International Journal of Multidisciplinary and Current Educational Research (IJMCER)
ISSN: 2581-7027 ||Volume|| 3 ||Issue|| 3 ||Pages 55-58 ||2021||

Discrepancy in a patient, shows weak expression in group B with mismatch transfusion with O; dilemma in a case report

Sonia Shormin Miah[1], Mostofa Ahmed Doha[2], Nadia Sharmin[3].

ABSTRACT: Safe blood transfusion is a pivotal part to maintain the safety of the patients. Detection of ABO blood groups through the agglutination technique is the basis of pre-transfusion safety. However, weak agglutination reactions, that is the weak expression of A or B antigens on the red cell surface may lead to discrepancy in blood grouping. Although all individuals of the same blood group have the same kind of agglutinogen in their red blood cells, the sensitivity of the cells to agglutination varies from individual to individual. Moreover, there is a wide variation in the amount of agglutinins in the serum of different individuals belonging the same blood group. The most reliable grouping is achieved if both these methods are used, so that they form a check on each other. Here is a case report showing a part of missing reaction in forward grouping that causes the discrepancy.

KEYWORDS: ABO blood grouping, weak antigenic expression, sub-groups of B.

I. INTRODUCTION:

ABO blood grouping and Rh typing has been established in1900 by the famous pioneer Karl Landsteiner. He has afforded this to ensure the safety of humankind. The human serum and cells of different individuals closely interact to confound the reactions of antigen and antibody. These antigens have inverse reciprocal relationship with the antibodies. That's being a prodigious part of our working. Some reactions cant conclude the detection whereas some has some unique pathology to overcome this type of situation. However, we confined this reaction into two parts: Forward and reverse. In forward grouping we would like to use commercial antisera and in reverse grouping A, B and pooled O cell to complete the reaction. When we face some dissimilar then we have to go for reconfirmation to resolve the part as well as in which cause has been legally causing this type of reactions.
Blood group discrepancy is not an uncommon issue to be afraid of, rather being a steady part to recognize the safety of the patients. Occasionally its closely correlated to the patients age, his brief clinical history, drug, vaccination, pregnancy and transfusion. The study of family tree is also become a part of this. Even in few cases patients' blood group has being draped by some disease condition or due to continuously mismatched blood transfusion.

Case report: A 47 years old female, preparing for hysterectomy and sent her sample sent to the department of Transfusion Medicine asking for group confirmation with compatibility test with two blood donors. She has also five reports of different blood centres. She was typed as O Rh D positive and respectively transfused with three units of O Rh D+ve blood. Now her minor cross matching was remaining incompatible with other O blood donors and she was referred to BSMMU for further evaluation and management. In laboratory, we have taken her sample in a vial with anticoagulant EDTA and another was fresh clotting sample to find out the discrepancy and cross matching incompatibility. All tests were run through the test tube method. The tests were performed according to the standard operating procedures (SOPs) in the transfusion medicine laboratory. Meanwhile, we first investigated any technical or clerical error that may offend the test results or interrupted the total technical procedure. We have a close observation of the color of the serum, either its haemolyzed, lipaemic or clear and also a look to the cells to see it's too sticky or easy to remove for cell washing for the procedural purpose. Next, after performing all the necessary steps we go through the result that has been documented at the first day:
Tube method:
Room temperature (at the first day)

	Forward grouping			**Reverse grouping**		**Rh typing**	
	-B	**-A**	**-AB**	**A cell**	**B cell**	**O cell**	**-D**	**A/C**
Patient (-)	-	(+-)	++	+-	+	++	-

(Therefore, reveals forward as B group and in reverse O group)
At 4^0C

Forward grouping			Reverse grouping			Rh typing	
-B	**-A**	**-AB**	**A cell**	**B cell**	**O cell**	**-D**	**A/C**
Patient (-)	**-**	**(+)**	**++**	**+-**	**+-**	**++**	**-**

Tube method : at 37^0C

-B	**-A**	**-AB**	**A cell**	**B cell**	**O cell**	**-D**	**A/C**
Patient (-)	**-**	**(+)**	**++**	**+-**	**++**	**++**	**-**

After 3 days, again her fresh blood sample was rechecked for grouping:

Forward grouping			Reverse grouping			Rh typing	
-B	**-A**	**-AB**	**A cell**	**B cell**	**O cell**	**-D**	**A/C**
Patient (+-)	**-**	**(+)**	**++**	**+-**	**+**	**++**	**-**

