Prevalence of A_2 and A_2B subgroups and anti-A_1 antibody in blood donors at tertiary care center in Bangladesh: A cross sectional study

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ABSTRACT

Context: A_2 and A_2B are rare phenotypes of ABO blood group system. The presence of anti-A_1 in A_2 and A_2B subgroups may cause hemolytic transfusion reaction if active at 37^0c.

Aim: To assess the prevalence of A_2 and A_2B subgroups along with anti-A_1 in blood donors of Bangladesh.

Setting and design: Cross sectional study in a tertiary care hospital, Bangladesh.

Subjects and Methods: The study included blood donor samples received at Transfusion Medicine Department. All samples were typed for ABO and RhD grouping using conventional tube technique. Group A and AB were further subtyped using anti-A_1 lectin. Detection of anti-A_1 antibody for A_2 and A_2B individuals was done using A_1 red cells.

Statistical analysis: The data were analyzed and tabulated by using Microsoft Excel and SPSS (version 26). Fisher's exact test was used for comparing categorical variables.

Results: Out of 1,696 healthy blood donors, blood group O was the highest. The overall phenotypic frequency of all blood groups was $O > B > A_1 > A_1B > A_2B > A_2$. A antigen (A and AB Blood Group) was present in 610 (36%). Of these, A_1 antigen was present in 599 (98.2%) donors and A_2 antigen was present in 11 (1.8%) donors. Of 468 donors with blood group A, 466 (99.6%) belonged to A_1 and 2 (0.4%) belonged to A_2. Out of 142 group AB donors, A_1B was found in 133(93.7%) and A_2B in 9 (6.3%). No anti-A_1 was detected in A_2 and A_2B subgroups.

Conclusion: Implementation of A_1 and A_2 grouping can prevent minor incompatibilities, thus ensuring safe blood transfusion.

Keywords: *A_2 subgroup, A_2B subgroup, Anti -A_1, Blood donors, Bangladesh.*

INTRODUCTION

Till July 2023, International Society of Blood Transfusion reported over 360 red cell antigens which are organized into 45 blood group systems[1]. These antigens may be proteins, glycoproteins or glycolipids and play a vital role in transfusion medicine, genetics understanding, inheritance pattern, forensic pathology, and medico-legal issues such as unmatched pregnancy and disputed paternity. Blood groups are hereditary where parents pass down their blood group allele to their children.

The ABO blood group was the first human blood group to be discovered by Karl Landsteiner in 1900, based on the presence or absence of A and B antigens on red blood cells and its Mendelian inheritance pattern by Bernstein in 1924[2]. At the beginning, three major blood groups A, B and O were identified and later in 1902, the fourth and

less frequent AB was discovered by DesCasterllo and Sturli. The ABO gene is located on chromosome 9 at 9p34.1–q34[3].ABO antigens are oligosaccharides found on the extracellular surface of red blood cell membrane. They are highly expressed on the surface of a variety of human cells and tissues including platelets, lymphocytes, vascula endothelium, intestine, cervices, urethra, mammary glands and in soluble form in secretions including saliva, tears, and breast milk.

Variation in A antigen expression was recognized early in the twentieth century. Polymorphisms in the genes coding for the A gene may lead to formation of diminished amounts of antigens on red blood cells with or without some qualitative changes. These are defined as subgroup of A. A_1 and A_2 are the most common (over 99%) subgroups of A. The A_2 gene has two nucleotides different from the A_1

gene, at nucleotide 467 (substitution of C to T) and nucleotide 1061 (deletion of C) within the exon 7 of ABO gene[4]. Approximately, 20% of individuals having A antigen belong to A_2 and thus, forming either A_2 or A_2B while rest 80% belong to either A_1 or A_1B subgroups[5]. Other less prevalent subgroups of A include A_3, A_x, A_{end}, A_y and A_{el}, A_1 and A_2 differ from each other both qualitatively and quantitatively. A_1 red cells express approximately five times more A antigen than A_2 red cells and both red cells react strongly with monoclonal anti-A reagents in direct agglutination tests. The distinction between these two subgroups is made by the reactivity with the lectin obtained from Dolichos biflorus seeds. The red cells of A_1 subgroup will agglutinate with this lectin but not A_2 red cells. Approximately 0.4% A2 and 25% of A2B individuals possess naturally occurring anti-A_1[6]. The thermal amplitude of this antibody is below 25°C and usually do not pose problem in transfusion. However, fewer cases of anti-A_1 reacting at 37° C have also been reported in the literature which can cause extensive destruction of A_1 cells leading to acute or delayed hemolytic transfusion reaction[7,8]. Thus, anti-A_1 active at 37°C is called as clinically significant. Moreover, the presence of anti-A_1 may interfere in routine blood grouping and cause discrepancies in forward and reverse grouping. An individual will forward type as group A and reverse type as a group O or forward type O and reverse type B[9]. Therefore, misidentifying the blood group can be lethal.

