Serology of blood group A2 in tertiary care hospital of Lahore

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	ABSTRACT
transfusion reactions as well knowledge and identification	re major subgroups of blood group A and have potential to cause a subgroup discrepancy and incompatible cross-matching. Prior on of ABO blood group subgroups is critical in blood transfusion and right donor at the right moment and place can be difficult in patient bing is required.
Methods: This Cross-secti Medical College, Lahore be (247) healthy whole blood was determined using anti- blood group A, classifying t	ional study was performed in Jinnah Hospital and Allama Iqbal etween March 2023 and August 2023. Two hundred and forty seven donors with blood group A were selected. ABO blood group status And anti-B antisera. Anti-A ₁ lectin was used to further 3 lbtype them into A ₁ and A ₂ categories. Furthermore reverse typing with incells and O cells was performed to detect anti-A ₁ antibodies.

Results: Among 247 individuals with blood Group A, 242 (98%) were typed as A_1 and 05 (02%) as A_2 . Of the A_2 blood samples, anti- A_1 antibodies were found in 1 (20%) of them.

Conclusion: Blood Group A_2 is a less frequent blood group in population of Lahore. Anti- A_1 antibodies are capable of causing fatal transfusion reactions as well as blood group discrepancy and incompatible cross-matching. Reverse typing and anti- A_1 lectin testing should both be performed as routine testing.

Key words: Serology, Blood group A2, Anti-A1 lectin, Anti-A1 antibodies

INTRODUCTION

The two most important blood group systems out of the 36 that the International Society of Blood Transfusion (ISBT) so far discovered are ABO and Rhesus. The ABO group classes: A, B, C (later renamed O after the German "Ohne," which means "without," or "Zero," "null"), and AB. Landsteiner received the physiology and medicine Nobel Prize in 1930 for his research. 1

Since these blood group systems are highly antigenic and produce antibodies that can cause hemolysis in vivo, the ABO and Rhesus blood type systems are regarded as having sinical significance. There are several inherited phenotypes (weak ABO subgroups) that express A or B weakly on red blood cells. Subgroups are a significant contributor to ABO blood group differences and incompatible cross match tests, notwithstanding their rarity. The majority of missense mutations, insertions, or deletions in the coding area, splicing sites, or regulatory components cause weak ABO groupings.²

Individuals with blood type A were further separated into A₁, A₂, and additional uncommon varieties including A₃, A_{el}, A intermediate t.t.), A_x, A_{Finland} (fin), A_m, A_{bantu}, A_{end}, A_y, and A_h (H partly deficient) as well as weak group A. Group A red blood cells that interact with both anti-A and anti-A₁ lectin were referred to as A₁. A₁ comprised around 80% of the total population of A blood cells, while A₂ made up the remaining 20%. Blood group A₂ were assigned to those that interact with a i-A antisera but didn't give any agglutination with anti-A₁ lectin. A decrease in the frequency of A antigen sites on RBCs and a corresponding rise in H antigen activity define subgroups that are weaker than A₂, which are uncommon.