So we have repeat this test for more three times with another monoclonal reagents (Tulip, Lorne and Biorex) to see the comparison and also with human serum too see the pattern of agglutination reaction at both $4^{0\,c}$,room temperature (25^{0C}), and 37^{0C}.We have observed that her blood group was probably Weaker than B may be B_w, B_3 or B_x. Moreover, she had a history of blood transfusion with O. Therefore, both forward and reverse typing shows mixed field appearance. After that we also see her antigen reaction with anti H to see either its B^h or AB^h .But it shows true agglutination with anti-H in presence of a control cell.Usually, B_3 or B_w gives comparatively weaker reaction or no reaction with anti-B but mixed field reaction with anti AB. Her serum was agglutinated with A cell, weak agglutination with B and O cell. In B_w or B_3 gives a mixed field appearance or no agglutination with anti B. But reactions with anti AB guided us that probably she is weaker than B. That is the issue for her serological typing of confirming the discrepancy. In this reverse typing shows 2+ agglutination with A cell and +-(mf) with B cell and O cell. This mixed field appearance is probably due to mismatched blood transfusion with O. Next Coombs test was performed which reveals direct shows positive reaction and indirect was negative. Here DCT was positive due to mismatched transfusion with O, as she has no significant history of disease e.g. Auto Immune Haemolytic Anaemia or drug and her auto control also shows negative result.

Rather this blood group was also performed using fresh human serum containing anti B, anti A and anti A, B with patients own cell to see the pattern of reactivity as well as the gradation of agglutination. The test result shows:

Forward grouping			Reverse grouping				
-B	**-A**	**-AB**	**A cell**	**B cell**	**O cell**	**-D**	**A/C**
Patient (+-)	**-**	**(+)**	**++**	**+-**	**+**	**++**	**-**

Secretor status guided as presence of B and H substance that has inhibited reaction between anti B and B cell. Elutes agglutinated with B and AB red cells did not agglutinate with A or O cell. So, in this part that she predicts as weaker than B or B_3. Meanwhile, cross matching was done with both B and O donor. It shows compatible with major and minor with blood group B but minor remains incompatible with blood group O. Therefore, she was later transfused with B and her transfusion remains uneventful. Then we asked her family members' blood group to know more about her family tree. Her husband was O and four siblings' were B. However, her parents was not alive therefore not possible to be noted.

II. DISCUSSION:

Subgroups usually possess decreasing antigenic sites on the red cells, therefore a reciprocal increase in H antigen activity [5, 6]. They are recognized when there is a discrepancy between the red cells and serum grouping**.** Subgroups of A are more common than B. Subgroups of B are usually recognized by variations in the strength of agglutination reaction using anti –B and anti-A, B. Inheritance of B subgroups are, similar to that of A, is considered to be a result of alternate alleles at the B locus. Criteria used for differentiation of weak B phenotype include the following serologic techniques:

Antigens and antibodies present in B sub group:

Variants of B (mistyped as O)	Antigenic pattern of reaction with anti B sera	Antibodies in serum
B_3	weaker reaction with presence of free cells	Anti A1, no anti B

B_w	mixed blood reaction or no reaction	Anti A1, no anti B
B_v	Do not agglutinate with anti-B sera	Anti A can agglutinate the Ax cells
B_x,	Do not agglutinate with anti B sera (serum and saliva contain group specific substance)	contains Anti A, Anti B
B_k,B_{el}	only seen in adsorption elution test	-----

The B_w phenotype generally results from the inheritance of a rare gene at the ABO locus and is characterized by a mixed field pattern of agglutination with anti- B and anti AB. Therefore, B_w secretors secreted B substance in their saliva. There is no anti B in the serum. In B_3 phenotypes also give weaker reaction with Anti-B but B_3 substance don't contain B substance in their saliva and the serum does not contain any anti B. Nevertheless, B_3 subgroup is the most frequent weak B phenotype. In a case study of Khatun A et al. describes a case report about B_x (subgroup of B) which is a very rare phenotype. It is wrongly typed as 'O' because the B antigen is very weakly present on red cell membrane, which evolves weak antigenic expression with anti-B. Most of the ABO blood groups antibodies are IgM in nature and detected best at 4^0C. B_x that contains weak anti-B too. After adsorption with anti-B an elute was prepared from patient's cells which agglutinated with B and AB cells but did not agglutinate with A or O cells. Elutes agglutinated with B and AB red cells did not agglutinate with A or O cells. B substance was present in his saliva, which inhibited the reaction between anti-B and B cells. However, in case of B_x subgroup typically demonstrate with weaker agglutination with anti-B and anti – AB and B glycosyltransferase is not detectable in the serum or in the red cell membranes of Bx phenotypes, but a weakly reactive anti-B usually is produced. B_x RBCs readily absorb and elute anti B. Secretor studies demonstrate large amount of H substance and some B substance that often can only detected by inhibiting agglutination of B_x cells with anti B. It's a rare allele of ABO locus.[10]

