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Prevalence and Causes of Direct Antiglobulin Test Positivity in Hospitalized Paediatric Patients: Experience from a Tertiary Care Centre in North India

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Abstract: ***Background: The direct antiglobulin test (DAT) is an essential immunohematological investigation used to detect in vivo sensitization of red blood cells by immunoglobulin G (IgG), complement components, or both. Although DAT is widely employed in the evaluation of immune-mediated hemolysis, positive results may also occur in several non-hemolytic clinical conditions, particularly in hospitalized paediatric patients. Limited data are available regarding the prevalence and causes of DAT positivity in the Indian paediatric population. Objective: To determine the prevalence of direct antiglobulin test positivity among hospitalized paediatric patients and to evaluate its association with various clinical etiologies. Materials and Methods: A hospital-based prospective cross-sectional study was conducted over one year (July 2024 to June 2025) at a tertiary care centre in North India. A total of 400 hospitalized paediatric patients meeting the inclusion criteria were enrolled. All samples were screened using a polyspecific DAT by the column agglutination technique. Samples with positive polyspecific DAT results were further evaluated using monospecific anti-IgG and anti-C3d reagents to characterize the type of red cell sensitization. Results: Of the 400 patients studied, 23 (5.75%) demonstrated DAT positivity. Among these, isolated C3d positivity was observed in 9 (39.1%) patients, isolated IgG positivity in 8 (34.8%), and combined IgG with C3d positivity in 6 (26.1%). DAT positivity was most frequently associated with infectious conditions (43.5%), followed by anaemia (21.7%), sepsis (17.4%), and miscellaneous disorders (17.4%). Pneumonia was the most common infection associated with DAT positivity. Conclusion: The prevalence of DAT positivity among hospitalized paediatric patients was 5.75%. Complement-mediated red cell sensitization was the predominant serological pattern, with infections- particularly pneumonia- representing the most frequent associated clinical condition. Monospecific DAT testing provides valuable diagnostic information by identifying the nature of red cell sensitization and facilitates accurate interpretation of positive DAT results in paediatric patients.***

Keywords: Direct antiglobulin test, DAT, paediatric patients, immunohematology, red blood cell sensitization, IgG, C3d, column agglutination technique.

1. Introduction

The direct antiglobulin test (DAT), commonly known as the direct Coombs test, is a fundamental immunohematological assay used to detect the in vivo coating of red blood cells (RBCs) with immunoglobulins and/or complement components. The test plays a pivotal role in the diagnosis of immune-mediated hemolytic disorders by identifying immunoglobulin G (IgG), complement component C3d, or both on the surface of circulating erythrocytes. A positive DAT indicates red cell sensitization but does not, by itself, establish the diagnosis of autoimmune hemolytic anemia (AIHA). Therefore, interpretation of DAT results should always be correlated with the patient's clinical presentation, hematological findings, and additional serological investigations.

The DAT is routinely performed using a polyspecific antihuman globulin (AHG) reagent containing antibodies against both IgG and complement (C3d). When the initial screening test is positive, further evaluation with monospecific anti-IgG and anti-C3d reagents is recommended to determine the nature of red cell coating. This distinction has important clinical implications. Isolated IgG positivity is commonly associated with warm autoimmune hemolytic anemia or alloantibody-coated donor erythrocytes following transfusion, whereas isolated C3d positivity is more frequently observed in complement-mediated disorders, cold-reactive antibodies, certain infections, and other non-immune conditions. Combined IgG and C3d positivity may indicate more complex immunological mechanisms requiring detailed serological evaluation.

Although the DAT is primarily employed in the investigation of hemolytic anemia, positive results have also been reported in several clinical conditions without overt hemolysis. These include bacterial and viral infections, sepsis, autoimmune disorders, malignancies, recent blood transfusion, and certain medications. Consequently, a positive DAT should be interpreted as a laboratory finding indicating red cell sensitization rather than definitive evidence of immune hemolysis.

In paediatric practice, the clinical significance of DAT positivity remains incompletely understood because children frequently present with infectious and inflammatory illnesses that may influence red cell immunology without producing clinically significant hemolysis. Furthermore, the prevalence and serological patterns of DAT positivity vary according to patient population, disease spectrum, laboratory methodology, and geographical region. Most published studies have focused on transfusion-dependent disorders such as thalassemia or on patients with autoimmune hemolytic anemia, whereas information regarding the prevalence of

DAT positivity among general hospitalized paediatric patients is relatively limited.

Identifying the immunological pattern of DAT positivity using monospecific reagents can provide valuable diagnostic information, assist in differentiating immune from non-immune causes of red cell sensitization, and guide appropriate clinical management. Knowledge of the underlying conditions associated with DAT positivity is also important for avoiding unnecessary investigations and ensuring accurate interpretation of laboratory findings.

2. Review of Literature

The direct antiglobulin test (DAT) remains one of the most important immunohematological investigations for detecting in vivo sensitization of red blood cells by immunoglobulins and complement proteins. Since its introduction by Coombs and colleagues, the DAT has become an indispensable diagnostic tool in transfusion medicine, autoimmune hemolytic anemia (AIHA), hemolytic disease of the fetus and newborn, and transfusion reaction investigations. More recently, its clinical significance has been explored in patients without overt hemolysis, particularly among hospitalized individuals with infectious, inflammatory, or autoimmune disorders.