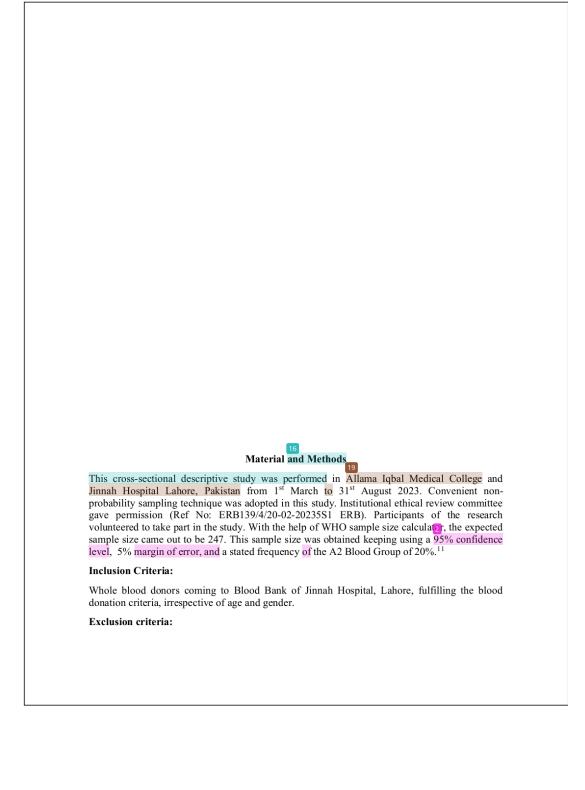
The origins of the A₁ and A₂ phenotypes have been a point of contention for many years. The A₁ and A₂ phenotypes are now understood to have genetic roots, with the A₂ phenotype being characterized by a transferase that sees effective than the A₁ transferase. The typical A₂ deletion in the coding area, which results in a protein with 21 additional amino acids, and other mutations in the peptide chain of the A₂ glycosyltransferases are likely to be to responsible for the inefficiency. The optimal pH, Km values, and ion requirements for the A₁ and A₂ transferases are also well known. With the A₁ phenotype expressing up to for means as many A epitopes as the A₂ phenotype, there is no question that the primary chemical difference between A₁ 201 A₂ is of a quantitative character. It has been hypothesized that the A-trisaccharide based on type 3 (Gal3GalNAc) and type 4 (Gal3GalNAc) chain glycolipids may be significant in differentiating the phenotypes. A₁ and A₂ differ from each other quantitatively, with the A₁ phenotype expressing up to four times as many A epitopes.

In 1% to 2% of individuals with A_2 there is an anti- A_1 found. Antibodies of the immunoglobulin (Ig) M class, which are active at temperatures below 25 °C and hence infrequently have clinical significance, are the most common form of an anti- A_1 . However, multiple studies have documented 15 ses in which fatal hemolytic transfusion reactions are noted becaus of Anti- A_1 antibodies. Due to the relative deficiency of A antigens on A_2B cells, persons with an A_2B phenotype are more likely to develop anti- A_1 than A_2 individuals.

There isn't a comparison research published in the literature that compares population of Lahore with the distribution of major subgroups of blood group A in the area. The current study was carried out to document the prevalence of blood group A subgroups among the people of ahore and to compare the results with those of other parts of Pakistan and certain other nations, with a view to generate data with multipurpose future utilities for the health planners and also see the common trend of the prevalence of various blood groups. ¹⁰

Awareness and distribution of A₁ and A₂ blood types are necessary for optimal blood bank management for the secure transfusion of blood and blood components and also due to differences in blood group predominance from race to race and area to region.¹¹ The most significant step of pretransfusion evaluation is ABO typing, and an ABO subgroup is a genetic variation of ABO phenotype that may produce ABO blood grouping difference. There are multiple reasons for acquired causes of blood group discrepancy as well, for example, blood diseases, malignancies and chemotherapy. Based on this, it is quite difficult to distinguish genetic causes of discrepancy from acquired ones in routine laboratories.⁵

It is therefore imperative to blain data on the geographic distribution of the aforementioned blood groups, which is why this study was conducted to establish the frequency of A₁ and A₂ types of blood among blood donors at a tertiary care hospital in Lahore and compare it to other studies.



Donors who did not fit into donor selection criteria.

Donors who did not give consent to participate in study.

Their details and information were kept private and were not accessible to anybody outside the team. Each participant was assigned a code number. DTA-anticoagulated venous blood was used to perform ABO and Rhesus right typing by test tube method using blood typing resents (Lorne Laboratories) according to directions provided by manufacturer. Additionally, left typing was carried out using A, B, along with O screening cells manufactured in-house. All Blood Group A samples were serologically subtyped using licensed Anti-A1 antisera (Lorne Laboratories) and labeled as A1 and A2.

The data was entered and assessed using the Statistical Package for Social Sciences (SPSS) version 22:00. For qualitative varables, like gender, ethnicity and serological findings frequency and percentages were estimated. Mean and standard deviation were determined for quantitative factors such as age.

RESULTS

All were male and no female. The median value of ages of the donors was 28 years (table I). Ethnically all were Punjabi.

Sesults of serological testing using Anti-A and Anti-A₁ lectin anti-sera are shown in table-II. Anti-A₁ antibodies were detected in 1 (20%) of individuals with blood group A_2 shown in figure I.

