INTRODUCTION

The ABO blood group system was first described in 1900’s¹ and its terminologies and blood typing procedure were developed later.² A and B are most significant antigens and antibodies in human transfusion practices which exist as three allelic forms of ABO, A, B and O. Gene of A and B, present on chromosome 9, encodes the corresponding glycosyltransferases while O gene does not code any functional enzyme.³ These enzymes add sugar molecules on glycoprotein and glycolipid on the surface of RBCs. In group-A, terminal monosaccharides are N-acetylgalactosamine and lactose in group-B. These antigens are present on the H-antigen which is synthesized by fucosyltransferases produced by a gene independent of ABO.⁴ It means that the O-gene does not code for any functional enzyme and RBCs possess an abundant amount of H-antigen on their surface but lacks the A and B antigen. In ABO blood grouping, the reciprocal antibodies are naturally present in the sera of most people who have had no exposure to human red cells. If ABO incompatible blood is transfused it can cause intravascular hemolysis.⁵ Testing to detect ABO blood group typing through agglutination is the basis of pre-transfusion testing. However, weak agglutination reactions may be obtained with reagent antibodies and are a result of weak expression of A and B antigens on red cell surface, which may cause a discrepancy in blood group typing. The variants of B blood group (Table I), with a weak expression have been previously reported.⁶ Present investigation was carried out to determine the blood group of a donor which showed discrepancy between red cells (forward) and serum (reverse) grouping.

CASE REPORT

The blood of a healthy male donor, 34 years of age with no history of disease and drug therapy was analyzed for blood group determination. The blood was reacted with commercially available monoclonal antisera against A, B and D along with the reagent red blood cells of a1, b and o type. No agglutination reaction was observed with known antisera of A and B while a strong reaction was seen with reagent a1 cells. Result so obtained was non-conclusive because known antisera agglutination reaction and known antigen (red blood cells) agglutination reaction are not complimentary. In order to resolve this discrepancy, further investigation using protocol as outlined by American Association of Blood Banking manual was adopted.⁷ In brief, donor blood was incubated with known antisera for varying time intervals at various temperatures. Incubating at low temperature enhances the reaction of antibodies (A and B), mostly IgM type. A parallel reaction with anti-H is performed to check the reactivity of donor’s red cells. Results so obtained are summarized in Table II.

A very weak agglutination reaction was observed with serum against known B red blood cells at 4°C after incubating for 30 minutes. Further confirmation was carried out by reacting donor and control (O red cells) cells, treated with bromelain, a proteolytic enzyme. No red cell agglutination was observed between donor cells and anti-A and anti-B.

Results were non-conclusive in determining the blood group type. Further evaluation was performed using the adsorption elution agglutination assay.⁸⁹ Reaction of last wash with known A1, B and O RBCs at IS, 37°C, and AHG were negative while strong agglutination reaction was observed with B red blood cells when reacted with elute at immediate spin (IS), 37°C and AHG (anti-human globulin) phase. The results from the present study indicated that the donor’s blood
group is a variant of B in ABO system, since the elute contained anti-B conforming the presence of antigen-B on donor's red cells.

**DISCUSSION**

The antigenic differences among the species are the basis for their recognition. It was Landsteiner who for the first time demonstrated that every individual carries a different antigen on the surface of its red blood cells.1 The ABO blood grouping system proved to be of vast importance. It is these antigens which play an important role in the transfusion practices. An ABO incompatible red blood cells transfusion is a leading cause of death from transfusion.10 Apart from A, B and O group, multiple phenotypes are also found among the humans. The subgroup typing is usually carried out when there is a discrepancy in blood group typing based on various methods: (a) degree of red cell agglutination with antisera, (b) presence or absence of agglutinins in serum, (c) presence of A or B and H substances in the saliva of secretors, (d) adsorption elution studies and (e) family studies. Moreover, ABO discrepancies may also be resolved using patient's age, diagnosis, medication, history of pregnancy, or recent transfusion. The time when the present case study was investigated no research has so far been reported on Pakistani individuals.

In the present case, the ABO discrepancy was found in a 34-year-old, healthy male blood donor. Errors arising as a result of commercial reagent usage and in-house reagent cells in reporting ABO blood grouping cannot be ruled out. This can be overcome by control runs in parallel with investigational testing. An identification of a weak reaction can be facilitated through increasing the incubation time, lowering the reaction temperature and treatment of red cells with enzymes, all these enhance the reactivity of antigen A and B with corresponding antibodies.9 Treating red blood cells with proteolytic enzyme degrades MNS antigens whereas not reacting with H antigens. Therefore, a strong positive reaction was obtained with O blood cells since they have H antigens on their surface. Furthermore, adsorption elution reaction of elute generated a positive agglutination with known B cells. In fact, it appears on the whole that weaker the antigen, the more potent is elute. Moreover, secretor or family (pedigree) studies should be carried out in such cases since red cells from individuals with variant A or B genes may carry poorly expressed antigens that may also arise as a result of any disease condition such as leukemia or malignancies. These secretor (Se) studies are of significance since the Se gene regulates the expression of H antigens which may alter the expression of antigen A or B.

It is concluded from the present investigation that the donor is a B variant phenotype and may have any of the four phenotypes (Bx, BM, Bel, B3) of blood group B. It is further recommended that salivary or secretor studies must be carried out to determine the frequency of phenotypes of blood group-B in Pakistani population.

**REFERENCES**