The prevalence of A subgroup varies between different populations. There is very limited published data about ABO blood groups distribution from Bangladesh, and only one report described the general frequency of A_1 and A_2 among patients[10]. To the best of our knowledge there is no published work in the literature regarding distribution of subgroups (A_1, A_2) among individuals with group AB from Bangladesh. Therefore, in this study, we intended to determine the prevalence of A_2 and A_2B subgroup in healthy blood donors and the presence of anti-A_1 antibody with reactivity pattern (if present) at tertiary care center.

SUBJECTS AND METHODS
Study Design
The present cross-sectional study was conducted over a period of three months, May to July 2023. The study included blood donor samples received at Transfusion Medicine Department. Blood donors were selected according to institutional protocol. Each donor was interviewed before donation. Donors with no clinical history of any hereditary blood disorders, no medication at the time of donation and physically fit, were accepted. Information regarding previous blood transfusion and donation was also obtained. After informed written consent, venous blood samples were collected in EDTA anticoagulated tubes and plain tubes. The study was conducted in compliance with the guidelines of the research and ethics committee of the institution.

a. Inclusion Criteria
1. Donors attending Evercare Hospital Transfusion Medicine Department.
2. Both genders.
3. Age more than 18 years.

b. Exclusion Criteria
1. Group B and O donors.
2. Samples with reactive transfusion transmitted infection including HBsAg, HCV, HIV, syphilis, and malaria.
3. Deferred donors.

c. Laboratory Techniques
- All samples were typed for ABO (Forward and reverse) and RhD grouping using conventional tube technique. Forward or cell grouping was based on an agglutination reaction between A and B antigen present on red blood cells with commercial monoclonal anti-A, anti-B antisera (Tulip Diagnostics; Goa, India) respectively. Reverse or serum grouping was based on an agglutination reaction between naturally occurring anti-A and anti-B antibodies in serum/plasma with in-house prepared pooled A cells, B cells and O cells.
- For blood groups positive for A antigen (Group A and AB) were tested with

commercial anti-A_1 lectin (Dolichos biflorus) to classify them under subgroup A_1, A_2, A_1B and A_2B. The test was done by tube method. The sample was considered as A_2 or A_2B subgroup if the agglutination was 4+ with anti A antisera but negative with anti-A_1 lectin.

- In addition to the reverse or serum grouping, detection of anti-A_1 antibody for A_2 and A_2B individuals was done using A_1 red cells. The thermal amplitude of the reacting anti-A_1 antibody (if present) was also determined in three different temperatures (4^0c, 22^0c and 37^0c).

- All the laboratory techniques were carried out according to the manufacturers' instructions and results were interpreted by a trained technologist under supervision of physician.

STATISTICAL ANALYSIS

The data were analyzed and tabulated by using Microsoft Excel and SPSS (version 26). Qualitative data were statistically expressed in the form of frequency and percentages. Fisher's exact test was used for comparing categorical variables. A P value less than 0.05 was considered significant.

RESULTS

Out of total 1,696 healthy blood donors, A antigen (A and AB Blood Group) was present in 610 (36%). Of this A_1 antigen was present in 599 (98.2%) donors and A_2 antigen was present in 11 (1.8%) donors. A_1 and A_1B were found in 466 (76.4%) and in 133 (21.8%) donors respectively, while A_2 and A_2B subgroups were found in 2 (0.3%) and 9 (1.5%) donors, respectively. It was observed that the occurrence of A_2 in AB blood group as A_2B was more than A_2 in A blood group and this difference was found to be statistically significant (Table 1). Anti A_1 antibody was not detected in any of the 11 samples with A_2 and A_2B blood group.

Out of the total donors having A antigen in blood, 37 (6.1%) were found to be RhD negative. Of these 35 (94.6%) were A_1 RhD negative and 2 (5.4%) were A_1B RhD negative and only 1 (2.7%) A_2 donor

was found RhD negative and no A_2B RhD negative donor was found. The frequency of ABO blood groups in all the study participants is shown in Table 2. Blood group O was the most prevalent. The overall phenotypic frequency of all blood groups was O>B>A_1>A_1B>A_2B>A_2 with percentage of 35.4%> 28.6%> 27.5%> 7.9%> 0.5%> 0.1% respectively.

