CASE REPORT

Non-Secretor "A" Subgroup with Acquired B Antigen, Naturally & Acquired Immune Anti-A1 Antibodies in a Patient with Carcinoma of Esophagus

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ABSTRACT

ABO blood group system is one of the most important in clinical transfusion medicine. Numerous ABO discrepancies are seen during routine blood banking and they should be properly sorted out for a safe blood transfusion. During usual blood grouping we found a patient with carcinoma of esophagus having "A" Sub Group, acquired B antigen along with a non-secretor status. The patient was referred to the blood bank of DDRRL, Dow University of health sciences, Karachi, Pakistan, for transfusion due to severe anemia.

The aim of this case report is to have a heightened awareness of blood sub-groups especially in patients with malignant diseases and performance of reverse grouping as a routine which is carried out in quality care transfusion services

The first choice of blood group for transfusion is the A_3 subgroup followed by O blood group. As the patient had already received A1 blood group, so in addition to the naturally occurring cold antibodies, he had developed warm anti A_1 antibodies so A_1 blood transfusion should be avoided in him.

Keywords: ABO blood grouping, Sub-groups A_3, Problems of RBC phenotype. Ca esophagus

INTRODUCTION

The ABO blood group system was the first to be discovered and till now remains the most important in transfusion practice. This is due to regular occurrence of Anti-A, anti-B & anti-AB antibodies, reactive at 37° C, capable of causing red cell destruction in persons whose red cells lack the corresponding antigens. There are over 70 ABO alleles reported to date highlighting the extensive sequence variation in the coding region of the gene[1].

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A_3 (subgroup of A) is a rare phenotype of blood usually due to allelic difference at "A" locus especially at 7th exon [2]. Many of these subgroups can be wrongly typed as 'O' because the "A" antigen is very weakly present on red cell membrane, which cannot be detected if weak anti-A is used in cell typing[3]. Most of the ABO blood group antibodies are 1gM in nature and detected best at 4°C. Many blood banks perform blood groups only by forward grouping and these results are not counter checked by reverse grouping. In addition some may use tile method in which weak antigens are not detected and as a result the persons are typed wrongly. As shown in table-1, the weak "A" subgroups are: A_2, A_3, A_x, A_{end}, A_m, Ay & A_{el}[4]. Only A_2, A3, A_x and A_{end} show agglutination with the anti-seras. "A2" subgroup shows +2-+3 agglutination with anti-A, A3 shows a mixed field agglutination with anti A, , while in A_{end} only 10 % cells show weak mixed field agglutination whereas A_x shows only weak reaction with AB antisera only. The other subgroups A_m, Ay & A_{el} do not show any

reaction with "A" antisera [5]. These groups may have naturally occurring cold antibodies but may develop immune anti-A1 antibodies reactive at 37° C if transfused with "A1" blood group[6]. Acquired "B" antigen is not an uncommon phenomenon especially in malignancies of gut and in severe infections[7]. The bacterial produce a deacetylase (enzyme) which chemically alters the terminal sugar of "A" antigens (N-acetyl-D-galactosamine) into D-galactosamine. Because the terminal sugar of the "B" antigen is galactose, anti-B antisera will cross react with the B-like D-galactosamine antigen [8]. As this phenomenon is *in vivo*, only group "A" people can develop an acquired B-like antigen. The condition is transient and disappears when the infection is cured and do not pose any transfusion risks except creating discrepancy in ABO grouping. These acquired B antigens are not secreted in saliva and this investigation is useful in differentiating it with rare B subgroups. In vitro, blood specimens can get an acquired B-like antigen if they are bacterially contaminated. This is because the membranes of some bacteria (e.g., E. coli and P. vulgaris) have determinants which are chemically similar to the B antigen [9] In this case, anti-B antisera actually reacts with the bacterial antigens which have attached to the red cells. In vitro, both group O and group "A" cells can acquire the B-like antigen. Important to note is that most examples of acquired "B" phenomenon detected in the blood bank happen in vivo to group "A" people only.

Case Report:

Blood sample of a 65-year-old man was received by our blood bank with a request for two compatible PRBCs. He was a diagnosed case of carcinoma of esophagus on palliative therapy. He was somewhere else labeled as "A" negative blood group and two weeks ago received one PRBC of "A" negative blood group. As a routine, his blood group was reconfirmed by repeat testing and a significant discrepancy was found as shown in table-2. As this patient had a discrepancy, so the negative reactions were also verified microscopically. It was found that in addition to mixed field reaction with anti "A" and "AB" sera, this patient had a microscopic positive reaction with anti B sera along with strong reaction with B known cells and a weak reaction with A cells on serum grouping. This patient was then tested with A1 lectin which was negative and anti-H which was positive. His reverse grouping was also tested at 4°C and 37° C and both were reactive. This raised the suspicion that probably the patient has developed anti A1 antibodies after transfusion on a background of naturally occurring anti A1 antibodies. To confirm the presence of acquired "B" antigen, the antisera "B" was acidified with HCl to keep pH just above 6.0. The sample did not give reaction with this acidified antisera. adsorption elution for B antibodies was also negative. His saliva did not contain any "A", "B" or "H" antigen.

