ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 11 | November - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY PREVALENCE OF SUBGROUPS OF A & DETECTION OF ANTI A1 ANTIBODIES IN PATIENTS ADMITTED IN A TEACHING TERTIARY CARE HOSPITAL, NORTH KARNATAKA REGION

Immunohaematology

Dr.Jeeva Priya .M.R* Assistant Professor, Department of Pathology, SDM College of Medical Sciences & Hospital, Dharwad. *Corresponding Author

Dr. Lakshmidevi K B Tutor, Department of Pathology, SDM College of Medical Sciences & Hospital, Dharwad.

Dr. Vidya N Ronghe Tutor, Department of Pathology, SDM College of Medical Sciences & Hospital, Dharwad.

Dr.Vidisha S Athanikar Professor & HOD, Department of Pathology, SDM College of Medical Sciences & Hospital, Dharwad.

ABSTRACT

Introduction: Landsteiner ABO system of blood groups is most important for transfusion medicine and has subtypes of A Antigen, A1 and A2, upon which further groups of A and AB have been classified. Of individuals with A antigen, approximately 20% belong to A2 while rest 80% belong to A1. Anti-A1 antibody, a cold agglutinin which destroys A1 cells is clinically significant when they react at 37°C, causing transfusion reactions.
Aims& objectives: To study and assess the prevalence of A1 and A2 subgroups in the patient population and to detect anti-A1 antibodies in A2 subgroups.
Materials & Methods: This was a two year retrospective study conducted in the admitted patient' s blood sample in the blood bank of Sri Dharmasthala Manjunatheshwara College of Medical Sciences &Hospital, Dharwad, North Karnataka. Over a period of 2 years, patients' blood group was typed using a column agglutination technique (gel typing), followed by standard tube method for anti-A1 antibodies. Anti-A1 lectin studies were done for all patients with groups A and AB. Based on serological reactivity the samples were classified into A1/A1B (with Rh positive/Negative), A2/A2B(with Rh positive/Negative) along with presence of anti-A1 antibodies reactive at room temperature and 370C.The statistical data was analysed.
Results: A total of 18,224 blood grouping were carried out. Among the 4556 group A samples, 99.1 % (n=4513) classified as A1, 0.9%(n=43) as A2. The majority of AB samples (n=1565) were of A1B type 91.1 %(n=1425) and the remaining8. 9% (n=140) are of A2B type. However, the proportion of A2B (9%) among AB samples was significantly higher than that of A2 in group A samples ($p < 0.0001$). The prevalence of anti-A1 antibodies among A 2 samples & A2B was 25.6% and 30% respectively.
Conclusion: Knowing the prevalence of common A subgroups (A1 and A2) and incorporating them in routine ABO grouping & crossmatching based on the anti-A1 antibody reactivity can prevent major transfusion reaction.

KEYWORDS

A1 ,A2 blood group, Hemolytic transfusion reaction,A1 lectin

INTRODUCTION:

Polymorphisms in the genes coding for the ABO blood group system may lead to diminished amounts of A or B antigens on red blood cells giving rise to subgroups in the system. The occurrence of weak variants due to heterogeneity of the A and B alleles poses a challenge for immunohaematology practice. A_1 and A_2 are the major subgroups of blood group A and they differ from each other both qualitatively and quantitatively.

ABO subgroups are distinguished by decreased amounts of antigens on RBCs and, in secretors, present in the saliva. Subgroups weaker than A_2 are not frequent, and are characterized by a decreasing number of A antigen sites on the RBCs and a reciprocal increase in H antigen activity. Other subgroups of A include A_{int} , A_3 , A_x , A_{end} , A_m , and A_{el} are met only rarely in transfusion practice, and the last four cannot reliably be identified on the basis of blood typing tests alone **[1].**Subgroups of A can result in discrepancy in ABO blood typing. Red cells from people with A_1 and A_2 subgroups both react strongly with monoclonal anti-A reagents in direct agglutination tests. The distinction between these two subgroups is therefore, made depending on the cells' reactivity with the lectin from *Dolichos biflorus* seeds.The *D. biflorus* lectin reacts specifically with cells of the A_1 subgroup, and will thus agglutinate A_1 but not A_2 red cells1**[2].** Anti-A_1 antibody appears as an atypical cold agglutinin in the sera of A_2 or A_2B individuals who lack the corresponding antigen. Weak subgroups of A can be defined as those of group A subjects whose erythrocytes give weaker reactions or are non-reactive serologically with anti-A antisera than do those of subjects with A_2 red blood cells. In the majority of cases, subgroups of A result from the expression of an alternate weak allele present at the ABO loci **[3].** The prevalence of A subgroups varies from place to place and with race. The observed frequencies of A_1 and A_2 subtypes are generally compatible with the Hardy- Weinberg equilibrium for the Mendelian inheritance of the allelic A_1 and A_2 genes.

