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Experience from an immunohematology reference testing center

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Abstract:

Blood Centres in India lack infrastructure to investigate immunohematology problems. Reference Testing Center (RTC) was established in 2014 to investigate Immunohematological problem as it is not possible for small blood centers to go for complete immunohematology work up due to lack of financial and technical resources in remote and rural areas. Objective of this study is to share our experience as RTC of past 6 years so that more RTC are established across Indian subcontinent. 1456 Discrepant samples received from various hospitals of South India for Immunohematology problems were analysed in 6 years. Maximum requisitions obtained in 2014 were more than 40 years of age and then 21-40 years of age group in 2015 and same was observed till 2020.75% of total samples received were for antibody identification followed by blood group discrepancy resolution, investigation of positive DAT, red cell phenotype and pre-natal evaluation & antibody titration. Single allo-antibodies were identified in 773 cases whereas multiple allo-antibodies were found in 118 cases. Most common single and multiple antibody found was anti D and Anti-D+C. Weak D subgroup was the most common blood group discrepancy.22 cases & 4 cases of Bombay and para-bombay were also investigated.

Keywords:

Immunohematology, reference testing center, transfusion

Introduction

Blood transfusion services has improved significantly in the past two decades but most of the blood centers in India lack specialized reagents, trained manpower, and technical infrastructure to investigate immunohematology problems such as antibody evaluation and blood group discrepancies which might lead to transfusion reaction, and other complications in patients. To prevent this, a detailed evaluation of red cell antibodies must be done in patients undergoing transfusion.[1,2] Increasing trend of immunohematology reference testing center (RTC) around the globe is seen to further investigation of the serological nature of a positive antibody screen or incompatible crossmatch cost-effectively.[3]

Immunohematology RTC was started at our blood center in 2014 in collaboration with ORTHO Clinical Diagnostics, India Private Limited and tests such as antibody evaluation, red cell phenotyping, resolution of blood group discrepancy, investigation of autoimmune hemolytic anemia, and direct antiglobulin test (DAT) positive cases were performed on samples sent to our center from adjoining areas so that safe transfusion can be provided to the patients in adjoining and remote areas as it is not possible for small blood centers to go for complete immunohematology workup due to financial and technical constraints in remote and rural areas. The objective

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of this study is to share our 6 years of experience as RTC.Setting of immunohematology RTC can solve discrepant and incompatible cases in and around the region which ultimately benefit patient care and prognosis. There is need of more RTC to come up across Indian subcontinent so that safe transfusion is provided to the patient.

RTC has received a total of 1456 patient samples in 6 years for immunohematology workup with females (986) much more than males (470). In 1st year, we received only 28 samples which increased to 278 samples in 2020. Maximum requisitions obtained in 2014 were >40 years of age which gradually shifted to 21–40 years of the age group in 2015, and the same pattern was observed till 2020 and was statistically significant [Table 1]. Around 75% of the total samples received were for antibody identification followed by blood group discrepancy resolution, investigation of positive DAT, complete red cell phenotype and prenatal evaluation, and antibody titration [Table 2]. Single alloantibodies were identified in 773 cases, whereas multiple alloantibodies were found in 118 cases. The most common single antibody found was anti-D with 341 out of 773 patients ($P < 0.001$ [S]), showed a significant increase over the years followed by anti-M, anti-E, anti-c, anti-Lea, and anti-Leb. We also detected anti-K, anti-P1, anti-Fya, anti-s, anti-Jka, anti-N, and anti-S [Table 3]. Multiple alloantibodies

Table 1: Demographic analysis

	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)	2020 (%)	*P*
Gender								
Male	13 (23.2)	38 (20.7)	50 (16.7)	62 (15.7)	99 (16)	122 (15.2)	86 (15.5)	0.316
Female	15 (26.8)	54 (29.3)	100 (33.3)	135 (34.3)	210 (34)	280 (34.8)	192 (34.5)	
Age (years)								
≤20	0	13 (7.1)	18 (6)	22 (5.6)	51 (8.3)	82 (10.2)	67 (12.1)	<0.001 (significant)
21-40	9 (16.1)	45 (24.5)	76 (25.3)	105 (26.6)	150 (24.3)	188 (23.4)	116 (20.9)	
>40	15 (26.8)	29 (15.8)	51 (17)	59 (15)	94 (15.2)	102 (12.7)	61 (11)	
unknown	4 (7.1)	5 (2.7)	5 (1.7)	11 (2.8)	14 (2.3)	30 (3.7)	34 (6.1)	