Cross match with "A1" blood group showed minimal incompatibility both in saline and AGT phase. Keeping in view the mixed field reaction with "A"& "AB" anti-seras, his blood group was assumed to be of "A3" phenotype. As this patient had immune anti-A antibodies and "A3" blood group was not available so his blood was cross matched with O negative blood group and he was transfused with this group without any transfusion reactions

Discussion:

"A" and "AB" have many sub-groups identified uptill now. "A" subgroups carry less amount and slightly different antigens due to allelic differences. These sub-groups secrete fewer amounts of antigens in the saliva. Sometimes they harbor naturally occurring anti A1 in them but as these are cold antibodies so they do not pose any transfusion risk, but can develop anti A1 if exposed to A1 blood group which are usually IgG and reactive at 37° C causing problems in compatibility testing and may produce immune transfusion reactions[10]. Subgroups of "A" are more commonly encountered than subgroups of "B" (AABB technical manual) which are B3, Bx & Bel. All subgroups are characterized by decreasing numbers of antigen sites on the red cells and reciprocal increase in H antigen activity. Subgroups are more often recognized when there

is a discrepancy between the red cells and serum grouping. Some blood banks perform only forward grouping and; these results are not confirmed by serum grouping. Moreover they perform grouping by slide method in which weak antigens are not detected and as a result the persons are typed wrongly. Acquired "B" antigen is not an uncommon finding and as the case here is sometimes found in malignances of gut[11] and may also be seen in severe bacterial infections when the bacterial enzymes convert the normal antigen to B antigen on the surface of red cell

When a subgroup is encountered it is always appropriate to determine the secretor status of that person, as these antigens are secreted in saliva, as more than 80 % individuals are secretors[12]. It is also necessary to check all "A" and "AB" groups with A1 lectin, as A2, sometimes A3 and usually other A sub groups can be missed. We also recommend confirming any negative reaction in cell and serum grouping by microscopic examination, as the antigen may be too weak to show a macroscopic reaction

Conclusions:

Usually subgroups are wrongly typed as "O", which may cause HTR (Hemolytic Transfusion reaction), leading to death. All negative reactions should be confirmed by microscopic examination. All "A" and "AB" groups should by subject to "A1" lectin and if negative should be checked in by anti-H antisera. Adsorption elution test and secretor status of the sub-group person can be helpful in confirming the subgroups.

It is worth mentioning that in cases of subgroups a proper three phase cross match should be done (Saline phase at room temp, 4^0 and 37^0 Celsius temp, ICT phase) to ensure correct blood to the right patient.

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Table-2 **Blood group reactions of patient**

	Forward grouping							Reverse grouping		
	Anti A	Anti B	Anti AB	Anti D	Anti H	A1 Lectin	Auto-control	A cells	B cells	O cells
Macroscopic	+1	Neg	+2	00/ Du negative	+ 3	Neg	Neg	+1	+4	Neg
Microscopic		+ 2		00 / 00		00	00			00

Table-1 Various reaction of "A" sub-groups

Sub group	Anti A	Anti B	Anti AB	Anti H	A1 lectin	A cells	B cells	Secretor status	"A" Transferase	Antigen: RBCs x 10^3	serum Antibody
A_2	+2	++mf	.wk/0	mf	0	+ / -	Yes	A, H	Yes	> 35	Anti B & anti A*
A_3	++mf	0	++mf	3+	0	+ / -	Yes	A, H	sometimes	35	
A_x,	Wk/0	0	2+	4+	0	always	Yes	A(trace), H	rarely	5	
A_{end}	mf/w	0	w/mf	4+	0		Yes	H	no	3.5	
A_m	0/wk	0	0/+	4+	0	No	Yes	A, H	yes	1	
Ay	0	0	0	4+	0	No	Yes	A, H	trace	1	
A_{el}	0	0	0	4+	+/-	yes	Yes	H	NO	0.7	

*(weak, rare & usually cold)

REFERENCES

1. Yip SP. Sequence variation at the human ABO locus. Ann Hum Genet. 2002;66(1):1-27.
2. Pipatvanichkul A, Permpikul P, Vejbaesya S, Chinswangwatanakul W. J Med Assoc Thai. 2011;94(3):379-85.
3. Cartron JP, Gerbal A, HughesJones NC, Salmon C. 'Weak A' phenotypes: relationship between red cell agglutinability and antigen site density. Immunology.1974;27(4):723-7.
4. Yamamoto F, Clusen H, White T, Marken J, Hakomori S. Molecular Genetic basis of the Histo-Blood Group ABO System. Nature. 1990;345(6272):229-33.
5. Heier HE, Namork E, Calkoska Z, et al. Expression of A antigens on erythrocytes of weak blood group A subgroups.Vox Sang . 1994;66:231236
6. Lee AH and Reid ME. ABO Blood Group System: A Review of Molecular aspects. Immuno-Haematology 2000;16:1-6.
7. Gerbal A, Ropars C. Rev Fr Transfus Immunohematol. 1976;19(1):127-44.
8. Denise M. Modern blood banking & transfusion practices, 5th edition, F A Davis, 2005 ch 6: p125
9. Dean L. Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2005. Chapter 5, The ABO blood group. Available from: http:// www.ncbi. nlm.nih.gov/ books/ NBK2267/
10. Economidou J, Hughes-Jones NC, Gardner B: Quantitative measurements concerning A and B antigen sites. Vox Sang 1967;12:321328
11. Beenu T, Karan S, Meenu B: Importance of weak ABO Sub-group. LabMedicine. 2003;36(1):32-4.
12. Denise M. Modern blood banking & transfusion practices, 5th edition, F A Davis, 2005 ch 6: p113