The frequency of the common A subgroups varies greatly among different populations. Approximately 80% of blood type A or AB are classified as A_1 or A_1B, the remaining 20% are either A_2 or A_2 B. A_2 and A_2B individuals may have anti-A_1 in their serum, which appears as an atypical cold agglutinin. Approximately 0.4% of A_2 and 25% of A_2B individuals have anti-A_1 in the serum, these antibodies become clinically significant when react at 37 °C and belong to IgG class and cause extensive destruction of A_1 cells **[4].**These antibodies can interfere in routine blood grouping and can give incorrect blood typing or can rarely cause haemolytic transfusion reactions. The red blood cells (A_2 or A_2B) from a donor having the same group should be selected for recipients having with clinically significant anti-A_1 antibodies. A_2 patients with an anti-A_1 reactive at 37°C should be transfused with group O or A_2 RBCs only. A_2B patients should receive group O, A_2, A_2B, or B RBCs **[5].** It is not a current practice to test A or B cells as part of a routine testing, though one could consider adding them to routine testing methods. Our hospital blood bank regularly carries out subgroup testing of A & AB blood groups for donors as well as for the patients. This study is carried out mainly on patients admitted for blood transfusion in our institute.

MATERIALS & METHODS:

This retrospective study was conducted in the Blood bank, Departmentt of Pathology, Sri Dharmasthala Manjunatheshwara College of Medical Sciences & Hospital, Dharwad for a period of 2 years from January 2017 to December 2018. A total of 18,224 patients' blood samples were analysed. Anticoagulated EDTA (ethylenediami netetraacetic acid) and clotted (plain) samples were used for testing. EDTA samples are used for forward grouping & serum from plain sample is used for reverse grouping. Blood grouping was done using the slide technique for initial blood group confirmation of patient's received sample. Forward or cell grouping was done using monoclonal antisera anti-A, anti-B and anti-D (Tulip Diagnostics, India) .After blood group confirmation with the request, column agglutination

technique is carried out using Biorad ABO/D + Reverse grouping gel card with monoclonal anti-A, anti-B and anti-D within the gel card for forward grouping and reverse grouping will be done with neutral gel for A_1 and B cells and control (ctrl) cells. Freshly prepared in house A_1, B cells & O pooled cells are used for reverse grouping. Gel card is properly labeled with patient's name & hospital registration number. Dispense 500 microlitre of LISS in a clean test tube & add 25 microlitre of patient's red cells. Add 10 microlitre of this 5% red cell suspension into the first 3 microtubes. Pippette 50 µL of A pooled cells to microtube 6, 50µL of B pooled cells to microtube 5 and 50µL of O pooled cells to Control (Ctrl) microtube. Centrifuge in ID centrifuge for 10 minutes & read the results. All the results were interpreted by a trained technologist & medical officer.

Samples of group A and AB were further tested with anti-A_1 lectin (Tulip Diagnostics; India) by tube method to classify them into A_1, A_2 subgroups. Whenever the agglutination was 4+ with anti-A antisera but negative with anti-A_1 lectin, the sample was considered as A_2 subgroup. The age of the patients was noted from the request forms. Newborn samples were not included in the study as they naturally have weak antigenic expression. A or AB group samples which showed agglutination with pooled A cells were tested with A_1cells to confirm the presence of anti-A_1 antibodies. The thermal amplitude of anti-A_1 was determined in each case with anti-A_1 antibodies by keeping test tubes for incubation at room temperature (RT) 22°C and 37°C. Agglutination reactions after incubation was examined by naked eye and also under microscope and graded according to AABB standards **[16].** A_2 blood group patients showing agglutination at room temperature are crossmatched with A_1/A_2 (depending on the inventory). A_2 B blood group patients showing agglutination at room temperature are crossmatched with A_1B/A_2B (depending on the inventory). A_2/A_2B patients showing reactivity at 37^0 C are segregated and crossmatched with only A_2/A_2B blood group only.

Data is analysed. Descriptive statistics is used to estimate the frequencies.