Table 2: Requisition category

	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)	2020 (%)	*P*
ABID	20 (71.4)	65 (70.7)	118 (78.7)	141 (71.6)	246 (79.6)	284 (70.6)	208 (74.8)	<0.001 (significant)
Resolution of blood group	7 (25)	16 (17.4)	21 (14)	30 (15.2)	42 (13.6)	96 (23.9)	36 (12.9)	
Investigation of positive DAT	1 (3.6)	9 (9.8)	10 (6.7)	15 (7.6)	12 (3.9)	16 (4)	28 (10.1)	
Complete red cell phenotype	0	0	0	4 (2)	4 (1.3)	5 (1.2)	5 (1.8)	
Other	0	2 (2.2)	1 (0.7)	7 (3.6)	5 (1.6)	1 (0.2)	1 (0.4)	

DAT=Direct antiglobulin test, ABID=Antibody identification

Table 3: Types of single alloantibodies

	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)	2020 (%)
Anti-D*	4 (25)	25 (39.7)	33 (31.1)	54 (43.2)	76 (39.8)	87 (52.7)	62 (57.9)
Anti-E	3 (18.8)	6 (9.5)	15 (14.2)	14 (11.2)	15 (7.9)	15 (9.1)	11 (10.3)
Anti-C	1 (6.3)	4 (6.3)	10 (9.4)	18 (14.4)	16 (8.4)	4 (2.4)	1 (0.9)
Anti-c	2 (12.5)	4 (6.3)	12 (11.3)	4 (3.2)	16 (8.4)	10 (6.1)	8 (7.5)
Anti-e	0	0	1 (0.9)	0	3 (1.6)	0	3 (2.8)
Anti-K	1 (6.3)	1 (1.6)	4 (3.8)	0	5 (2.6)	1 (0.6)	0
Anti-Fya	0	1 (1.6)	2 (1.9)	3 (2.4)	3 (1.6)	6 (3.6)	2 (1.9)
Anti-Fyb	0	1 (1.6)	0	1 (0.8)	0	0	0
Anti-Jka	0	0	3 (2.8)	3 (2.4)	2 (1)	0	2 (1.9)
Anti-Jkb	0	0	0	0	1 (0.5)	0	1 (0.9)
Anti-Lea	0	4 (6.3)	4 (3.8)	4 (3.2)	9 (4.7)	11 (6.7)	3 (2.8)
Anti-Leb	2 (12.5)	4 (6.3)	5 (4.7)	3 (2.4)	12 (6.3)	3 (1.8)	2 (1.9)
Anti-M	3 (18.8)	7 (11.1)	12 (11.3)	17 (13.6)	31 (16.2)	22 (13.3)	10 (9.3)
Anti-N	0	1 (1.6)	0	1 (0.8)	0	0	0
Anti-S	0	1 (1.6)	3 (2.8)	1 (0.8)	0	1 (0.6)	2 (1.9)
Anti-s	0	2 (3.2)	0	0	0	1 (0.6)	0
Anti-P1	0	2 (3.2)	2 (1.9)	2 (1.6)	2 (1)	3 (1.8)	0
Anti-Kpa	0	0	0	0	0	1 (0.6)	0
Total	16 (100)	63 (100)	106 (100)	125 (100)	191 (100)	165 (100)	107 (100)

*For anti D – $P<0.001$ (significant). Anti-D showed a significant increase over the years. $\chi^2=140.742$ with 102° of freedom, $P=0.007$ (significant)

were also identified in 118 samples and anti −D + C was found in over 50% of the cases followed by anti −E + c and anti − Lea + Leb [Table 4]. Many blood group discrepancies were investigated and resolved in 6 years by both tube technique and column agglutination technique according to the standard operating procedure of the blood center. The weak D subgroup was the most common blood group discrepancy seen followed by the A2 subgroup in 31 cases from 2014 to 2020. Out of all blood group discrepancies, 22 cases and four cases were of Bombay and para-Bombay type [Table 5].