In another study from Chourasia R et al. they have shown that subgroups of B have been reported to be more common in India in comparison to French population. They have shown three case reports. Two cases of B_m and one case of B_w which was done on the degree of agglutination reaction with the monoclonal Anti-B, monoclonal Anti-AB, monoclonal Anti-H antisera, the saliva-secretor status, the adsorption-elution studies with polyclonal Anti-B antisera and the presence or absence of ABO isoagglutinin's in the serum subgroups can be classified as B_3, B_x, B_m, and B_{el}, plus B_w for those that do not fit any of the other four categories [3,4]. They have also featured the problems in resolving the discrepancy. Afterwards, led to further adsorption-elution studies with polyclonal human antisera of group B, group A, and group O. Individuals cell was then taken for adsorption at 4 °C for 1 h; heat elution at 56 °C for 10 min and then elute test against 3 sets each of reagent red cells (A and B). To ascertain their secretor status, saliva samples were collected and further tested by haemagglutination inhibition assay. In all 3 cases, cell grouping was consistent with group 'O' and serum grouping was consistent with group 'B', in immediate-spin at 22 °C. Adsorption-elution technique with appropriate valid controls yielded only "Anti-B" on elution. Thus, indicating the presence of weaker variants of B group. Saliva sample could only be tested for two cases (i.e. 1 donor and 1 patient); of these 2 cases, presence of both B and H substances was detected in 1 case, whereas the other was found to be a non-secretor . The secretor status for 2 and donor couldn't be ascertained as the donor was unable to come back to our center for providing the saliva sample[13,14].

III. CONCLUSION:

Although of considerable academic interest, solving blood grouping discrepancy is still a limited practice though it has worthy clinical importance as most of the laboratories still perform only forward grouping during issuing their reports. Anomalous grouping results primarily to the occurrence of any atypical reaction should be encountered sooner or later and must be dealt with according to the resolving procedure as we discussed above. Nevertheless, molecular analysis of the genetic markers proves to be a useful tool for resolution of such grouping discrepancies. The serologically classified subgroups of 'B' thus found at our center, required molecular confirmation through genetic analysis that might have revealed the underlying mutations. However, still it is not possible due to our limitations to take all necessary steps.

REFERENCES:

1. Sherman M, Bain V, Villeneuve JP. The management of chronic viral hepatitis: A Canadian consensus conference 2004. Can J Gastro Enteral 2004; 18: 715-28
2. Brown KE, Hibbs JR, Gallinella G. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte p antigen). N Eng J Med 1994; 330: 1192-6.
3. 1. Daniels G. Human blood groups. 3. New York: Wiley; 2013.
4. 3. Harmening D. Modern blood banking and transfusion practices. 6. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2012.
5. Haataja S, Tikkanen K, Liukkonen J. Characterization of a novel Bacterial adhesion specificity of a streptococcus suis recognizing blood group P receptor oligosaccharides. J Biol Chem 1993; 268: 4311-17 6. Spitalnic PF and Spitalnic SL. The P Blood Group System: Biochemical, Serological and Clinical aspects. Transfuse Med Rev 1995; 9: 110-22.
7. Kennedy MS, Waheed A, Moor J. ABO discrepancy with monoclonal ABO reagents caused by ph dependent autoantibody. Immuno-Haematology 1995; 11: 71-3.
8. Mollison PL, Engelfriet CP, Contrera M. Blood Transfusion in Clinical Medicine. 10th ed Oxford: Blackwell Scientific Publication 1997; 114-117.
9. Kennedy MS, Waheed A, Moor J. ABO discrepancy with monoclonal ABO reagents caused by ph dependent autoantibody. Immuno-Haematology 1995; 11: 71-3.
8. Mollison PL, Engelfriet CP, Contrera M. Blood Transfusion in Clinical Medicine. 10th ed Oxford: Blackwell Scientific Publication 1997; 114-117.
10. Silva MA. Standards for Blood Banks and Transfusion Services, 23rd edition Bethesda MD; AABB 2005; 293-294. 8. Oriol R, Candelier JJ, Mollicon R. Molecular Gecetics of H. Vox Sang 2000; 78 (suppl 2): 105-8.
11. KhatunA1 , BiswasJ 2 , HabibullahMM 3 , Islam A 4 , ShillN 5 et al. B Subgroup: Bx blood Group in a Patient : A Case Report Bangabandhu Sheikh Mujib Medical University Journal 5(1) · June 2012 *with* 719 Reads,DOI: 10.3329/bsmmuj.v5i1.11032
12. Brown KE, Hibbs JR, Gallinella G. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte p antigen). N Eng J Med 1994; 330: 1192-6.
13. Haataja S, Tikkanen K, Liukkonen J. Characterization of a novel Bacterial adhesion specificity of a streptococcus suis recognizing blood group P receptor oligosaccharides. J Biol Chem 1993; 268: 4311-17
14. Chaurasia R, Rout D, DograK, Coshic P, and Chatterjee Discrepancy in Blood Grouping: Subgroups of B-Challenges and Dilemma, Indian J Hematol Blood Transfus, 2017 Dec; 33(4): 628–629. PMID: 29075085