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A PROSPECTIVE STUDY ON BLOOD GROUPING AND DETECTION OF RARE GROUPS AMONG VOLUNTARY DONORS

Pathology

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ABSTRACT

Bombay phenotype is rare blood type which was first reported in 1952 in Bombay, India and is associated with the ABO and H blood group systems. The Para-Bombay phenotype is very rare. Only a few cases of Para-Bombay were reported in India till now. This entity is characterized by the absence of H, A and B antigens on the red cells but their presence in saliva and secretions of gastrointestinal and genitourinary tracts. We planned this study to detect the rare blood group during our routine blood grouping and typing. Forward grouping and reverse grouping were done if any discrepancy between them the secretor status of the patient was determined and depending on the secretor status the blood group was reported. Out of all the blood samples we received we had one patient whose forward and reverse group were mismatching and secretor status was performed and we found it to be a Para Bombay blood group. This study highlights the importance of proper blood grouping (forward and reverse both) and use of anti-H reagent for the identification of Para-Bombay phenotype, otherwise this donor would have been mislabeled as Group O. In routine blood banking, besides blood grouping detailed serological tests such as secretor status should be performed

KEYWORDS

Blood group , Forward grouping , secretor status , Bombay blood group , para Bombay blood group .

1.INTRODUCTION:

Bombay blood group is a rare blood group present in about 0.0004% (about 4 per million) of the human population, though in some places such as Mumbai locals can have occurrences in as much as 0.01% (1 in 10,000) of inhabitants.[1] Given that this condition is very rare, any person with this blood group who needs an urgent blood transfusion will probably be unable to get it, as no blood bank would have any in stock. Persons with rare Bombay phenotype (hh) do not express H antigen , the antigen which is present in blood group O. As a result, they cannot make A antigen or B antigen on their red blood cells, whatever alleles they may have of the A and B blood-group genes, because A antigen and B antigen are made from H antigen. For this reason people who have Bombay phenotype can donate red blood cells to any member of the ABO blood group system but they cannot receive blood from any member of the ABO blood group system but only from other people who have Bombay phenotype. Another related phenotype is para-Bombay which is extremely rare, with only a very few cases being reported and none of the published studies have reported the prevalence of para-Bombay phenotype in India.[2] The para-Bombay phenotype is characterized by the absence of ABH antigens on red blood cells (RBCs) with the presence of ABH substances in body secretions or by the weak expression of ABH antigens on RBCs with the absence or presence of substances in body secretions. In contrast, Bombay phenotypes have an absence of ABH antigens on both, the RBCs as well as in body secretions. We planned this study to detect the rare blood group during our routine blood grouping and typing.

2. AIM :

Detection of rare blood group during blood grouping and typing

3. METHODS AND MATERIALS:

Study was carried out in the blood bank, government sivagangai medical college, sivagangai. Samples for blood grouping from the hospital and blood donor samples from replacement and blood camps were taken for the study.

Blood grouping and typing:

ABO and Rh typing was done by antigen antibody agglutination test by commercially available standard antisera i.e. anti A, anti B Anti D and Anti H after validation at blood bank. Venous blood was collected from the patents from cubital vein and added to plain and EDTA added tubes which were then used to determine the blood group. Blood groups were done by slide agglutination method both forward grouping and reverse grouping were done, Reverse grouping was done with pooled cells of Group A , group B and Group O cells . Both forward and reverse grouping were verified and then the group was declared for the patient.

Determination of Secretor status:

