



Role of Coombs' Test in analysis of Immunohematological Cases

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ABSTRACT: Anti human globulin test (AHG) comprising of both Direct Coombs' Test (DAT) and Indirect Coombs' Test (IAT) can be used to detect RBC sensitized with Ig G auto and alloantibodies, complement occurring in vivo and vitro. The present study was conducted in 204 cases on which 360 tests comprising of both DAT and IAT were carried out. Out of 29 positive Coombs' cases, 11 were positive for DAT, 16 were positive for IAT and 02 had both DAT and IAT positivity. Maximum cases DAT and IAT positive cases were autoimmune hemolytic anemia (AIHA) and Rh incompatibility respectably. In our set up 5.6 % cases were DAT positive and 9.3 % cases were IAT positive. Any immune-hematological work up is incomplete without the intervention by anti-globulin tests which should be mandatory for the better management of patients.

KEY WORDS: Coomb, hemolytic anemia

INTRODUCTION:

Coombs' test also known as Anti Human Globulin test (AHG) was described in 1945, by Coombs and associates for detection of weak and nonagglutinating Rh antibodies in serum. Direct antiglobulin test (DAT) was described by Coombs and coauthors in 1946 describing use of AHG to detect in vivo sensitization of RBC of babies suffering from hemolytic disease of new born (HDN).¹The Antiglobulin test can be used to detect RBC sensitized with Ig G alloantibodies ; Ig G autoantibodies and complement components occurring in vivo or in vitro. Indirect Antiglobulin

Test (IAT) refers to the use of AHG in detecting in vitro sensitization of RBC as two-stage technique. The present study deal with the evaluation of DAT and IAT in all the immunohematological cases keeping in view to the past and present medical history and presentations.

MATERIALS AND METHODS:

The present study was carried out in the Department of Transfusion Medicine on 204 patients whose blood samples were sent for Coombs' test. Female patients constituted

maximum number of cases 138 followed by 50 male patients and 16 babies. (Fig 1)

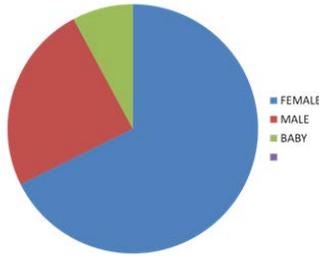


Fig. 1 Coombs' test performed on different groups
The age group constituting maximum number of 46 cases was in the range of 21 to 30 years.(Fig 2)

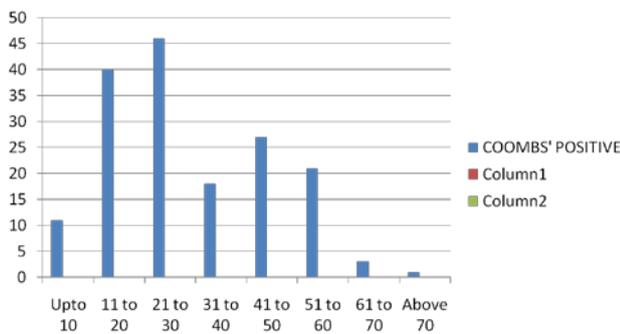


Fig.2 Different age groups on whom Coomb test has been performed

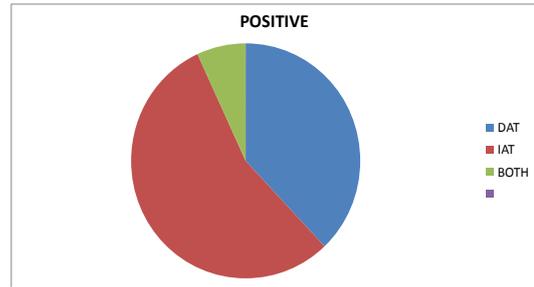


Fig .3 Positive numbers of different Coombs' tests
For DAT, 1-2 drops of EDTA anticoagulated patients' red cells were washed three times with isotonic saline in test tubes . After decanting the supernatant completely following the third wash, to the dry botton Polyspecific AHG was added and incubated in the room temperature for 5 minute. The tubes were centrifuged at 1000rpm for 1 minute. Immediately after gentle re-suspension of the agglutination, macroscopic examination was done using lighted agglutination viewer. When the result was negative, we examined for the agglutination under microscope. To negative result interpreted microscopically, one drop of Ig G- coated Coombs Control Cell was added to the tube and centrifuged lightly for 1 minute at 1,000 rpm. It was immediately re-suspended gently and examined macroscopically for agglutination. A positive reaction at this state

confirmed a negative test. If the result was negative after addition of the Ig G coated Coombs Control Cells, the test was reported to be invalid and was repeated.

