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PREVALENCE OF BOMBAY BLOOD GROUP AMONGST THE HEALTHY BLOOD DONORS AT A TERTIARY CARE CENTRE IN WESTERN RAJASTHAN, INDIA

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Abstract
Introduction: The prevalence of Bombay (Oh Phenotype) is not precisely known in Rajasthan state. Because of the fact that Bombay blood group is clinically significant, we decided to conduct a study at our centre to determine the prevalence of Bombay blood group among blood donor population in Western Rajasthan.
Methodology: This blood bank based prospective study was carried out amongst the blood donors over a period of 11 months, i.e., from February 2019 to December 2019. Total 30,000 donor samples were screened for ABO-RhD blood grouping and antibody screening. Auto control, indirect antiglobulin test (IAT) and Bombay blood group (Anti-H lectin) tests were run on all the O blood group samples showing agglutination with O reagent cells (in reverse grouping). Donors negative on Anti-H testing were tested for saliva A, B and H antigens and interpreted.
Results: Out of the total 30,000 donors, the maximum number of donors had blood group B+ (32.76%), followed by O+ (29.9%), A+ (20.41%), AB+ (8.06%), B- (3.11), O- (3.02%), A- (1.93%) and AB- (0.8%). Prevalence of Bombay blood group among study population was calculated to be 0.003%.
Conclusion: Bombay phenotype is a rare blood group but not uncommon. If serum grouping is not performed, it is misdiagnosed as blood group O. Therefore both forward and reverse blood grouping should be done on 100% samples.
Keywords: Bombay, Anti-H lectin, Serum grouping, Blood donors.

Introduction

The first person found to have the Bombay phenotype had an interesting blood type that reacted to other blood types in a way never seen before. The serum contained antibodies that reacted with all red blood cell normal ABO phenotypes. The red blood cells appeared to lack all of the ABO blood group antigens and to have an additional antibody (Anti-H) that was previously unknown.[1]

It is important to correctly type individuals who are Bombay phenotypes because these individuals require autologous blood donation or blood from another Bombay individual. Unavailability of this rare phenotype may sometimes prove fatal due to delay in the transfusion and/or required surgery. Thus, it is recommended that all blood group donors and patients should be routinely screened by both forward and reverse grouping for screening of Bombay phenotype to reduce the risk of hemolytic transfusion reaction resulting from issue of O blood group to Bombay blood group recipients.[2]

Bombay phenotype is extremely rare (Autosomal Recessive phenotype), since it occurs in 1 in 10,00,000 individuals in Europe, 1 in 2,50,000 in Caucasian[3,4] and about 1 in 10,000 individuals in India[5,6] (Prevalence being 1 in 7600 in Maharashtra state and 1 in 4600 in Ratanagiri district of Maharashtra).[6] Although in some places of Mumbai (formerly Bombay) the prevalence of this phenotype is as high as 0.01 % prevalent. High incidence of Bombay phenotype reported in Orissa of eastern India, among Kutia Kondh tribe.[7] Another study from northwestern Orissa reported an average of 1 in 278 Bombay phenotype among Bhuyan tribal population.[8]

The prevalence of Bombay blood group (Oh Phenotype) in Rajasthan state, Western India, is not precisely known. So, we decided to conduct a study on donor population to determine the prevalence of Bombay blood group at our centre.

Subjects and Methods:

This blood bank based prospective study was carried out amongst the voluntary blood donors over a period of 11 months, i.e., from February, 2019 to December, 2019 in the Department of Immuno-Haematology & Transfusion Medicine, S.P. Medical College & Associated Group of Hospitals, Bikaner (Rajasthan). Individuals were selected for blood donation as per donor selection criteria according to Drugs & Cosmetics Act, 1940 & Rules, 1945.

Individuals selected for blood donation were included in the study after taking informed consent. 3-4 ml venous blood sample from all the healthy blood donors was collected in EDTA (ethylene diamine tetra acetic acid) anticoagulated tube in bar coded test tubes.

Routine (ABO-RhD) blood grouping of blood donors was done on **fully automated immuno-hematology analyzer** (Neo Galileo machine) by **direct hemagglutination assay** using Galileo Grouping Microplates and ABODFULL2 (i.e., 8 well ABO-RhD blood grouping) assay was run using commercially available reagents as per the instrument operator manual. To rule out presence of unexpected/irregular antibodies, antibody screening was performed on fully automated Immuno-Haematology analyzer (Galileo Neo, Immucor) by using commercially supplied Pooled screening cells on micro-well plates. Auto control and indirect antiglobulin (IAT) tests were run using Matrix Octoplus AHG (Coombs) Gel Cards (Column Agglutination Technology) on all the O blood group samples showing agglutination with "O" cells (in reverse grouping).

