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## INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/21407

DOI URL: <http://dx.doi.org/10.21474/IJAR01/21407>



### RESEARCH ARTICLE

## SEROLOGY OF BLOOD GROUP A<sub>2</sub> IN TERTIARY CARE HOSPITAL OF LAHORE

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### Manuscript Info

#### Manuscript History

Received: 14 May 2025

Final Accepted: 17 June 2025

Published: July 2025

#### Key words:-

Serology, Blood group A<sub>2</sub>, Anti-A<sub>1</sub> lectin, Anti-A<sub>1</sub> antibodies

### Abstract

**Background:** A<sub>1</sub> and A<sub>2</sub> are major subgroups of blood group A and have potential to cause transfusion reactions as well as blood group discrepancy and incompatible cross matching. Prior knowledge and identification of ABO blood group subgroups is critical in blood transfusion and transplantation. Finding the right donor at the right moment and place can be difficult in patient care. So necessary blood typing is required.

**Methods:** This Cross-sectional study was performed in Jinnah Hospital and Allama Iqbal Medical College, Lahore between March 2023 and August 2023. Two hundred and forty-seven (247) healthy whole blood donors with blood group A were selected. ABO blood group status was determined using anti-A and anti-B antisera. Anti-A<sub>1</sub> lectin was used to further subtype blood group A, classifying them into A<sub>1</sub> and A<sub>2</sub> categories. Furthermore, reverse typing with in-house prepared A<sub>1</sub> cells, B cells and O cells was performed to detect anti-A<sub>1</sub> antibodies.

**Results:** Among 247 individuals with blood Group A, 242 (98%) were typed as A<sub>1</sub> and 05 (02%) as A<sub>2</sub>. Of the A<sub>2</sub> blood samples, anti-A<sub>1</sub> antibodies were found in 1 (20%) of them.

**Conclusion:** Blood Group A<sub>2</sub> is a less frequent blood group in population of Lahore. Anti A<sub>1</sub> antibodies are capable of causing fatal transfusion reactions as well as blood group discrepancy and incompatible cross-matching. Reverse typing and anti-A<sub>1</sub> lectin testing should both be performed as routine testing.

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### 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 system was discovered in 1900 by Karl Landsteiner. He found four blood 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.<sup>1</sup>

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 clinical significance. There are several inherited phenotypes (weak ABO subgroups) that express A or B weakly on red blood cells. Subgroups are a significant rarity. The components

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Individuals with blood type A were further separated into  $A_1$ ,  $A_2$ , and additional uncommon varieties including  $A_3$ ,  $A_{el}$ , A intermediate (int.),  $A_x$ ,  $A_{Finland}$  (fin),  $A_m$ ,  $A_{bantu}$ ,  $A_{end}$ ,  $A_y$ , and  $A_h$  (H partly deficient) as well as weak group A.<sup>3</sup> Group A red blood cells that interact with both anti-A and anti- $A_1$  lectin were referred to as  $A_1$ .  $A_1$  comprised around 80% of the total population of A blood cells, while  $A_2$  made up the remaining 20%. Blood group  $A_2$  were assigned to those that interact with anti-A antisera but didn't give any agglutination with anti- $A_1$  lectin.<sup>4</sup> 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  $A_2$ , which are uncommon.<sup>5</sup>

The origins of the  $A_1$  and  $A_2$  phenotypes have been a point of contention for many years. The  $A_1$  and  $A_2$  phenotypes are now understood to have genetic roots, with the  $A_2$  phenotype being characterized by a transferase that is less effective than the  $A_1$  transferase. The typical  $A_2$  deletion in the coding area, which results in a protein with 21 additional amino acids, and other mutations in the peptide chain of the  $A_2$  glycosyltransferases are likely to be responsible for the inefficiency.<sup>6</sup> The optimal pH, Km values, and ion requirements for the  $A_1$  and  $A_2$  transferases are also well known. With the  $A_1$  phenotype expressing up to four times as many A epitopes as the  $A_2$  phenotype, there is no question that the primary chemical difference between  $A_1$  and  $A_2$  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.  $A_1$  and  $A_2$  differ from each other quantitatively, with the  $A_1$  phenotype expressing up to four times as many A epitopes.<sup>7</sup>

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 cases in which fatal hemolytic transfusion reactions are noted because of Anti- $A_1$  antibodies.<sup>8</sup> 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.<sup>9</sup>

There isn't comparison research published in the literature that compares the 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 Lahore 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.<sup>10</sup>

Awareness and distribution of  $A_1$  and  $A_2$  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.<sup>11</sup> 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.<sup>5</sup>

It is therefore imperative to obtain data on the geographic distribution of the aforementioned blood groups, which is why this study was conducted to establish the frequency of  $A_1$  and  $A_2$  types of blood among blood donors at a tertiary care hospital in Lahore and compare it to other studies.