RESULTS:

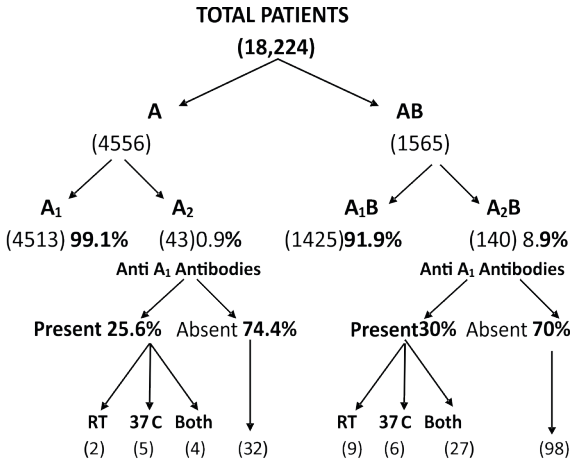

Fig1: Chart Showing The Results Of Initial Blood Grouping

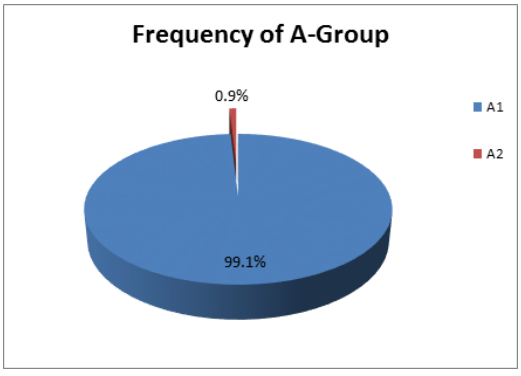

Fig2: Distribution Of A_1 & A_2 Blood Groups Amongst The Study Population

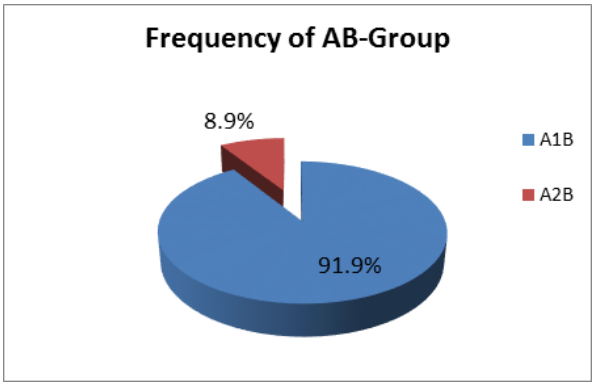

***Fig3:* Distribution Of A_1b & A_2b Blood Groups Amongst The Study Population**

Table1: Table Showing The Distribution Of A And Ab Blood Groups Among The Study Population.

ABO Blood Group	Frequency	%
A	4556	74
AB	1565	26
Total	6121	100

Table2 : Table Showing The Distribution Of Subgroups Of A And Ab Blood Groups Among The Study Population.

Blood Group	Sub Group	Frequency	%
A	A1	4513	99.1
	A2	43	0.9
AB	A1B	1425	91.1
	A2B	140	8.9

A total of 18,224 patients' samples were typed for ABO, Rh group in our blood bank attached to a tertiary care hospital. Among the 4556 group A samples, 99.1 %(n=4513) classified as A_1, 0.9 %(n=43) as A_2. The majority of AB samples (n=1565) were of A_1B type 91.1 %(n=1425) and the remaining8. 9% (n=140) are of A_2B type. However, the proportion of A_2B (8.9%) among AB samples was significantly higher than that of A_2 in group A samples ($p < 0.0001$).

The prevalence of anti-A_1 antibodies among A_2 samples was 25.6% (n=11). Out of the 11 units, 18 %(n=2) showed reactivity in only room temperature (RT), 45% (n=5) showed reactivity at 37°C and 36 %(n=4) showed reactivity at both room temperature (RT)& 37 °C.

The prevalence of anti-A_1 antibodies among A_2B samples was 30%. Out of the 42 units, 21 %(n=9) showed reactivity in only room temperature (RT), 14% (n=6) showed reactivity at 37°C and 64 %(n=27) showed reactivity at both room temperature (RT)& 37 °C.

DISCUSSION:

The blood group A can be sub-classified as A_1, A_2 and weak A subgroups (A_x, A_3, A_{end}, etc.) based on red cell agglutinability and various serological reactions.