Table 1: Distribution of A_1 and A_2 subgroups among A and AB blood groups

ABO phenotypes	Total (N)	Sub group	Frequency	%	*P* value
A	468	A_1	466	99.6	
		A_2	2	0.4	0.000
AB	142	A_1B	133	93.7	
		A_2B	9	6.3	

Table 2: Frequency of ABO blood groups in all study participants

ABO blood group	Study group [n (%)]
A	468 (27.6%)
B	485 (28.6%)
AB	142 (8.4%)
O	601 (35.4%)

DISCUSSION

The frequency of ABO blood groups varies markedly amongst different population around the globe. Few studies about frequency of ABO and RhD blood grouping have been carried out among Bangladeshi population. In our study population blood group O has been found to be the most common blood group which is in agreement of various studies around the world[11-14]. Blood group A is mainly found in Central and Northern Europe and B is most frequent in Central Asia. In global perspective blood group O is the most frequent.[15] Blood group AB is the lesser reported group in almost all population. We also reported the phenotypic frequencies in the order O>B>A>AB.

From 1,696 blood donors, 27.5% was A_1 and 7.9% was A_1B, and A_2 and A_2B was 0.1% and 0.5% respectively. Our study showed that A_1 was more common in A group and A_1B more common in AB which was in agreement with study done in parts of Sudan, Southern and Northern India, Northern Pakistan.[6,13,16,17] Amongst all donors having A

antigen, group A was 76.7% and AB was 23.3% which was almost same as study in North Karnataka region and Sudan[5,6]. We found frequency of A_2 among A, 0.4% and A_2B 6.35% in AB blood group. A study from north Karnataka reported A_2 and A_2B to be 1.1% and 10.3% respectively[5]. While in South India frequency of A_2 and A_2B was 3.01% and 1.43% respectively[18]. Bangera et al. found the prevalence of A_2 and A_2B 1.3 and 12.7%, respectively[19]. In a pilot study done in Rayalaseema region, values of A_2 and A_2B in were 4.1% and 19.2% and Mahapatra S et al. showed comparatively higher frequency of A_2 and A_2B as 5.8% and 31.5%[20,21]. In another study done by Sharma DC et al. A_2 and A_2B were found to be 8% and 8.6% respectively[22]. Our present study showed lower values as compared to these studies.

In case of subgroups among A and AB, A_1 was the highest (76.4%) and A_2 was lowest (0.3%). Various other studies showed A_2B was the rarest[23,24]. In general population, A_2B subtype is found in 0.9% to 1% individuals[25]. Our study revealed A_2B among all donor is 0.5%. It is worth mention that we found A_2B phenotype in group AB was much higher than A_2 phenotype in group A. The similar finding was noticed in South India, in blacks, and Japanese population except for Caucasian[13,26,27]. To explain the excess of serological blood type A_2B, dominance of a strong B gene that would suppress A_1 antigen activity has been postulated. Ogasawara et al. explained this by different expressions of the allele R101 which was uncommon in individual with the A_2 phenotype, but common with A_2B phenotype. R101 is expressed as phenotype A_1 in *R101/*O heterozygous individuals, but as phenotype A_2 in *R101/*B heterozygotes causing the high A_2B frequency[28].

The Rh frequencies varies within any group of population. Around 10% Asian population carries Rh negative gene and in Europe it is almost 35-45%[29]. There are scarce literature showing the prevalence of A_2 and A_2B along with Rh negative status. No A_2B negative was found in our study population and A_2 negative was found to be 0.06%. In North Karnataka region in India, it was found to be 0.004%[5].

Despite A_2 and A_2B are rare subgroups, still they are important because anti-A_1 antibodies may be found which can cause discrepancies in ABO grouping and cross-matching, and lead to lethal hemolytic reaction. In our study, the prevalence of anti-A_1 antibodies among total A_2 and A_2B samples was 0%. Other studies also found similar findings[9,30]. But several other studies reported various frequencies of anti-A_1 in A_2 and A_2B subgroups[13,17].

From a transfusion perspective, A_2 and A_2B individuals should be transfused with same blood groups. However, due to rarity, these individuals can be transfused with O group packed red cells considering it the next compatible group.

A major limitation of this study is the small sample size, and it can be continued prospectively to get large data from which more statistically significant analysis can be revealed. We also could not distinguish between A_2 and other weaker A subgroups serologically in this study.

Blood group is crucial in blood transfusion, stem cell transplantation as well as organ transplantation. To the best of our knowledge, this is the first study on prevalence of both A_2 and A_2B among Bangladeshi population. Awareness of A_1 and A_2 subgroup prevalence at tertiary care hospital could help in improving inventory management and prevent incompatible transfusions. Most of the time identification of a rare blood group is coincidental when a routine pre-transfusion testing, or pregnancy follow-up is performed. Therefore, A_1 and A_2 grouping in ABO typing is vital to ensure safe blood transfusion.

CONCLUSION

Implementation of A_1 and A_2 grouping can prevent minor incompatibilities, thus ensuring safe blood transfusion. Although we did not find any anti A_1, but still, we suggest testing for anti-A_1 with its thermal range in all patients with A subgroups before transfusion.

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