## Material and Methods:-

This cross-sectional descriptive study was performed in Allama Iqbal Medical College and Jinnah Hospital Lahore, Pakistan from 1<sup>st</sup> March to 31<sup>st</sup> August 2023. Convenient non-probability sampling technique was adopted in this study. Institutional ethical review committee gave permission (Ref No: ERB139/4/20-02-2023/5S1 ERB). Participants of the research volunteered to take part in the study. With the help of WHO sample size calculator, the

expected sample size came out to be 247. This sample size was obtained keeping using a 95% confidence level, 5% margin of error, and a stated frequency of the A2 Blood Group of 20%.<sup>11</sup>

#### **Inclusion Criteria:**

Whole blood donors coming to Blood Bank of Jinnah Hospital, Lahore, fulfilling the blood donation criteria, irrespective of age and gender.

#### **Exclusion criteria:**

**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. EDTA-anticoagulated venous blood was used to perform ABO and Rhesus right typing by test tube method using blood typing reagents (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-A<sub>1</sub> antisera (Lorne Laboratories) and labeled as A<sub>1</sub> and A<sub>2</sub>.

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

#### **Results:-**

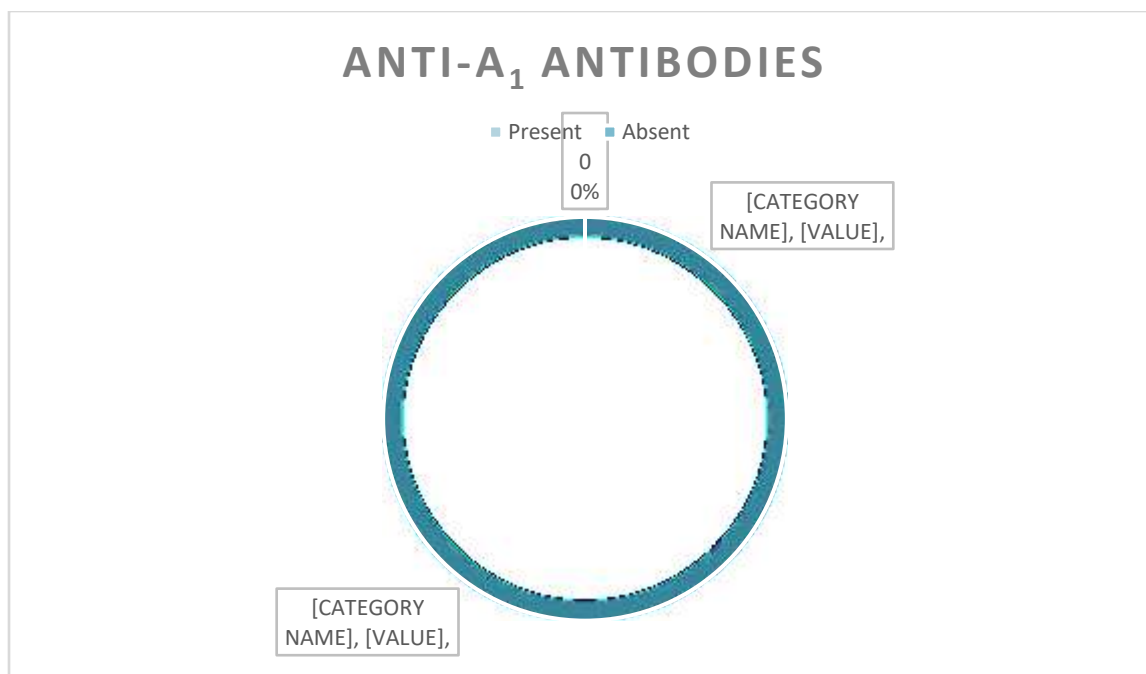
All were male and no female. The median value of ages of the donors was 28 years (table I). Ethnically all were Punjabi. Results of serological testing using Anti-A and Anti-A<sub>1</sub> lectin anti-sera are shown in table-II. Anti-A<sub>1</sub> antibodies were detected in 1 (20%) of individuals with blood group A<sub>2</sub> shown in figure I.

