

## A STUDY ON SIGNIFICANCE OF COOMBS TEST AND ANTIBODY SCREENING IN PATIENTS WITH CHRONIC ANAEMIA IN A TERTIARY CARE HOSPITAL



## Immunohaematology

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## ABSTRACT

Most of the inpatients admitted in our hospital for the evaluation of anemia needs blood transfusion as part of the treatment. A total of 719 cases from medical ward received blood transfusion from the period of January 2016 to December 2016. Irrespective of the provisional diagnosis of case, we performed Direct Coombs test, Indirect Coombs test, Autocontrol and 3 cell antibody panel from the samples received for cross matching. Of that 532 case were females, 187 cases were male patients. Our transfusion protocol included identification of presence of antibodies including the following antibodies: A, B, C, c, D, E, e, Lea, Leb, K, Fya, Fyb, Jka, Jkb, M, N, S, s, by using 3 cell panel. Screening for antibodies was performed before transfusion, according to standard antiglobulin techniques. If screening was positive, the antibody was identified by means of panels of reagent red cells, identification was confirmed by a regional reference laboratory. We identified most of the female patients between the age group 20 to 30 with chronic anemia were found to have DCT positive hemolytic anemia. Few patients had only DCT positive without abnormal antibodies and some had DCT negative AIHA.

## KEYWORDS

Chronic anemia, Auto immune hemolytic anemia, Coombs test

## INTRODUCTION

Women are at risk of iron-deficiency anemia and older adults of both sexes have a greater risk of anemia because of the underlying causes like nutritional deficiency, kidney disease or other chronic medical conditions. Mostly anemia divided into three groups, Anemia caused by blood loss, Anemia caused by decreased production of red blood cell and Anemia caused by destruction of red blood cells. Not all the anemia are corrected by blood transfusion alone. Though the etiology might differ, some patients with auto immune hemolytic anemia will worsen the state of anemia with blood transfusion due to lysis of RBCs. On this back ground we proceeded with doing DCT, ICT, Auto control and 3 cell panel for all the blood samples of patients with anaemia with hemoglobin less than 6 gms who required blood transfusion for the correction of anemia.

Interestingly our results demonstrated that auto antibodies were found in all the age groups including neonates, and alloantibody in few samples. The frequency of hemolytic anemia that we found among the adult patients is similar to or higher than previously reported rates. Our interest is to identify the auto antibodies and alloantibodies before transfusion in order to restrict the transfusion in risky patients and to follow rational use of blood, thereby to improve the standard of safe and quality blood transfusion.

## RESULTS:

A total of 719 samples were received with the provisional diagnosis of anemia and among the samples 632 were females and 87 were male. The duration was one year from January 2016 to December 2016. 28 samples were positive for DCT, 11 cases were positive for ICT, 28 samples had positive auto control. Cell 1 was positive in 8, Cell 2 was positive in 11, cell 3 was found positive in 9 cases.

Cell 1 was identified as Antibody C, Cell 2 was identified as Antibody E, Lewis b.

**Table-1 Total Number Of Anemia Cases Positive For Direct Coombs Test**

| Age    | Male | Female | Total |
|--------|------|--------|-------|
| 0-1    | -    | 2      | 2     |
| 10 -20 | -    | 7      | 7     |
| 21-30  | 1    | 7      | 8     |
| 31-40  | -    | 3      | 3     |
| 41-50  | -    | 1      | 1     |
| 51-60  | 4    | 1      | 5     |
| 61-70  | 1    | -      | 1     |
| 71-80  | 1    |        | 1     |
| Total  | 7    | 21     | 28    |

**Table – 2 Total Number Of Cases Positive For DCT, ICT & AUTO Control**

|                      |    |
|----------------------|----|
| DCT                  | 28 |
| ICT                  | 11 |
| Auto control         | 28 |
| DCT + Autocontrol    | 28 |
| DCI+ICT+ Autocontrol | 5  |
| ICT + Autocontrol    | 1  |

**Table-3 Total Number Of Antibody Identified Positive For Isolated Cell 1, Cell 2, Cell 3**

| Total cases screened | Cell 1 | Cell 2 | Cell 3 |
|----------------------|--------|--------|--------|
| 719                  | 8      | 11     | 9      |

**Table – 4**

| Coombs test          | Reactive | Presence of Antibody | Antibody Identified |
|----------------------|----------|----------------------|---------------------|
| DCT + Autocontrol    | +        | +                    | Antibody E,C        |
| DCI+ICT+ Autocontrol | +        | +                    | Antibody D, S, Le b |
| ICT + Autocontrol    | +        | +                    | Antibody N          |