If any mismatch in the forward and reverse grouping then the group will be confirmed by doing the salivary substance secretion test 1 ml of unstimulated saliva was obtained from each subject using the Navazesh method Saliva was transferred into a sterile test tube that was then sealed with nonabsorbent gauze and placed in a boiling water bath for 10 min. It was then centrifuged at 1,700 rpm for 10 min and the supernatant was separated by decantation. The upper layer of centrifuged saliva was used to identify secretors and nonsecretors. Analysis of secretor status was carried out by Wiener agglutination testing. The test serum and the saliva were diluted with a saline solution at a ratio of 1:10. The following antiserum was poured into test tubes numbered I–IV: test tube I = 1 drop of saliva + 1 drop of anti-B serum; test tube II = 1 drop of saliva + 1 drop of anti-A serum; test tube III = 1 drop of saline solution + 1 drop of anti-B serum, and test tube IV = 1 drop of saline solution + 1 drop of anti-A serum. After 10 min at room temperature (20°C), 1 drop each of 2–3% A 2 erythrocyte solution in saline solution was added to test tubes II and IV. One drop each of 2–3% B-erythrocyte solution in saline solution was added to test tubes I and III. All of the test tubes were shook and kept at room temperature. The results were recorded after 1 h. The test was based on the ability of saliva to inhibit the agglutination reaction of blood groups, such as antibody A without Ag A in the saliva → no agglutination (secretor), but mixture of antibody A and Ag A on the erythrocyte → agglutination (nonsecretor).

Agglutination observed was recorded in this order
1+ multiple small sgglutinate with hazy supernatant
2+ multiple small agglutinate with clear supernatant
3+ 2-3 large agglutinate with clear supernatant
4+ single large agglutinate with clear supernatant

After determination of secretor or non-secretor status the group of the patient will be confirmed in case of any discrepancy between forward and reverse grouping.

4.RESULTS:

Table No 1: Forward And Reverse Group

Forward grouping (cell grouping)				
	Anti- A	Anti- B	Anti -H	Anti – D
Observation	0	0	0	4 +
Interpretation	O Rh (D) positive , H antigen Negative			
Reverse grouping (serum grouping)				
	Acell	B cell	O cell	
Observation	4+	4+	0	
Interpretation	O group , Anti- H absent in serum			

Table No :2 Secretor Status Determination

Detection of secretor status in Saliva			
	A cell	B cell	O cell
Observation	4+	4+	0
Interpretation	Presence of substance H in saliva – secretor		

Hemagluttination inhibition test	A cell	B cell	O cell
Test	4+	4+	0
Control	4+	4+	4+

Out of all the blood samples we received we had one patient whose forward and reverse group were discordant so we performed the secretor status for that patient Forward grouping no agglutination in the anti –a and anti –b and also no agglutination in the anti – H so we thought it to be Bombay blood group. We performed the reverse grouping and we found agglutination with pooled a cells and pooled b cells but no agglutination in the pooled o cells .We found forward and reverse grouping were discordant so we came to conclusion that the patient had Bombay variant blood group ,We decided to check the secretor status of the patient by detection of substance in the saliva and performed the hemagultination inhibition test and we found that the patient had secretion of H substance in the saliva Rh typing : it was found that patient had positive in Rh typing. Family members were screened and they all were O positive.

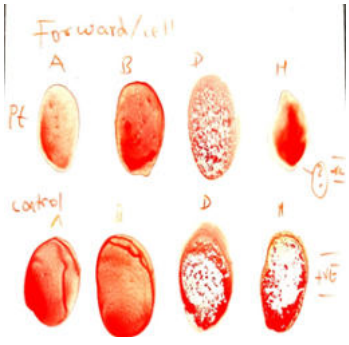

Figure 1: Forward Grouping

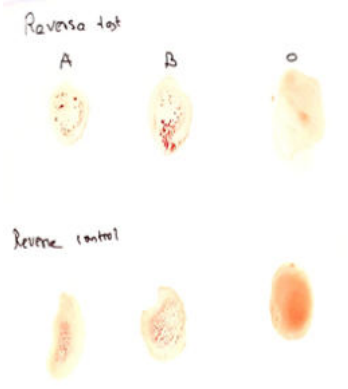

Figure:2 Reverse Grouping

Figure 3: Saliva After Heating And Centrifuge

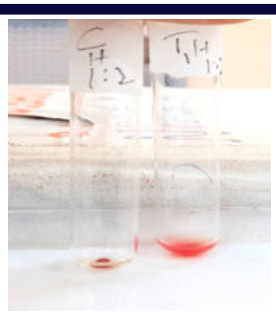

Figure 3: Hemaglutination Inhibition Test

5.DISCUSSIONAND CONCLUSION :

