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Original Research Article

A Study on Role of Coombs Test in Children with Autoimmune Hemolytic Anemia

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Abstract

Background: Autoimmune haemolytic anemia (AIHA) in children is diagnosed by identifying clinical and laboratory evidence of hemolysis and confirming with a direct antiglobulin (Coombs) test (DAT).

Introduction: AIHA is an immune disorder caused by autoantibodies directed against red cell surface antigens, resulting in premature red cell destruction. AIHA is classified as warm, cold, or mixed type. Warm AIHA, mediated mainly by IgG antibodies reactive at 37°C, is the commonest form; it may be primary or secondary to drugs or underlying diseases. Cold AIHA involves IgM antibodies that react at lower temperatures (4–18°C) with complement fixation.

Materials and Methods: This observational study was conducted in the Department of Pathology, Indira Gandhi Institute of Child Health, Bengaluru, from July 2023 to June 2024. A total of 310 children (aged 1–18 years) clinically and hematologically suspected of AIHA were evaluated. Diagnostic workup included peripheral smear examination and DAT by gel card technique (Matrix™ AHG test card). Positive cases underwent antibody typing with monospecific DAT. Results were interpreted based on agglutination patterns.

Results: Among 310 children, 127 (40.96%) were male and 183 (59.04%) female. Warm AIHA constituted 271 (87.41%) cases, followed by mixed (10%), cold (1.63%), and Coombs-negative (0.96%) types. Primary AIHA accounted for 38.7% and secondary for 61.3%. Secondary causes included Systemic Lupus Erythematosus (SLE) (34.21%), Evans syndrome (23.68%), infections (21.06%), autoimmune hepatitis (10%), drug-induced (7.89%), and neoplasms (3.16%).

Conclusion: The gel card Coombs test proved valuable in diagnosing pediatric AIHA and accurately identifying autoantibody type due to its high specificity.

Keywords: Autoimmune Hemolytic Anemia, Direct Coombs Test, Anti-Human Globin, Direct Antiglobulin Test.
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Introduction

Autoimmune haemolytic anaemia (AIHA) is an immune disorder, mainly caused by the presence of auto-antibodies directed against antigens on the surface of red blood cells, leading to premature destruction of the cells. The annual incidence of AIHA in children is reported to be 1–3 cases/100,000 patients. AIHA can be primary (37% Idiopathic) or secondary (63%) may be due to autoimmune diseases, drugs, infections or primary immune deficiencies [1].

AIHA can also present with thrombocytopenia, which is defined as Evans syndrome (ES) [2]. AIHA is classified into warm, cold or mixed subtypes. This thermal-based classification is based on the optimal autoantibody-red blood cells (RBC) reactivity temperatures. Most children suffer from pallor, tiredness, jaundice, dark urine, fever or abdominal pain.

Along with clinical symptoms and signs, children suspected with AIHA are assessed mainly with laboratory investigations such as Complete blood

count (CBC), Reticulocyte count, Serum total bilirubin levels, Haptoglobin levels and direct coombs test (DCT) [3]. Positive DCT is the diagnostic hallmark of AIHA [4]. Warm active antibodies are Immunoglobulin G (IgG) type, may or may not fix complement, have greater affinity at 37ºC (IgG or IgG + C3d). Cold active antibody are IgM type, fix complement and have greater affinity at lower temperature of 3-4 ºC. Initial testing is done by polyspecific DAT, which contain anti IgG and anti C3d (antisera), followed by a monospecific DAT to identify the type of immunoglobulin i e, IgG or Immunoglobulin M (IgM) or Immunoglobulin A (IgA) and complement coating the red blood cells [5]. AIHA is primary when there is no underlying condition and secondary to an infection, immune dysregulation and in neoplastic conditions. Hence, this study was under taken to assess the role of coombs test in children with AIHA and classified based on primary and secondary causes along with identification of type of AIHA based on the type of antibody.

Aims and Objective of the Study: To assess the role of Coombs test in children with AIHA and to identify type of AIHA.