In present study, as shown in Table 1 the prevalence of A_1 and A_1B was 99.1% and 91.1% and A_2 and A_2B was 0.9% and 8.9% respectively of all the admitted patients' samples which had come for crossmatching in our blood bank. These results were similar to the study done by Shastry S et al., which showed 1.07% A_2 and 8.99% A_2B respectively **[6].**Similar studies are done by Bangera IS et al., in which prevalence of A_2 and A_2B in groups A and AB respectively was 1.3% and 12.7%. Similar studies were done by Mahapatra S et al., showed A_2 and A_2B as 5.8% and 31.5% **[7].**

The neonatal samples were totally exempted from the study, because ABO antigens are not fully developed at birth, the red cells of neonates who are genetically group A_1 may not react or react only weakly with anti-A_1 lectin. A_1 adults have approximately 0.8×10^6 antigen sites per red cell. A_2 adults have approximately 0.24×10^6 antigen sites per cell**[17]**

Our study findings in the North Karnataka population(South India) are similar to those in blacks and Japanese, in whom the prevalence of A_2B

is significantly higher than the A_2 subgroups ($p<0.0001$). To explain the high frequency of A_2B in black populations, the presence of a strong B gene that would suppress A_1 antigen activity has been postulated **[8,9].** In Hiroshima the proportion of A_2 among A types is 0.17%, whereas the proportion of A_2B among AB types is 1.14%; for Nagasaki, the proportions are 0.08% and 2.44%, respectively. The excess of serological blood type A_2B in these populations is attributed partially to the suppressed synthesis of A_1 substance by the coexisting B enzyme in heterozygous AB individuals **[8].** According to Yoshida *et al.*, there are other unidentified factors that also contribute to the large imbalance of serological A subtypes in the Japanese. In order to understand the genetic basis of this "excess" of A_2B, Ogasawara *et al.* examined ABO alleles in individuals with A_2-related phenotypes. Alleles were identified by means of polymerase chain reaction single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses **[9].** The frequencies of A_2-related alleles were clearly different between the A_2 and A_2B phenotypes.

In the studies done at International level in Sudan out of 76 cases of A, A_1 was present in 93.42%, A_2 in 6.58% cases. Out of 24 cases of AB, A_1B was present in 91.67% and A_2B was present in 8.33%. **[10].**In Japan (Hiroshima) out of 3564 cases of A, A_1 was present in 99.83%, A_2 in 0.17% cases. Out of 976 cases of AB, A_1B was present in 98.86% and A_2B was present in 1.14%. Our study is similar to those in Blacks and Japanese, who showed more prevalence of A_2B than A_2 subgroup.

The presence of strong B gene would suppress A_1 antigen activity explaining to the high frequency of A_2B in Black populations **[11].** A_2 and A_2B are rare subgroups. But still they are important because anti A_1 antibodies occur in sera of A_2 groups and more common in A_2B subgroups and can be encountered during clinical practice causing difficulties in blood typing, haemolytic transfusion reaction and complicate organ transplantation.

A_2 and A_2B individuals may have anti A_1 in their serum. Approximately 0.4% of A_2 and 25% of A_2B individuals have anti-A_1 in the serum. Anti-A_1 antibody usually agglutinates cells only up to 25°C and are of no clinical significance. However, anti-A_1 can occasionally be a clinically significant antibody when it reacts at 37°C and the extensive destruction of A_1 cells has also been reported **[12].**We detected anti-A_1 in 25.6% of A_2 and in 30% of A_2B individuals. 45% of anti-A_1 of A_2 individuals and 14% of anti-A_1 of A_2B patients showed significant reactivity at 37^0C.

Although rare, Padmashri et al still reported cases on transfusion reactions in patients with anti-A_1 antibodies**[13].** Naturally the anti-A_1 antibody is not detected in A_2 individuals. However because of sensitization like transfusion and pregnancy patients might develop anti-A_1 antibodies reactive at $37\ ^0$C might result in delayed hemolytic transfusion reaction.