For IAT, to 2 drops of patient's serum 1 drop of 5% saline-suspended reagent group O cells is added in the test tubes and mixed. The test tubes were then centrifuged at 1000 rpm for 1 minute and were observed for hemolysis and agglutination. The tubes were incubated at 37°C for 30 to 60 minutes followed by centrifugation and observation was done for hemolysis and agglutination. Again, red cells were washed for three times with saline; completely decanting the final wash. AHG was added to the dry red cell button and was mixed well. The test tubes were centrifuged and were observed for agglutination. Results were graded and were recorded. Validity of the negative results was confirmed by addition of IgG-coated red cells.

RESULTS:

In 204 patients' number of DAT and IAT performed were 188 and 172 respectively total constituting 360 Coombs' tests. Out of 29 positive

Coombs' cases, 11 were positive for DAT, 16 were positive for IAT and 02 had both DAT and IAT positivity. (Fig 3) Maximum cases (8) DAT positive cases were autoimmune hemolytic anemia (AIHA) followed by 2 cases with Systemic Lupus Erythematosus (SLE) and one case had history of multiple transfusion. Out of 16 positive ICT cases, maximum cases constituting 9 in number had Rh incompatibility followed by 5 cases of multiple transfusions, 2 cases of hemolytic anemia, and one case each with rheumatoid arthritis and squamous cell carcinoma. Two cases positive for both DAT and IAT were diagnosed one as hemolytic anemia and other with Acute Vascular Necrosis of hip with sickle cell disease having multiple transfusions.

DISCUSSION:

Antiglobulin test detects red cell antibodies which are bound and do not produce direct agglutination. AHG reacts with human antibodies and complement bound to red cells and also unbound to cells, free in serum. AHG sera can be used for performing DAT and IAT. DAT demonstrate in-vivo sensitization of red cells and

is performed on the patients' washed red cells to which AHG is to be added. IAT demonstrates in-vitro reactions between red cells and antibodies which can be performed on serum of patients from which red cells are washed to remove unbound globulins.²

DAT is primarily used for investigation of hemolytic transfusion reactions, hemolytic disease of the fetus and newborn (HDFN), autoimmune hemolytic anemia (AIHA), and drug-induced immune hemolysis. The predictive value of positive DAT is 83% in a patient with hemolytic anemia, and only 1.4% in a patient without hemolytic anemia.³ IAT is used for antibody detection, antibody identification, crossmatching and blood group phenotyping.

DAT is performed by testing freshly washed red cells directly with Antiglobulin reagents containing anti-IgG and anti-C3d. False negative or weaker result can be obtained if the washed red cells are allowed to sit before testing with anti-IgG or delay in the reading. False positive results can arise in degradation of specimens causing non-specific binding of DAT

reagents. Causes of false positivity in Antiglobulin test include over-centrifugation, under agitation, prolonged delay in testing, clotted specimen, reagent issues and spontaneous agglutination.^{4,5}

Causes of false negative results include neutralization of AHG reagent by failure to wash cells inadequately, interruption in testing, improper reagent storage, over-centrifugation.²

Positive DATs are reported in 1:1000 up to 1:14,000 blood donors and 1% to 15% of hospital patients.⁶ In our set up 5.6 % cases are DAT positive and 9.3 % cases are IAT positive. Maximum number of DAT was positive in autoimmune hemolytic anemia followed by SLE and multiple transfusions. ICT was found to positive in maximum number in Rh incompatibility followed by multiple transfusion, hemolytic anemia, rheumatoid arthritis and squamous cell carcinoma. Whereas both DAT and IAT were positive in hemolytic anemia.

CONCLUSION:

Maximum number of positive result of DAT and IAT in immunohematological cases in our study strongly recommend the routine use of AHG test

in the work up of these cases as well Coombs' cross match for the better management of the patients.

REFERENCES:

1. Coombs RR Historical note; past, present and future of the Antiglobulin test. *Vox Sang* 1998; 74: 67-73
2. Leger RM. The Positive Direct Antiglobulin test and Immune-Mediated hemolysis. In Roback JD, Grossman BJ, Harris Teresa, Hillyer CD, editors. *Technical Manual*. 17th ed. New York: American
3. Kalpana HS, Garraty G. Predictive value of direct antiglobulin test results. *Diagnostic Med* 1985; 8: 29-32
4. South SF. Use of the direct antiglobulin test in routine testing. In: Wallace Ma LJS 9th Ed, *Current Applications and Interpretations of the Direct Antiglobulin Test*. American Association of Blood Banks Arlington, VA, USA. 1988; 25-45
5. Vovak D, Downie DM, Moore BP, Ford DS, et al. Replicate tests for the detection and correlation of errors in anti-human globulin (AHG) tests:

optimum conditions and quality control. *Haematologica* 1988; 21: 3-16

6. Petz LD, Garraty G. *Immune hemolytic anemias*. 2nd ed. Philadelphia: Churchill-Livingstone, 2004