Materials and Methods

This study was conducted at the Department of Pathology of Indira Gandhi Institute of Child Health, Bengaluru, Karnataka, South India over a period of one year after obtaining approval from Institutional Ethics Committee: IGICH/ACA/EC/05/2023-24. Children between the age of one and eighteen years suspected with autoimmune hemolytic anemia referred to the department of pathology. Children with hemoglobin of less than 11 g/dL, reticulocyte count of more than 2%, peripheral smear findings showing autoagglutins, polychromatophils, nucleated red blood cells, spherocytes, microspherocytes and serum findings (raised serum total bilirubin and serum lactate dehydrogenase) were enrolled in the study. This was followed by Direct coombs test

Flowchart of Methodology

Inclusion Criteria: Subjects newly diagnosed with AIHA by peripheral smear examination aged from 1year to 18 years.

Exclusion Criteria: Subjects with other haemolytic anaemias, history of recent blood transfusion.

Results

Of the 310 cases studied, the age of the children ranged from 1-18 years, AIHA was more common in the age group of 5-12 years in 106 (34.19%) cases, 103 (33.23%) cases in 12-18 years and 101 (32.58%) cases in the age group of 1-5 years as depicted in Table 1.

Table 1: Showing distribution of study population according to age & gender

Demographic	Category	Number of Cases (%)
Age Group	1-5 years	101 (32.58%)
	5-12 years	106 (34.19%)
	12-18 years	103 (33.23%)
Gender	**Males**	**127 (40.96%)**
	Females	**183 (59.04%)**
Female: Male Ratio	1.4:1	

In the present study out of 310 cases, 3 cases belong to coombs negative AIHA. Polyspecific DAT showed 4+ reactivity in 43 cases, 3+ in 95 cases, 2+ in 130 cases and 1+ in 39 cases, which showed 2+ reactivity and Negative in 3 cases. Monospecific DAT, Only IgG showed 4+ reactivity in 39 cases, 3+ in 75 cases, 2+ in 122 cases, 1+ in 35 and we found both IgG and C3d were positive in 31 cases, the presence of only C3d in 5 cases as depicted in Table 2 & 2A.

Table 2: Showing Distribution of study population in various age group by Polyspecific and Monospecific AHG test

Strength of reactions	Polyspecific DAT		
	1-5years (%)	**5-12 years (%)**	**12-18 years (%)**
4+	23 (22.77)	9 (8.49)	11 (10.6)
3+	26 (25.74)	34 (32.07)	35 (33.9)
2+	40 (39.60)	52(49.06)	38 (36.8)
1+	12 (11.89)	11 (10.38)	16 (15.5)
Negative	-	-	03 (2.9)
Total	**101 (100.00)**	**106(100.00)**	**103(100.00)**

Table 2A: Showing Distribution of strength by Monospecific AHG test

Strength of reactions	Monospecific DAT			Total
	Only IgG +	Both IgG + C3d +	Only C3d	
4+	39	2	02	43
3+	75	17	03	95
2+	122	8	-	130
1+	35	4	-	39
Negative	-	-	-	3
Total	**271**	**31**	**05**	**310**

Based on thermal classification of AIHA in this study, among 310 cases the most common type was warm AIHA in 271(87.41%) cases followed by mixed AIHA in 31(10.00%) cases, there were 5(1.63%) cases of cold AIHA and 3 cases of coombs negative AIHA were found as depicted in TABLE 3. In this study, we found that out of 310 cases, 120(38.70%) cases were due to primary cause and rest 190 (61.30%) cases due to secondary cause as depicted in TABLE 3. Among those 120 primary causes of AIHA cases, 101 (84.16%) cases belong to warm type of AIHA, 15(12.5%)cases belongs to mixed type of AIHA, cold AIHA and coombs negative AIHA was seen in 2(1.67%) cases and among 190 secondary causes of AIHA 170(89.47%) cases belongs to warm type of AIHA, 16(8.43%) belongs to mixed type AIHA, there were 5 (1.6%) cases of cold AIHA and 3 (0.1 %) case of coombs negative AIHA was found as depicted in TABLE 3.

Table 3: Showing distribution of cases among study population based on based on thermal classification of AIHA

Sl no	Type of AIHA	Primary cause (%)	Secondary cause (%)
1	**Warm AIHA (IgG)**	101 (84.16)	170 (89.47)
2	**Mixed AIHA (IgG +C3d)**	15 (12.5)	16 (8.43)
3	**Cold AIHA (C3d)**	02 (1.67)	03 (1.58)
4	**Coombs negative AIHA**	02 (1.67)	01 (0.01)
Total no of cases		120 (100.00)	190 (100.00)

The most common etiology of secondary AIHA in this present study was due to SLE in 65(34.21%) cases, followed by ES in 45(23.68%) cases, infection in 40(21.06%) cases, autoimmune hepatitis in 19(10.00%), due to drug induced in 15(7.89%) cases and least is due to neoplasm in 6(3.16%) cases as depicted in TABLE 4.

