibodies that attach to Red cells resulting in their destruction causing anemia. AIHA could be primary or secondary to some other cause. The positive DCT is hallmark for diagnosis of AIHA. **Aim of Study:** To perform clinical and hematological study of AIHA and from available data to derive necessary conclusion for accurate diagnosis of AIHA. **Material and Methods:** The prospective study was carried out in blood bank at tertiary care hospital in positive DCT cases from suspected AIHA cases. The detail history was taken, clinical presentation studied and necessary laboratory investigations like peripheral smear examination, Reticulocyte count, Serum bilirubin, Serum LDH etc done and all data tabulated and conclusion was made for diagnosing AIHA accurately. DCT was performed both manually and using gel card technology. **Conclusion:** Females and age group between 20 to 30 have more predilections for developing AIHA. Secondary cases are more common and severity of presentation was aggressive in primary cases as compared to secondary. Such type of study helpful in diagnosis AIHA accurately and delay in treatment can be avoided and awareness can be created .

Key words : Autoimmune hemolytic anemia, DCT, Primary AIHA, Secondary AIHA.

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Introduction

Autoimmune hemolytic anemia characterized by production of antibodies that attach to Red cells resulting in their destruction causing anemia. Antibodies are of two types depending on their avidity for red cells at low temperature 4'C (cold antibodies) or at body temperature 37'C(Warm antibodies) [1].

Warm antibodies that react at 37'C are IG G type and antibody coated red blood cells sequestrated in spleen and liver, resulting in extra vascular hemolysis. While cold antibodies that are effective at 4'C are IG M type and can activate complement and leads intravascular hemolysis [1,2]. As hemolysis occurs in autoimmune hemolytic anemia, it will show positive coomb's test (Direct coomb's test) which is the diagnostic test for it. [3] Coomb's et al. in 1945, showed that AHG test useful in detecting antibodies to human globulin &

complement that coat RBC in hemolytic anemia [4]. The clinical features include: weakness, dyspnea on exertion, fatigue, jaundice, splenomegaly, Pallor, dizziness, fever, bleeding, weight loss, abdominal pain etc [1,5].The incidence has been discussed in discussion portion of this article.

Aim of Study

To perform clinical and hematological study of AIHA and from available data to derive necessary conclusion for accurate diagnosis of AIHA.

Materials and Methods

The prospective study carried out on patients that are referred to blood bank at tertiary care hospitals for investigating suspected cases AIHA.

The study was conducted from 1st January 2015 to June 2016.

The tests that were performed are Direct coomb's test (DCT) and Indirect coomb's test (ICT) by both manual as well as Gel card technique. The ICT positive cases investigated further by doing antibody titration.

The detail history along with clinical features taken for all patients and physical examination was also done. Patients with Rh and ABO incompatibility and blood transfused patient in last six month were excluded from this study.

After performing DCT and ICT, laboratory investigations like CBC, retic count, peripheral smear examination, serum LDH estimation and bilirubin estimation were done. All records were tabulated and final conclusion was made regarding clinico-hematological study of AIHA.

Results

Total 60 DCT positive cases were studied and investigated. The mean age was found to be 27 year and among this 38 patients were female and 22 were males.

Among these 60 cases etiologically 34 cases were due to secondary to other causes while 26 cases were primary AIHA.

Table No-.1: Etiology of Autoimmune hemolytic anemia

Primary AIHA cases	Secondary AIHA cases	Tuberculosis	HIV	Connective tissue disorders like SLE	AML	CML	CLL	Drug induced	ITP	Malaria
26	34	07	04	03	02	03	01	06	05	03

In primary cases of autoimmune hemolytic anemia the common presentation was dyspnea on exertion and fatigue. In secondary cases of AIHA common presentation was weight loss, bleeding and joint pain.

All studied patient showed pallor except two cases. Splenomegaly was more common in primary cases.

Table No.2: Clinical Feature and Physical Examination Findings

Symptoms		Primary cases (no. of cases showing following findings)	Secondary cases (no.of cases showing following findings)	Total cases of specific complaints
	Fatigue	13	05	18
	Dyspnea on exertion	08	06	14
	Joint pain	04	09	13
	Bleeding	05	10	15
	Weight loss	03	12	15
	Dizziness	03	03	06
	Fever	04	04	08
Physical Examination				
	Pallor	26	32	85
	Splenomegaly	21	12	33

The anemia was graded in severity according to hemoglobin reports as below : Those having HB between 10 to 12 mg/dl considered as mild case, Those having HB between 7 to 10 were labeled as moderate degree anemia and those having HB value less than 7 were considered as severe degree anemia.