All the O blood group samples showing agglutination with "O" cells (in reverse grouping) were tested for Bombay blood group by gel column agglutination technique, using commercially available Immucor Anti-H lectin (Buffered saline extract of seeds from Ulex europaeus). ABO-RhD grouping was also reconfirmed. Subjects showing negative reaction with Anti-H lectin were subjected to Saliva Inhibition Test to look for A, B and H antigens in saliva secretions (secretor status). Agglutination of indicator red cells by antibody in tubes containing saliva indicates that the saliva does not contain the corresponding antigen. The failure of known antibody to agglutinate indicator red cells after incubation with saliva indicates that the saliva contains the corresponding antigen.

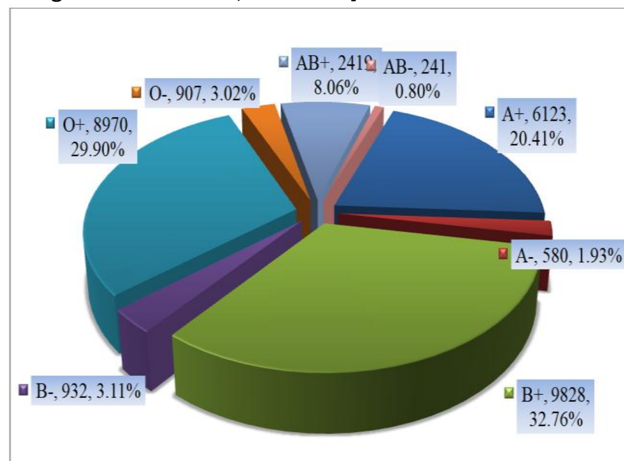
The collected data and results were evaluated and presented in tables and figures in accordance with purpose of the study. It was a prevalence study and direct comparison with previous studies was made. All the data was statistically analyzed and tests of significance were applied using **MS-Excel** and **PRIMER** software.

Observation and Results:

Table 1: Sex distribution among the blood donors

Sex	No. of donors	Percentage out of total donors
Male	29580	98.6%
Female	420	1.4%
Total	30000	100%

In present study, we observed that maximum donors were males (29580/30000; 98.6%) and females were only 1.4% (420/30000). Among ABO blood groups, donors with blood group B (10760/30000; 35.87%) were maximum followed by O, A and AB; as shown in Chart 1. Rh D positive donors were 91.13% and D negative donors were 8.87%. Statistical test of significance did not establish any significant correlation between the occurrences of two blood group systems among the blood donors. [Chi square = 2.285 with 3 degrees of freedom; P = 0.704].



Pie Chart 1: DISTRIBUTION OF ABO AND RH (D) BLOOD GROUPS

AMONG THE BLOOD DONORS

Table 2: Observation and results of serological reactions performed for the detection of Bombay blood group

Anti A (Series 1)	**Neg**
Anti B (Series 3)	**Neg**
Anti D (Series 5) (IgM+IgG)	**4+**
Anti D Novaclone (IgG+IgM)	**4+**
Monoclonal Control	**Neg**
A1 cell	**2+**
B cell	**2+**
O cell	**2+**
Indirect Antiglobulin Test	**4+**
Antibody screen & Auto-control	**Neg**
Anti H	**Neg**
Saliva Test (Result)	**2+**
Saliva Test (Interpretation)	**Non-secretor of A, B & H**

Table 2 shows the results of serological reactions performed for the detection of Bombay blood group on a sample of a 23 years old male donor (who was further identified as Bombay blood group). ABO-RhD blood group of this donor was detected as O+ on forward and reverse grouping but there was a positive reaction in reverse O cell. The same sample (plasma) gave a 4+ reaction on indirect antiglobulin test (IAT) while a negative reaction on antibody screening and auto-control, ruling out the possibility of irregular antibodies and autoantibodies.

Cell suspension of that IAT positive sample gave a negative reaction with Anti H, establishing the absence of H antigens on the donor sample RBCs. Saliva inhibition test was performed on donor's saliva which showed a positive reaction indicating the absence of A, B and H substances in

saliva (i.e., being a non-secretor), thus confirming the blood group as Bombay and ruling out para-Bombay.

Discussion:

The present study proved that occurrence of Bombay blood group is not uncommon among the blood donors of Western Rajasthan. During the study period, a total of 30,000 blood donor samples were typed for ABO-RhD, antibody screening, Bombay and weak D testing. Out of the total donors (30,000) included in the study; 29,580 (98.6%) were male donors and 420 (1.4%) were female donors. The prevalence of Bombay blood group in our study, in a mixed population covering urban and rural areas of Bikaner district and Western Rajasthan, was found to be 0.003% which is in accordance with the studies done by Das PK et al[9] in Tamil Nadu and Periyavan S et al[10] in Karnataka (0.004% and 0.005% respectively).