The idea of setting immunohematology RTC at our regional blood center came from the American Red Cross initiative of the setting of RTC.[4] Which was supported by Ortho Clinical Diagnostics, India Private Limited. This study talks about our experience of the same. In 2014, we had to face many challenges to make physicians understand the idea of immunohematology which is evident from the fact that we only received 28 samples in 2014 which gradually increased to 302 till 2019. In 2020, we received 278 requisitions, the dip in requisitions was due to the COVID-19 pandemic. One of the main reasons for decreased requisition was that the cases such as bone marrow transplants and cadaveric transplants were postponed for indefinite time till the pandemic condition improves. Courier services were also halted for some time which also impacted our requisitions. Globally, the health-care system halted due to the COVID-19 pandemic so were our requests from the surrounding areas.[5,6]

Table 4: Combinations of multiple alloantibodies

	2014	2015	2016	2017	2018	2019	2020
Anti-D+C	1	4	5	16	15	8	13
Anti-E+c	1	0	3	3	3	2	1
Anti-E+s	0	2	0	0	0	0	0
Anti-Fyb+Lea	0	1	0	0	0	0	0
Anti-E+C	0	0	2	0	0	0	0
Anti-C+e	0	0	1	0	1	0	0
Anti-D+C+Jka	0	0	1	0	0	0	0
Anti-E+c+K	0	0	1	0	0	0	0
Anti-c+K	0	0	1	0	0	0	0
Anti-E+P1	0	0	1	0	0	0	0
Anti-Fya+Jka	0	0	1	0	0	0	0
Anti-Lea+Leb	0	0	1	1	4	5	0
Anti-C+Fyb	0	0	0	1	0	0	0
Anti-E+Jka	0	0	0	1	0	0	0
Anti-E+Fya	0	0	0	1	0	1	0
Anti-c+Jka	0	0	0	0	1	2	0
Anti-K+M	0	0	0	0	1	0	0
Anti-M+Jka						1	0
Anti-c+Lea						1	0
Anti-D+C+E						1	0
Anti-c+M+Lea						1	0
Anti-Fya+S						1	0
Anti-Leb+N						1	0
Anti-D+E						1	0
Anti-E+K						0	1
Anti-C+Jkb+Leb						0	1
Anti-Jak+Fyb						0	1
Anti-E+Fyb+S+M						0	1
Anti-D+C+M						0	1

The maximum immunohematology cases we received in the past 6 years were of female gender and in 20–40 years of the age group, i.e., childbearing age as pregnancy is one of the biggest risk factors of alloimmunization other than transfusion and transplantation and the most common alloantibody found was anti-D (44.11%). Anti-D alloimmunization is one of the leading causes of hemolytic disease of the fetus and newborn followed by anti-C.[7] Hence, routine antibody screening during pregnancy must be done followed by titer if there is an antibody.[8] Distant hospitals in rural areas can only adopt this practice in a routine if we have more reference laboratories coming in India. Findings and learnings have been depicted in the result section of this study but an interesting finding we came across is the identification of 22 cases of the Bombay blood group and four cases of the para-Bombay blood group. Counselling, screening, and motivating these patients for future donation can be a precious donor pool for future pan India and can be a boon for Bombay and para-Bombay blood group patients.[9,10]

Future Prospects and Learning

The establishment of RTC not only helps in improving the blood transfusion services in a region by solving the immunohematology cases and providing compatible blood and blood components to patients but by also maintaining a rare blood group donor inventory. We would like to improve our services and also help other

Table 5: Blood group discrepancy

	2014 (%)	2015 (%)	2016 (%)	2017 (%)	2018 (%)	2019 (%)	2020 (%)	*P*
Weak D	0	4 (50)	2 (18.2)	2 (12.5)	5 (0)	8 (38.1)	11 (61.1)	<0.001 (significant)
A2 subgroup	0	3 (37.5)	6 (54.5)	9 (56.3)	8 (0)	3 (14.3)	2 (11.1)	
Weak A variant	0	1 (12.5)	1 (9.1)	2 (12.5)	2 (0)	0	0	
Weak B variant	1 (100)	0	0	0	0	0	1 (5.6)	
Bombay	0	0	1 (9.1)	2 (12.5)	7 (0)	9 (42.9)	3 (16.7)	
Para-Bombay	0	0	1 (9.1)	1 (6.3)	0	1 (4.8)	1 (5.6)	

blood centers located in metro cities and tertiary care settings to come up and establish RTCs so that quality and safe blood be provided to the patients in remote areas.

Limitations

1. Transportation of samples from remote areas was a problem in 1st year but then was sorted out as process flow established with time
2. From March 2020, transportation was again a problem due to the pandemic.

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Conflicts of interest

There are no conflicts of interest.

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