Heimlich et al reported a case of acute hemolytic transfusion reaction in A_2 blood group patient with anti-A_1 antibodies who died shortly after transfusion of blood group A red blood cells RBCs. Directly after starting transfusion, acute dyspnea was observed, while other clinical signs for a transfusion reaction were absent. Analysis revealed that the anti-A_1 was present as a high titer IgM class immunoglobulin that induced complement deposition on A_1 RBCs. The anti-A_1 reacted in a wide temperature amplitude upto 37^0 C with A_1 RBCs, while weak agglutination was observed with A_2 RBCs at room temperature**[14].**

In this study conducted by Aruna Anbukarsu et al in Malaysian population, they have demonstrated the utility of gene sequencing technique in identifying A subgroup blood type, particularly the A2 subtype to validate the serological findings and reported the absence of 1061C deletion, a common SNP in A2 blood phenotype**[15].**

CONCLUSION:

Hemolytic transfusion reactions based on anti-A_1 remain a relatively rare phenomenon, which is covered by infrequent and heterogeneous case reports in literature. But still, clinically significant anti-A1 antibody reactive at 37^0C is found in our study population. As we don't want to take risk on our patients, we have our blood bank policy of providing A_2 /A_2B blood for those patients showing reactivity at 37^0C. Donor is screened as well for A subgroups & inventory is separately maintained.

Awareness of rare blood groups and subtypes will help in improving inventory management in the blood bank. Development of better techniques to identify rare blood type can help shifting the focus to recruiting minor blood group donors to increase the pool of rare blood in the blood bank.

Financial Support & Sponsorship:
Nil.

Conflicts Of Interest:
Nil.

Acknowledgements:
We thank Dr. Col U. S. Dinesh, Registrar, Shri Dharmasthala Manjunatheshwara University for his guidance and support.

REFERENCES:

1. Franchini M, Liumbruno GM (2013) ABO blood group: old dogma, new perspectives. ClinChem Lab Med 51(8): 1545-1553.
2. Cartron JP, Gerbal A, Hughes–Jones NC, et al. 'Weak A' phenotypes: relationship between red cell agglutinability and antigen site density. Immunology 1974; 27: 723-7.
3. Heier HE, Namork E, Calkoska Z, et al. Expression of A antigens on erythrocytes of weak blood group A subgroups. Vox Sang 1994; 66: 231-6.
4. Rudmann SV (1995) Textbook of Blood Banking and Transfusion Medicine. (1st edn) WB Saunders, USA, p.73-75.
5. Boorman KE, Dodd BE, Loutit JF, et al. Some results of transfusion of blood to recipients with "coldagglutinin". Br Med J 1946; 751-4.
6. Shastry S, Bhat S. Imbalance in A2 and A2B phenotype frequency of ABO group in South India. Blood Transfusion. 2010;8(4):267-270
7. Mahpatra S, Mishra D, Sahoo D: Study of prevalence of A2, A2B along with Major ABO Blood groups to minimize the transfusion reactions: 5: (3) IJSR March 2016, 189-190
8. Yoshida A, Dave V, Hamilton HB. Imbalance of blood group A subtypes and the existence of superactive B gene in Japanese in Hiroshima and Nagasaki. Am J Hum Genet 1988; 43: 422-28.
9. Ogasawara K, Yabe R, Uchikawa M, et al. Different alleles cause an imbalance in A2 and A2B phenotypes of the ABO blood group. Vox Sang 1998; 74: 242-7.
10. Ahmed M E, Nada Y A, Hala A M, Sulafa A H; Frequency of the A2- subgroup among blood group A and blood group among students of faculty of medicine and health sciences at Alimam Almahadi University, White Nile, Sudan. Hematol Transfus Int J 2015; 1(4):00022.
11. Voak D, Lodge TW, Reed JV. A possible explanation for the expression of A2B phenotypes observed in some populations. Vox Sang 1970; 18: 471-4.
12. Chaudhary R, Sonkar A. High titer immunizing anti-A1 in an A2B patient resulting in hemolytic transfusion reaction. Transfus Bull 2004;12:2004-8.
13. Padmasri, R., Bhatara, U. & Iyengar, R.S. (2014). A rare case of A2+ve blood group in an obstetric emergency. Journal of Clinical and Diagnostic Research, 8(2), 181-2.
14. Floris Helmich, Inge Baas, Peter Ligthart et al. Acute hemolytic transfusion reaction due to a warm reactive anti-A1. Transfusion 2018;00;00–00
15. Aruna Anbukarsu, Mohd Nazif Samat @ Darawi and Mohd Nazil Salle Absence of 1061C deletion in A2 blood subgroup validated through gene sequencing in the Malaysian population. Life Sciences, Medicine and Biomedicine, Vol 3 No 5 (2019) 27.
16. Brecher ME, editor. Technical Manual 14th Edition. Bethesda,Maryland. American Association of Blood Banks 2002.
17. M. Dense, "Harmening," Test Book on Modern Blood Banking and Transfusion Practices, 3rd Edition, Jaypee Brothers Medical Publishers LTD, New Delhi.