No consanguinity was reported in Bombay blood group donor's parents in our study. However study done by Anju Verma et al[11] in Andhra Pradesh had shown an increase in the prevalence of this phenotype (0.048%). This increase in their prevalence may be attributed to the screening of the family members of oh phenotypes. Study done by Geetha et al[12] (Bangalore, 2011) had prevalence of 0.016%, which was attributed to consanguinity (among parents of 4 out of 6 cases). The prevalence of Bombay phenotype reported in various studies has been compared in Table 3.

Table 3: Prevalence of Bombay phenotype in various studies

S. No.	Study	Total (n)	O group	Bombay	% Prevalence
1	Balgir RS,[8] 2007, Orissa (Bhuyan tribe)	244 379		2 1	0.819 0.26
2	Balgir RS,[7] 2005, Orissa (Kutia Kondh tribe)	254		2	0.787
3	Verma A et al,[11] 2011, Tirupti A.P.	26638	4021	7M+6F	0.048
4	Kavitha A et al,[13] 2016, Chennai	8903	338 (33%)	2	0.022
5	Rudrappan RB & Veeran K,[14] 2016, Chennai	11512		2	0.017
6	Geetha RL et al,[12] 2011, Bangalore	37117	15032 (40.5%)	2M+4F	0.016
7	Mahapatra et al,[15] 2018, Cuttuck	76204 49604 Donors 26600 Patients	 19383 10108	 4M 4M+4F	0.015 0.008 0.03
8	Mallick et al,[16] 2015, Puducherry	35497 (Both donors & recipients)	14164	3	0.008
9	Talukder et al,[17] 2014, Kolkata	56465 28934 Donors 27531 Patients		6 2 4	0.011 0.007 0.014
10	Singh A et al,[18] 2019, Lucknow (Consanguinity in 2 cases of Bombay)	177888		5M+1F	0.003
11	Rao C et al,[19] 2014, Karnataka	43103 (14798 Donors, 28305 Patients)	18103, 42%	2	0.005
12	Present Study (Bikaner, 2019)	30000	9877, 32.92%	1M	0.003%

The study done by Balgir RS[7] (Orissa, 2005) reported an incidence of Bombay phenotype 1 in 33 among the Kutia Kondh tribe, 1 in 127 among Kondh tribe and 1 in 1,244 among the tribal populations of Orissa. Since the population size of Kutia Kondh tribe was relatively small (around 5000 individuals) and the practice of endogamy was strictly followed, therefore, the inbreeding and consanguinity amongst them was not ruled out which may be one of the major factors for the combination of recessive rare alleles like Bombay phenotype among the Kutia Kondh tribe. Prevalence is higher in Andhra Pradesh as compared to the other parts of India. This is probably because of consanguinity among parents. The author found 10 (77%) cases that had history of consanguineous marriage among parents.[11] In western states of India its incidence is high. Gorakshakar et al[6] (1987) found 1 in 4500 incidence in rural population of Maharashtra. Bhatia and Sathe[20] (1974; Mumbai) found incidence 1 in 7600 in the urban population of Mumbai.

Regarding the distribution and spread of Bombay phenotype in different states of India, the Oh phenotype is more common in state of Western and Southern parts of India as compared to other states. Of the 179 cases reported by Sathe et al[21] (1988) from the Institute of Immunohematology (formerly Blood Group Reference Centre, Bombay), Mumbai, 112 (62.6%) cases belonged to the state of Maharashtra alone. The frequencies reported from other locations are depicted in Table 4 below.

Table 4: DISTRIBUTION OF BOMBAY (OH) PHENOTYPE CASES REPORTED FROM DIFFERENT STATES OF INDIA (DATA FROM SATHE ET AL[21] 1988).

S.No.	State	No. of Bombay phenotype cases
1	Andhra Pradesh	8
2	Bihar	2
3	Goa	6
4	Gujarat	5
5	Karnataka	14
6	Kerala	4
7	Madhya Pradesh	4
8	Maharashtra	112
9	North India (unclassified)	2
10	Orissa	1
11	Pondicherry	1
12	Rajasthan	2
13	South India (unclassified)	1
14	Tamil Nadu	2
15	Uttar Pradesh	5
16	Not Known	10
Total		179

Conclusion:

Prevalence of Bombay blood group among study population was calculated as 0.003%. Female donors being very small in number in present study, population surveys for rare blood groups should be conducted with bigger sample sizes. Bombay blood group is a rare blood group. If serum grouping is not performed, it is misdiagnosed as blood group O. Therefore both forward and reverse blood grouping should be done on 100% samples. Rare blood group registry should be maintained by the blood banks; such donors should be educated about their blood group status and its importance. Blood Banks should be sensitized for the importance of rare blood groups like Bombay blood group and it should be performed on hundred percent donors and patients to avoid wrong blood transfusions.

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