Table 4: Showing distribution of study population based on etiology in secondary AIHA cases

Sl No	Etiology in secondary AIHA	No of cases	Percentage
1	SLE	65	34.21
2	Evans syndrome	45	23.68
3	Infection	40	21.06
4	Autoimmune hepatitis	19	10.00
5	Drug induced	15	7.89
6	Neoplasm	6	3.16
Total		190	100.00

Discussion

In this study, of 310 children with AIHA, females constituted 59.04% (n = 183) and males 40.96% (n = 127), yielding a female-to-male ratio of 1.4:1 and demonstrating a clear female predominance.

This gender distribution is consistent with observations reported by Arora S et al., Abdel-Salam A, Aladjidi N, and Naithani R. [1,4,5,9] In contrast, Sanchez et al documented a male predominance (72%), highlighting potential geographic, genetic, or referral-related variations in disease patterns. As our study population included children and adolescents aged 1–18 years—comparable to most published pediatric cohorts—the observed female predominance further reinforces the recognized autoimmune bias toward females even within pediatric age groups. [7]

Table 5: Table showing distribution of study population in children with the type of AIHA in various study

Studies	Type of AIHA	Present study	Arora S et al [1] 2021	Thatikonda K B et al [3] 2021	Sanchez M N et al [7] 2021	Barcellini W et al [8] 2014
Number of cases in %	Warm AIHA (IgG)	87.41	72.72	28.00	76.00	60.00
	Mixed AIHA (IgG +C3d)	10.00	18.19	15.00	0	8.00
	Cold AIHA (C3d)	1.63	0	35.00	24.00	27.00
	Coombs negative AIHA	0.96	9.09	6.00	4.00	5.00

With respect to serological classification, warm AIHA was overwhelmingly predominant in our cohort (87.41%), followed by mixed AIHA (10.00%), cold AIHA (1.63%), and DAT-negative AIHA (0.96%).

The predominance of warm AIHA aligns with findings reported by Sanchez M N7 and Barcellini W, 8, both of whom identified warm antibody-mediated hemolysis as the most common subtype in pediatric and mixed populations. However, these findings are discordant from Thatikonda K B (2021), who reported cold AIHA as the most common subtype (35%), followed by warm (28%) and mixed (15%) forms. Such discrepancies may reflect difference in patient demographics, environmental triggers, underlying etiologies, or diagnostic methodologies. [3] Present study showed that the incidence of warm type of AIHA was the highest (87.41%), followed by mixed type AIHA (10.00%) and cold type AIHA (1.63%) with DAT negative AIHA in (0.96%) cases. DAT-negative AIHA was identified in 0.96% of cases, including two primary and one secondary AIHA cases. These cases are likely attributable to low-affinity IgG antibodies or IgA antibodies undetectable by routine DAT techniques. The low incidence in our study parallels rates described in prior literature, underscoring the importance of clinical correlation and extended immunohematologic evaluation in suspected cases with negative routine testing.

AIHA was further subclassified into primary and secondary forms. In our study, secondary AIHA (61.30%) predominated over primary AIHA (38.70%), corroborating findings from study by Abdel-Salam A and Aladjidi N. [5,9] Whereas it was discordant with findings reported by Thatikonda K B and Naithani R, where primary AIHA accounted for 64% and 65% of cases, respectively. These variations likely reflect differences in referral patterns, underlying disease prevalence, and regional epidemiology. [3,9]

Table 6: Table showing comparison of secondary causes in various studies

Studies		Present studies	Thatikonda K B et al [3] 2021	Abdel-Salam A et al [4] 2023	Aladjidi N et al [5] 2011	Naithani R et al [9] 2009
No of cases	SLE	**34.21**	10.00	24.00	**-**	22.00
	ES	**23.68**	6.00	-	37.00	22.00
	Infection	**21.06**	10.00	20.00	10.00	22.00
	Autoimmune hepatitis	**10.00**	-	-	-	-
	Drug induced	**7.89**	-	4.00	-	-
	Neoplasm	**3.16**	6.00	**-**	-	-