**Table – 5 Antibody Screening With 3 Cell Panel (Reacell 1,11,111)**

| Antibody panel | Reactive state | Antibody identified      |
|----------------|----------------|--------------------------|
| Cell1          | 8              | Antibody C, K, Jk a      |
| Cell 1&2       | 3              | Antibody D, S, Le b      |
| Cell 1 & 3     | 2              | Antibody e, M, Fy b      |
| Cell 1,2,3     | 5              | Antibody k, Le b         |
| Cell 2         | 2              | Antibody E               |
| Cell 2&3       | 1              | Antibody c, N, Fy a, Jkb |

719 samples received from Medical ward for blood transfusion were taken into study to rule out auto antibodies, allo antibodies and antibody screening was done by using standard Matrix Gel column technology and ReaCell 3 cell antibody panel.

Among the 719 samples 28 cases were turned to be positive for Direct Coombs test, predominantly females between the age group of 20 -30 yrs. 4 of male patients between the age of 50 -60 are commonly positive for DCT. Interestingly 2 neonates with the age of 15 days and 5 days were positive for DCT and Autocontrol. (Table 1).

Most of the cases presented with the blood picture of Pancytopenia and hematological evaluation resulted in favour of hemolytic anemia. The positive DCT signifies the destruction of RBCs of various reasons and the associated positivity for autocontrol adds value in diagnosing the hemolysis. Our study noted that more than 95% of cases were positive for DCT & Autocontrol. (Table2)

The identification of antibodies were done by using Matrix gel column and ReaCell panel, we noted that 8 cases were reactive for Cell 1, 11 cases were reactive for Cell 2 and 9 cases were reactive for Cell 3 (Table 3)

Antibody E, C was identified in association with positive DCT & Autocontrol, Antibody D, S, Le b was in association with positive DCT, ICT & Autocontrol. Antibody N was identified in association with positive ICT & Autocontrol (Table 4)

The significant antibodies were Antibody C, e, K, E and M. Neonatal cases were presented with Antibody e, this caused the hemolytic disease of newborn (Table 5)

## DISCUSSION

Nowadays the evaluation of anemia in clinical practice has changed over. The appropriate scenario of treating anemia cases has been depending upon the diagnosis of right cause at a right time. With this idea in background, in our study we identified that among the anemia due to various reasons like nutritional deficiency, chronic underlying diseases and malignancy more than 5 % of the people suffer from Immune hemolysis and which should be addressed at the earliest [1,2]. Serological characterization of autoantibody helps to differentiate various types of Autoimmune hemolytic anaemia (AIHA) and gives a better assessment to the clinician regarding the likely course of disease and the form of treatment to be given [3,4,5].

In one case the same Antibody e in the mother and neonate indicates that the presence of maternal antibody e' was the reason for immune hemolytic anemia in neonate where the neonate undergone exchange transfusion. This maternal antibodies causing severe hemolytic disease of the newborn has been reported in many studies [3].

Rh incompatibility and ABO incompatibility causing Hemolytic disease of newborn (HDN) has been reported in studies, but majority has been presented in Rh negative mother delivering a Rh positive baby [6,7]. Only few studies had reported HDN even in Rh positive mother. In our study the mother was B+ and baby was O+ with severe HDN with the Hemoglobin 2 gms/dl and serum bilirubin was 29 gms wherein the neonate was admitted in NICU, twice undergone exchange transfusion and the bilirubin was reversed.

The adult male patients presented with the pancytopenia was identified positive for DCT, ICT, Autocontrol and antibodies. The medical records revealed the history of previous transfusion and blood transfusion was not supported for the correction of anaemia and they were started with Steroids [4,5].

The DCT positivity correlates with the presence of clinical symptoms. Regularly, strongly positive DCT cases are encountered with no sign of hemolysis, while severe hemolysis can be seen even in patients with a negative DCT score also [8,9,11,12,13]

Hence before blood transfusion the screening of Antibody identification and Coombs test was helped in all the age group patients including Neonates Antenatal patients, adult male and females in identifying hemolytic anemia and abnormal antibodies. Such screening will surely give an alarm regarding the immune status of the patient [10,13,14,15].

## CONCLUSION

Therefore, it can be concluded that AIHA is not uncommon and requires advanced, efficient immunohematological screening and transfusion support. Detailed characterization of autoantibodies is important, as there is a relationship in hemolysis and antibody characteristics. All blood banks should have the facilities to perform the necessary investigations required to diagnose the hemolytic cause of anaemia to issue the safest blood component in AIHA.

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