	Age (years)
Median	28
Minimum age	20
Maximum Age	40
IQR	28 (24-30)

Table I: Ages of healthy blood donors

Blood group	Frequency (number)
Aı	98% (n=242)
A ₂	02% (n=05)

Table II: Results of serological testing with anti-sera.

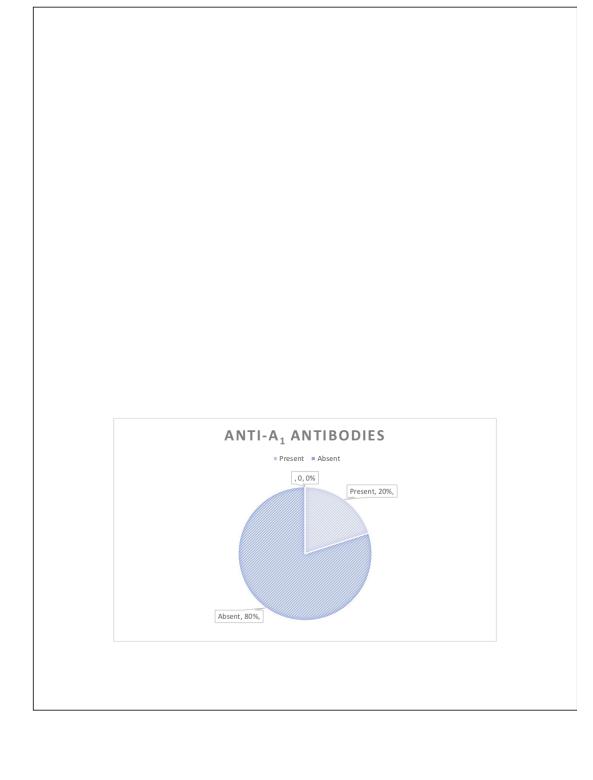


Figure I: Frequency of Anti-A1 antibodies

Discussion

This is the first study of its kind conducted in Lahore stating frequency of blood group A₂ and anti- A₁ antibodies among them. This study was conducted on 247 healthy, whole blood donors. Among them, 242 (98%) were typed as blood group A₁ and remaining 05 (02%) as A₂. Only 1 (20%) among A₂ blood group individuals were known to develop anti- A₁ antibodies.

In my current study I have observed that major part of blood donations are made by male individuals as compared to females. Primary cause of this tendency is illiteracy, cultural customs and lack of motivation. Additionally, they are frequently deemed unsuitable due to anemia, reduced body weight, and a propensity to result in TRALI (transfusion associated acute lung injury). The median age of healthy donors is 28 years. Around 50% of healthy blood donors were from age group 24-30 years as depicted by Inter-quartile range (IQR). Co-morbidities including hypertension, diabetes, and surgery are known to induce older people to donate less.

This is the first study of its kind in Lahore that has reported the frequency of blood group A₂ and anti-A₁ antibodies in 21st century. Frequency of blood group A₁ and A₂ determined in this study came out to be 98% and 2%. Recent study conducted in Lahore in 2024 showed frequency of A₂ 2.2% which is close to my current study. ¹² Recent studies conducted in twin cities of

Rawalpindi-Islamabad showed frequency of A_2 around 13.8% and 20% which are in clear deviation from this study.¹³ ¹⁴ One possible reason for this difference could be multiple ethnicities residing in twin cities while in my study Punjabi is the dominant ethnicity. Difference in sample size could be another possibility for this deviation.

India has done lot of research on A_2 frequency. In Great Swalior region of India, frequency determined was 8% among adults and 16% in neonates. A pilot study done in Rayalaseema region of India showed frequency of 4%. In South India, estimated frequency is 1.07% that is similar to study under discussion.

In Chinese Han population, calculated frequency is 1.1%.¹⁷ In Japanese population of Hiroshima and Nagasaki, frequency is very low estimated to be 0.17% and 0.08% respectively.¹⁸ These studies show less frequency of A_2 as compared to this study.

In Kuwaiti population, frequency of A_2 is 8%. A study conducted in White Nile region of Sudan showed frequency of A_2 around 7%. Both these studies are showing clear difference and are high as compared to study under discussion.