Table No-3: Severity of Anemia in AIHA

	Mild anemia	Moderate anemia	Severe anemia
Primary AIHA Cases	03	04	15
Secondary AIHA Case	06	18	08

The other parameters like reticulocytosis, nucleated RBC, Polychromasia etc.were tabulated as below:

Table No.4 : various other parameters in AIHA

Parameters	Primary AIHA	Secondary AIHA
Reticulocytosis	18	12
Nucleated RBC	16	13
Polychromasia	04	03
Thrombocytopenia	05	02
Raised LDH	17	16
Increased bilirubin	22	19

Among all DCT positive 60 cases, 10 patients had shown positive ICT also. In our study warm antibody detected cases were 70% while others were 30%.

Discussion

In Choudhary et al.study[6] most common clinical feature was pallor followed by fever and jaundice. In our study pallor and jaundice were also common and correlate well but fever was present in only 5 cases in our study. In Choudhary et al. study splenomegaly was present in 81% and most of cases of AIHA were primary while in our study secondary cases were more as compared to primary and splenomegaly was present in 33 cases (55%).

In study done by Serrano J [7] warm antibodies were present in 74.5% while cold antibodies were present in 19%. In our study also warm AIHA cases were 70% which correlate well with this study. In Sokol et al.study [8] 95% cases of warm AIHA showed positive DCT test suggesting that DCT can be implied as diagnostic test for AIHA[9]

Usually AIHA affect red blood cells mainly in which antibodies form against it. Hence White blood cells and platelet should be normal, But in our study platelet count decreased by autoimmune destruction suggesting antibodies might form against platelet as well like in Evans syndrome but affection is less than red blood cells.

In our study autoimmune hemolytic anemia dived into primary and secondary cases but some researchers had divided AIHA in four subtypes including idiopathic, associated cases, secondary, accompanying cases [7]. The study regarding such detail classification needs to be done and evaluated accordingly.

The DCT test considered as effective test for diagnosis of AIHA but if antibodies titers are low than some time DCT can be false negative. Some studies had been done regarding this and found that threshold for IG G molecules should be between 100 to 500 in number to give positive DCT.[10,11].

The several reasons why DCT can be false negative in few cases are as follow.

1. There might be low affinity binding antibodies so that while doing cell washing with normal saline antibodies might washed off. Use of low ionic strength solution LISS can eliminate this problem.

2. Sometime antibody titer might low as discussed above to give positive reaction. For that ELISA and Flow cytometry like higher investigations needs to be done. If antibodies are of IG M or IG A type than it cannot be detected by routine DCT test [12].

In our study to detect positive cases accurately, for reliable result, for stable result and to increase

sensitivity we have used gel card testing along with manual DCT. The study regarding usefulness of gel card has been done and it proves that gel card having more sensitivity as compared to manual method [13].

According to some studies incidence per year of warm AIHA estimated to be approximately one per 75000 populations. The onset of presentation is usually insidious and not acute in most of cases. Features in primary AIHA are mainly related to anemia but in secondary cases symptoms from underlying disease may be preceding [14].

In our study to perform DCT we have used polyspecific cooms reagent containing anti IG G and Anti C3. Whenever IG M antibodies are present in AIHA patient they fix the complement so that by anti complement action in coombs sera indirectly IG M will be detected. IG A antibodies have never been found without presence of IG G and IG M type antibodies.

Hence in cooms serum no need for use of Anti IG M and Anti IG A as per WHO manual for transfusion medicine [15].

In our study ICT test was also done to detect free antibodies in plasma or serum which might be present in plasma or serum in few cases because there is a dynamic reaction between RBC and plasma for antibodies and this reaction is reversible. Hence ICT testing also needs to be done [16].

In our case drug induce hemolytic anemia cases were six among all 34 cases of secondary AIHA cases. The common drug we have encountered is ceftriaxone which correlate with study done by Arndt PA et al [17].

Clinical picture in drug induced hemolytic anemia varies according to degree of hemolysis. The history regarding drug exposure therefore should always be elicited in hemolytic anemias. Widespread use of cephalosporin group drug usage for acute infections might be the reason for common drug encountering in drug induced autoimmune hemolytic anemias.

AIHA cases are treated with corticosteroid particularly prednisolone. In steroid non responder cyclophosphamide, azathioprine and cyclosporine like drug given. In selected cases splenectomy can be done. In 30% case benefit from intravenous IgG is also noted [1]

Conclusion

With taking consideration of age, sex, clinical history, physical examination and DCT with laboratory investigation findings help in diagnosis of AIHA. The diagnosis of AIHA should be made accurately as it needs different treatment and special monitoring with clinical follow up. So this type of study should be done to create awareness.

From our study it can be concluded that female having more chance of AIHA (as compared to male) and secondary causes are more common in AIHA with age group between 20 to 30 year have more predilection. It can be concluded from this study that Severity of anemia, jaundice, clinical presentation, degree of hemolysis, splenomegaly are aggressive in primary cases as compared to secondary cases.

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