Yadav et al. reported a significant prevalence of DAT positivity among multiply transfused thalassemia patients in northern India and demonstrated that positive DAT results were associated with increased transfusion requirements, elevated serum ferritin concentrations, and previous transfusion exposure. Their findings highlighted the importance of monospecific DAT testing for distinguishing IgG-mediated and complement-mediated red cell sensitization and emphasized its role in transfusion management.

Systematic reviews and meta-analyses have further confirmed that alloimmunization represents one of the major complications of chronic transfusion therapy. Although DAT positivity and alloimmunization frequently coexist, they represent distinct immunological phenomena. A positive DAT reflects red cell sensitization occurring in vivo, whereas alloimmunization refers to the production of antibodies directed against foreign red cell antigens following transfusion. Consequently, DAT positivity should not be interpreted as definitive evidence of alloimmunization without additional serological investigations such as antibody screening, antibody identification, and elution studies.

The serological characteristics of DAT-positive patients have also been extensively investigated. Monospecific testing has demonstrated three principal patterns of red cell coating: isolated IgG, isolated complement (C3d), and combined IgG with complement. IgG-positive DAT is commonly associated with warm autoimmune hemolytic anemia and transfusion-related alloantibodies, whereas isolated C3d positivity is more frequently observed in complement-mediated immune reactions, cold-reactive antibodies, infections, and certain non-immune conditions. Combined IgG and C3d positivity may indicate more complex immune mechanisms requiring comprehensive immunohematological evaluation.

The available evidence therefore indicates that DAT positivity is influenced by multiple clinical and immunological factors and cannot be regarded as synonymous with hemolytic disease. Accurate characterization using monospecific anti-IgG and anti-C3d reagents is essential for understanding the mechanism of red cell sensitization and improving clinical interpretation. In view of the limited data available for hospitalized paediatric patients in India, the present study was undertaken to determine the prevalence of DAT positivity and evaluate its association with various clinical etiologies in a tertiary care setting.

3. Aim and Objectives

Aim

To determine the prevalence of direct antiglobulin test (DAT) positivity among hospitalized paediatric patients and to evaluate its association with various clinical etiologies at a tertiary care centre in North India.

Objectives

1) To characterize DAT-positive samples using monospecific anti-IgG and anti-C3d reagents following an initial positive polyspecific DAT.
2) To assess the association between DAT positivity and various clinical conditions, including infections, sepsis, anaemia, and other underlying disorders.

4. Materials and Methods

Study Design

A hospital-based prospective cross-sectional observational study was conducted to determine the prevalence of direct antiglobulin test (DAT) positivity.

Study Setting and Duration

The study was carried out in the Department of Transfusion Medicine in collaboration with the Department of Paediatrics at a tertiary care teaching hospital in North India. The study was conducted over a period of one year, from **1 July 2024 to 30 June 2025**.

Study Population

The study included hospitalized paediatric patients fulfilled the predefined inclusion and exclusion criteria.

Sample Size

A total of **400 hospitalized paediatric patients** were enrolled consecutively during the study period.

Inclusion Criteria

- Hospitalized paediatric patients (<15 yrs) requiring laboratory evaluation during the study period.
- Patients whose parents provided informed consent for participation.

Exclusion Criteria

- Patients with inadequate or haemolysed blood samples.
- Patients or guardians who declined participation in the study.

Sample Collection
Approximately 2–3 mL of peripheral venous blood was collected aseptically in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes following standard phlebotomy procedures. All samples were properly labelled and transported promptly to the immunohematology laboratory for analysis.

Laboratory Methods
Direct antiglobulin testing was performed using the **column agglutination technique (CAT)**.

Initially, all patient samples were screened using a **polyspecific antihuman globulin (AHG) reagent**. Samples demonstrating a positive polyspecific DAT were subsequently tested using **monospecific anti-IgG and anti-C3d reagents** to determine the specific pattern of red cell sensitization.The strength of agglutination reactions was graded as **1+, 2+, 3+, or 4+** according to standard immunohematological guidelines.

Data Collection
Clinical and laboratory information, including patient demographics, primary diagnosis, and relevant clinical findings, was collected from hospital records using a structured data collection form. DAT-positive patients were further classified according to their underlying clinical conditions, including infections, sepsis, anaemia, and other miscellaneous disorders.

Outcome Measures
The primary outcome was the prevalence of DAT positivity among hospitalized paediatric patients.

Secondary outcomes included:Distribution of monospecific DAT reactivity patterns and frequency of various grades of DAT positivity.

Statistical Analysis
Data were entered into Microsoft Excel and analysed using appropriate statistical software. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range, depending on data distribution. Categorical variables were presented as frequencies and percentages. Descriptive statistical methods were used to summarize the study findings. Where applicable, associations between categorical variables were assessed using the Chi-square test or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Ethical Considerations
The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from the parents or legal guardians of all participating children before enrolment. Patient confidentiality was maintained throughout the study, and all collected data were used solely for research purposes.