	Age (years)
<b>Median</b>	28
<b>Minimum age</b>	20
<b>Maximum Age</b>	40
<b>IQR</b>	28 (24-30)

**Table I: Ages of healthy blood donors**

Blood group	Frequency (number)
A <sub>1</sub>	98% (n=242)
A <sub>2</sub>	02% (n=05)

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



**Figure I: Frequency of Anti-A<sub>1</sub> antibodies**

### Discussion:-

This is the first study of its kind conducted in Lahore stating frequency of blood group A<sub>2</sub> and anti- A<sub>1</sub> antibodies among them. This study was conducted on 247 healthy, whole blood donors. Among them, 242 (98%) were typed as blood group A<sub>1</sub> and remaining 05 (02%) as A<sub>2</sub>. Only 1 (20%) among A<sub>2</sub> blood group individuals were known to develop anti- A<sub>1</sub> antibodies.

In my current study I have observed that major part of blood donations is 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 A<sub>2</sub> and anti- A<sub>1</sub> antibodies in 21<sup>st</sup> century. Frequency of blood group A<sub>1</sub> and A<sub>2</sub> determined in this study came out to be 98% and 2%. Recent study conducted in Lahore in 2024 showed frequency of A<sub>2</sub> 2.2% which is close to my current study.<sup>12</sup> Recent studies conducted in twin cities of Rawalpindi-Islamabad showed frequency of A<sub>2</sub> around 13.8% and 20% which are in clear deviation from this study.<sup>13,14</sup> 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<sub>2</sub> frequency. In Great Gwalior region of India, frequency determined was 8% among adults and 16% in neonates.<sup>15</sup> A pilot study done in Rayalaseema region of India showed frequency of 4%.<sup>16</sup> In South India, estimated frequency is 1.07% that is similar to study under discussion.<sup>8</sup>

In Chinese Han population, calculated frequency is 1.1%.<sup>17</sup> In Japanese population of Hiroshima and Nagasaki, frequency is very low estimated to be 0.17% and 0.08% respectively.<sup>18</sup> These studies show less frequency of A<sub>2</sub> as compared to this study.

In Kuwaiti population, frequency of A<sub>2</sub> is 8%.<sup>19</sup> A study conducted in White Nile region of Sudan showed frequency of A<sub>2</sub> around 7%.<sup>20</sup> 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<sub>2</sub> around 0.18%.<sup>5</sup> In Caucasians frequency of A<sub>2</sub> is 0.5%.<sup>21</sup>

Like the present study, the frequency of A<sub>2</sub> is found to be similar in Saudia Arabia, calculated to be around 2%.<sup>4</sup> Similarly, A study published in 2022 in Dhaka documented frequency of A<sub>2</sub> around 1%.<sup>22</sup> 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%.<sup>23</sup>

Subgroups of blood group A<sub>2</sub> develop Anti-A<sub>1</sub> antibodies and this can cause 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<sub>2</sub> individuals developed anti-A<sub>1</sub> antibodies making frequency of 20%. A study conducted in twin cities of Pakistan showed frequency of anti-A<sub>1</sub> antibodies to be 14% which is similar to my current study.<sup>24</sup> 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.<sup>8</sup>

A study in Iran on frequency of anti-A<sub>1</sub> antibodies among A<sub>2</sub> individual was conducted on sample size of 245 and were not able to detect any anti-A<sub>1</sub> antibody.<sup>25</sup> Another study published in 2022 in Jazan, Saudia Arabia on 446 sample size was not able to detect any anti-A<sub>1</sub> antibodies among A<sub>2</sub> individuals.<sup>4</sup> 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.<sup>22</sup>

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°C 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-A<sub>1</sub> antibodies have potential to cause fatal transfusion reaction, so testing with Anti-A<sub>1</sub> lectin and reverse grouping should be made part of routine testing.

Individuals with Blood group A<sub>2</sub> should be transfused with A<sub>2</sub> or red cells of blood group O in case of non-availability of A

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