Among secondary AIHA cases in our study, systemic lupus erythematosus (SLE) emerged as the leading etiology, accounting for 34.21% of cases. This observation is in agreement with studies by Abdel-Salam A and Thatikonda K B, reinforcing the well-established association between pediatric AIHA and SLE. Evans syndrome (ES) was the second most common cause (23.68%), characterized by the sequential or simultaneous occurrence of autoimmune hemolytic anemia with immune thrombocytopenia and/or neutropenia. Although Aladjidi N reported a higher proportion of ES (37%) in secondary AIHA, our findings still underscore its substantial contribution to disease burden. [3,4,5,9]

Infectious aetiologies accounted for 21.06% of secondary AIHA cases. Warm and mixed AIHA were most commonly associated with retroviral (HIV) infection, hepatitis A, and Plasmodium vivax malaria, while cold AIHA was predominantly linked to Mycoplasma and Cytomegalovirus infections. These findings highlight the heterogeneity of infectious triggers and their immunopathogenic roles in hemolysis. In certain published series, infection has been identified as the leading cause of secondary AIHA, emphasizing regional variability in etiological distribution.

Drug-induced AIHA represented 7.89% of secondary cases, most commonly associated with zidovudine and dapsone. Although this proportion is notable, it remains lower than that reported by Abdel-Salam A. Childhood malignancies accounted for 3.16% of cases, including acute lymphoblastic leukemia, acute myeloid leukemia, and myelodysplastic syndromes. This rate is lower than the 6% reported by Thatikonda K B, but nonetheless emphasizes the importance of malignancy screening in pediatric AIHA. [3,4]

Interestingly, autoimmune hepatitis was identified in 10.00% of secondary AIHA cases in our cohort—a finding that appears underreported or absent in many comparable studies. This distinctive observation may reflect differences in diagnostic vigilance or regional autoimmune disease patterns and warrants further investigation.

Overall, our study confirms that pediatric AIHA demonstrates a strong female predominance, overwhelming dominance of warm antibody-mediated hemolysis, and a higher incidence of secondary over primary AIHA. Systemic autoimmune diseases—particularly SLE and Evans syndrome—constitute the leading etiologies of secondary AIHA, followed by infections, drug exposure, and malignancy. The etiological spectrum observed in our cohort largely aligns with existing literature, while also revealing unique regional variations that contribute valuable insights to the understanding of pediatric AIHA.

Conclusion

This study concluded that coombs test plays an important role in diagnosing autoimmune haemolytic anemia.

AIHA is not an uncommon problem in children in the recent years especially children. Majority of the cases are due to secondary causes, was seen in children belonging to adolescent age group. Hence, children presenting with severe anaemia with/without leucopenia or thrombocytopenia with organomegaly, coombs test should be performed to rule out AIHA.

Monospecific DAT by gel card technique was most effective in thermal classification of AIHA. It aided in early diagnosis, classification and prompted to administer appropriate treatment which improved the prognosis.

Summary

This study evaluated the role of the Direct Coombs (DAT) test in 310 pediatric patients diagnosed with autoimmune hemolyticanemia (AIHA) and characterized the disease spectrum.

AIHA showed a clear female predominance (59.04%) with a female-to-male ratio of 1.4:1. Warm AIHA was the most common subtype, accounting for 87.41% of cases, followed by mixed AIHA (10.00%) and cold AIHA (1.63%). DAT-negative AIHA was rare.

Polyspecific DAT demonstrated predominantly moderate (2+) reactivity. Monospecific testing revealed IgG as the principal autoantibody, while C3d positivity was less frequent. Combined IgG and C3d positivity was observed in a subset of cases, reinforcing the immunohematological heterogeneity of AIHA.

Secondary AIHA (61.30%) was more common than primary AIHA (38.70%). Among secondary causes, systemic lupus erythematosus was the leading etiology (34.21%), followed by Evans syndrome (23.68%), infections (21.06%), autoimmune hepatitis (10.00%), drug-induced causes (7.89%), and neoplasms (3.16%).

Overall, the findings highlight the predominance of warm AIHA in children, the diagnostic utility of DAT—particularly IgG detection—and the significant contribution of secondary autoimmune disorders, especially systemic lupus erythematosus, in pediatric AIHA.

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