In Thai population, a study conducted in 2017 determined frequency of A_2 around 0.18% .⁵ In Caucasians frequency of A_2 is 0.5%.²¹

Like the present study, the frequency of A_2 is found to be similar in Saudia Arabia, calculated to be around 2%. Similarly, A study published in 2022 in Dhaka documented frequency of A_2 around 1%. Both these studies are showing results similar to study under discussion.

The highest frequency observed so far is in African countries and is estimated to be around $40\%.^{23}$

Subgroups of blood group A₂ develop Anti-A₁ antibodies and this can care transfusion reactions, blood group discrepancy and incompatible cross-match testing. These antibodies are of IgM type mostly and are reactive on temperature up to 25°C and rarely cause significant hemolytic transfusion reactions. But in certain situations, antibodies of IgG type are formed and are known to cause severe hemolytic transfusion reactions at body temperature as documented by multiple studies.

In this study, 1 in 5 A_2 individuals developed anti- A_1 antibodies making frequency of 20%. A study conducted in twin cities of Pakistan showed frequency of anti- A_1 antibodies to be 14% which is similar to my current study. ²⁴ However this finding is surprising as it is modestly high as compared to other studies.

A study conducted in South India show frequency of 1.8% which is significantly low as compared to study under discussion.⁸

A study in Iran on frequency of anti-A₁ antibodies among A₂ individual was conducted on sample size of 245 and were not able to detect any anti-A₁ antibody.²⁵ Another study published in 2022 in Jazan, Saudia Arabia on 446 sample size was not able to detect any anti-A₁ antibodies among A₂ individuals.⁴ According to authors, large sample size should be dealt to measure its frequency.

A study conducted and published in Dhaka, Bangladesh has documented frequency of 0.5% which is again in clear contradiction and is very low as compared to my current study.²²

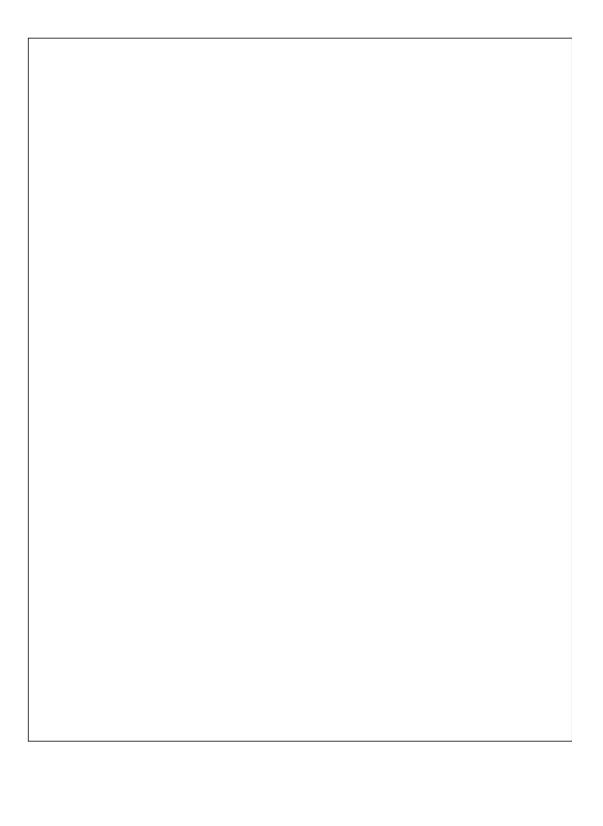
This study is prepared to best of author's knowledge, however there are certain limitations to it. The major limitations could be small sample size. The reverse grouping should be performed on 4°C, 22°C and 37° because these antibodies can be reactive at colder temperature as well as body temperature. A molecular characterization of the subtypes would have been useful in this regard, but not possible in this study. The disparity between our findings and those reported from other areas might be related to ethnic differences among the Gwalior and nearby region.

Conclusion

Because frequency varies by area, understanding the distribution of blood types is critical for optimal blood bank management. For accurate findings, multi-center studies including larger sample sizes should be conducted. Anti- A₁ antibodies have potential to cause fatal transfusion reaction, so testing with Anti- A₁ lectin a reverse grouping should be made part of routine testing. Individuals with Blood group A₂ should be transfused with A₂ or red cells of blood group O in case of non-availability of A₂.

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