Bombay phenotype is rare blood type which was first reported in 1952 in Bombay[3], India and is associated with the ABO and H blood group systems. In Bombay phenotype, both red cells and secretions are deficient in H, A, and B antigens. In routine blood testing, cell grouping shows characteristics of O group. Serum grouping reveals the presence of anti-H activity in the serum in addition to the natural ABO antibodies. The anti-H present is of clinical significance since it can activate complement and cause hemolysis. As their blood is compatible only with the Bombay phenotype cells, getting blood unit for transfusion is not easy. Bombay phenotypes have a relatively higher incidence in India. Its prevalence is reported as 1: 4600 in Ratnagiri district[4], 1: 7600 in Mumbai[5], 1 in 10,000 in India[6], and 1/1,000,000 individuals in Europe[7],[8].

Another related phenotype is para-Bombay which is extremely rare, with only a very few cases being reported and none of the published studies have reported the prevalence of para-Bombay phenotype in India. However, in the Chinese population, more number of para-Bombay than Bombay phenotype cases have been reported. The para-Bombay phenotype is characterized by the absence of ABH antigens on red blood cells (RBCs) with the presence of ABH substances in body secretions or by the weak expression of ABH antigens on RBCs with the absence or presence of substances in body secretions. In contrast, Bombay phenotypes have an absence of ABH antigens on both, the RBCs as well as in body secretions[9]. As H antigen is the precursor for the A and B antigens, it is expressed on all red cells except in the rare Bombay and para-Bombay phenotype showing its absence or deficiency. The ABO genes determine the presence of A and B antigens, whereas the H antigen is a result of α-(1,2)-fucosyl transferase (FUT)genes. FUT1 (H gene) determines the presence of H antigen on the RBCs and FUT2 (Se gene) in body secretions. FUT1 forms the H antigen which is preferentially expressed in erythroid tissues and vascular endothelial cells by fucosylation of the type 2 chain oligosaccharides on red cell glycoproteins and glycolipids. FUT2 recognizes type 1 chain precursors to form H type I antigen in secretions and tissues such as secretory glands and digestive mucosa. Bombay phenotype is characterized by the absence of ABH blood group antigens both on the surface of RBCs and in saliva resulting from both silenced mutationsin FUT1 (h/h)and FUT2 (se/se)genes.[7] The para-Bombay phenotype results from a silenced FUT1 gene (h/h) but an active FUT2 (Se/Se or Se/se) gene to synthesize H Type I antigen (and A/B antigens) in the secretions (H-deficient secretors) that may be adsorbed onto RBCs from the plasma or from a mutated FUT1 gene resulting in great diminished enzyme activity to produce low amounts of H Type II antigen (and A/B antigens) on the surface of RBCs, which could only be detected by adsorption and elution technique.[8] We had one patient with very rare blood group and her history was 21 female patient with 22 weeks of gestation admitted for anemia evaluation. She had history of one abortion and she had no history of consanguineous marriage .she had not been transfused with blood or blood product previously. Her hemoglobin was 6.5 grams. No history of bleeding disorders and she was not on any medication. She was diagnosed to be o positive blood group by private lab.Her family member blood grouping was also performed her parents were O positive and her brother was O positive , husband also O positive all had 4+ agglutination with Anti –h in the forward grouping The Para-Bombay phenotype is very rare.[9] Only a few cases of Para-Bombay were reported in India till now .[10] This entity is characterized by the absence of H, A and B antigens on the red cells but their presence in saliva and secretions of gastrointestinal and genitourinary tracts.[11-13] Proper identification of this phenotype is very important; otherwise this particular blood group may be mislabelled as group O.

This case of para-Bombay phenotype was detected as a result of discrepancy in cell and serum grouping. This study highlights the importance of proper blood grouping (forward and reverse both) and use of anti-H reagent for the identification of para-Bombay phenotype, otherwise this donor would have been mislabeled as Group O. In routine blood banking, besides blood grouping detailed serological tests such as secretor status should be performed.

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