5. Results

A total of **400 hospitalized paediatric patients** were enrolled in the study during the study period. All samples were subjected to direct antiglobulin testing (DAT) using a polyspecific antihuman globulin reagent by the column agglutination technique.

5.1 Prevalence of DAT Positivity

Among the 400 patients evaluated, **23 patients were DAT positive**, giving an overall prevalence of **5.75%**. The remaining **377 patients (94.25%)** were DAT negative.
Of the 23 DAT-positive patients, **13 (56.52%)** were males and **10 (43.48%)** were females.

5.2 Grading of Polyspecific DAT Reactivity

The strength of agglutination observed in DAT-positive samples varied as follows:

DAT Reaction Grade	Number of Cases (n=23)	Percentage (%)
1+	6	26.09
2+	11	47.83
3+	4	17.39
4+	2	8.69

Grade **2+** reactivity was the most frequently observed pattern, accounting for nearly half of all DAT-positive cases.

5.3 Monospecific DAT Findings

All polyspecific DAT-positive samples were further evaluated using monospecific anti-IgG and anti-C3d reagents.

Monospecific DAT Pattern	Number of Cases	Percentage (%)
Isolated C3d positivity	9	39.13
Isolated IgG positivity	8	34.78
Combined IgG + C3d positivity	6	26.09

Complement-mediated sensitization (isolated C3d positivity) was the most common serological pattern identified among DAT-positive patients.

5.4 Clinical Distribution of DAT-Positive Patients

The clinical conditions associated with DAT positivity are summarized below.

Clinical Condition	Number of Cases	Percentage (%)
Infections	10	43.48
Anaemia	5	21.74
Sepsis	4	17.39
Miscellaneous disorders	4	17.39

5.5 Overall Findings

The study demonstrated that DAT positivity was relatively uncommon among hospitalized paediatric patients, with an overall prevalence of **5.75%**. Complement-mediated red cell sensitization was more frequent than isolated IgG sensitization. Most DAT-positive patients were diagnosed with infectious illnesses, particularly pneumonia, followed by anaemia and sepsis. These findings indicate that DAT positivity in hospitalized children is not confined to

autoimmune haemolytic disorders and may also occur in a variety of infectious and inflammatory conditions.

6. Discussion

The present prospective cross-sectional study evaluated the prevalence of direct antiglobulin test (DAT) positivity among hospitalized paediatric patients and examined its association with various clinical conditions. Among the 400 children included in the study, 23 were DAT positive, corresponding to an overall prevalence of **5.75%**.

Evaluation of agglutination strength demonstrated that **2+ reactions** were the most frequent, accounting for nearly half of all positive cases. Weak (1+) reactions were also common, whereas strong (3+ and 4+) reactions were observed less frequently. The intensity of DAT reactivity reflects the degree of red cell sensitization but does not necessarily correlate with the severity of hemolysis.

Monospecific DAT testing revealed that **isolated C3d positivity (39.13%)** was the predominant pattern, followed by **isolated IgG positivity (34.78%)** and **combined IgG with C3d positivity (26.09%)**. These findings emphasize the diagnostic value of monospecific testing after an initial positive polyspecific DAT. Isolated C3d positivity suggests complement-mediated red cell sensitization, which may occur in association with infections, cold-reactive antibodies, immune activation, or other inflammatory conditions. In contrast, isolated IgG positivity is more commonly associated with warm-reactive autoantibodies or alloantibody-coated erythrocytes, whereas combined IgG and C3d positivity may indicate more complex immunological mechanisms.

The present study demonstrated that **infectious diseases accounted for the largest proportion of DAT-positive cases (43.48%)**, with pneumonia being the most frequently encountered infection. These findings support previous reports that acute infections can induce transient red cell sensitization through activation of the immune and complement systems. Cytokine release, immune complex formation, and complement activation during infection may result in deposition of immunoglobulins or complement proteins on erythrocytes without producing clinically significant hemolysis.

The findings of the present study highlight the importance of performing monospecific DAT following a positive polyspecific screening test.

The present study has certain limitations. It was conducted at a single tertiary care centre, which may limit the generalizability of the findings to other populations. The number of DAT-positive patients was relatively small, reducing the statistical power for subgroup analyses. In addition, antibody elution studies and extended immunohematological investigations were not performed routinely; therefore, the specific antibody responsible for red cell sensitization could not be confirmed in all cases. Future multicentre studies involving larger patient populations and comprehensive immunohematological characterization are warranted to further define the clinical significance of DAT positivity in hospitalized paediatric patients.

7. Conclusion

The present study demonstrated that the prevalence of direct antiglobulin test (DAT) positivity among hospitalized paediatric patients was **5.75%**. Monospecific DAT evaluation revealed that isolated **C3d positivity** was the predominant serological pattern, followed by isolated **IgG positivity** and combined **IgG with C3d positivity**.

The routine use of monospecific anti-IgG and anti-C3d reagents following an initial positive polyspecific DAT provides valuable information regarding the mechanism of red cell sensitization and improves diagnostic accuracy.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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