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Research Article

Correlation of Tube Agglutination with the Gel Microagglutination Technique for ABO and Rh Blood Group Determination

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Abstract

Blood groups are used to divide human blood into different types, according to the presence or absence of certain markers present on the surface of red blood cells, which we call antigens. There are many types of systems used in order to determine the different types of blood groups, but the most relevant at a clinical level is the ABO system, followed by the Rhesus (Rh) system, of which there are some techniques to make the determination, two of them are the tube technique and the gel microagglutination technique, which are the ones that will be evaluated throughout this project. The objective of this work is to make a comparison between the results of both techniques to find out whether or not there is a discrepancy between the results. The study was carried out on a total of 110 patients treated at the Hospital General de Zona (HGZ) #46 of the Instituto Mexicano del Seguro Social (IMSS) in Gómez Palacio, Durango. The patients were taken from the admissions to the blood bank area in the laboratory, both techniques were performed on each patient to identify their blood group with 100% exactly the same results in both techniques, however, it has been observed that, unlike the technique in tube, the gel technique, was able to identify a small population of O positive erythrocytes in a patient with blood group A positive. In terms of frequency, the results are: The most frequent blood group was group O positive with 64 individuals representing 58.18% of the total subjects in the study, followed by group A positive with 27 individuals representing 24.54%. B positive with 12 individuals is equivalent to 10.90%, AB positive with 3 individuals is equivalent to 2.72%, O negative with 3 individuals is equivalent to 2.72%, finally the groups with zero frequency were the groups B negative and AB negative with 0 individuals and which represents 0%. With these results, evidence has been obtained that demonstrates better diagnostic performance in the gel microagglutination technique than in the tube agglutination technique, by also showing the presence of small populations of other blood groups, however, 100% of the blood group identification results was the same in both techniques.

Keywords: Blood group, ABO, Rh, frequency, gel microagglutination, tube agglutination

Introduction

An individual's blood type, once established at birth, remains constant throughout life. Therefore, blood types can be successfully used to establish an individual's identity [1]. In the 20th century, Karl Landsteiner demonstrated that there were antigenic particles in the erythrocyte membrane, which led him to investigate the existence of "natural" antibodies in serum with specificity contrary to these antigens, thus developing knowledge of the ABO system. Landsteiner's studies did not stop with the discovery of the functioning of the ABO system, but also of the Rh system, thus revolutionizing immunopathology [2].

Human ABO blood group antigens are expressed on the surface of red blood cells and a variety of human cells and tissues [3]. They are also the most frequently studied genetic markers in a large group of people. There are four common blood types in the ABO system: O, A, B, and AB. Blood type frequencies vary across different racial/ethnic groups [4]. From a clinical point of view, red blood cell group systems are of vital importance among the listed blood cell group systems, as they are responsible for immunological compatibility between donor and recipient during a blood transfusion. Antigens of the ABO system are also considered tissue antigens and are therefore of particular importance in organ transplantation and epidemiology [5].

A thorough understanding of the basic principles of immunology regarding the antigen-antibody reaction is essential if one wants to understand the immunology of blood groups. On the one hand, blood groups are antigens and can lead to the production of specific antibodies if inoculated in the form of blood into a different person. Some antibodies exist physiologically when the person lacks the corresponding antigen (natural antibodies) [6].

According to data from the World Health Organization, approximately 118,5 million blood donations are made worldwide. Forty percent of these donations are in high-income countries, home to 16% of the world's population. Approximately 13,300 blood donation centers in 169 countries reported collecting a total of 106 million donations. The number of units collected at donation centers varies by income group. The median annual number of donations per center is 1,300 in low-income countries, 4,400 in lower-middle-income countries, and 9,300 in upper-middle-income countries, compared to a median of 25,700 in high-income countries [7]. Therefore, blood typing and the different types of identification techniques are currently very important due to the high demand for blood and blood component transfusions resulting from the increase in hematological and degenerative diseases, traffic accidents, obstetric patients, and other conditions. The objective was to compare the tube agglutination technique with the gel microagglutination technique for determining ABO and Rh blood groups.

Material and Methods

The study was descriptive, retrospective, and cross-sectional. It was conducted at the General Hospital of Zone 46 (HGZ46) of the Mexican Social Security Institute (IMSS), specifically in the blood bank area. Based on the patients who received transfusions in the blood bank area at HGZ 46 in Gómez Palacio, all patients admitted to the emergency department and who were hospitalized on the floor with scheduled surgeries were considered. All of these patients, with their corresponding request, required blood type studies for subsequent crossmatching. Therefore, the universe and sample constituted a total of 110 individuals, corresponding to 100% of the patients in the study. Table 1 shows the study variables.

Blood sample collection

Blood samples were collected by the hospital's nursing staff, as requested by the attending physician. The samples were placed in Vacutainer tubes containing ethylenediaminetetraacetate (EDTA), which prevents blood clotting. A total of 110 samples were received and analyzed. Patients whose blood type was needed for crossmatching for a possible future blood transfusion.

Methods used for the determination of ABO and Rhesus blood group systems (D).

Tuve technique

Qualitative test. Tests with Anti-A (murine monoclonal) and Anti-B (murine monoclonal) serum are required to determine whether or not human red blood cells possess A and/or B blood group antigens. Agglutination is considered a positive test result and indicates the presence of the corresponding antigen; conversely, the absence of agglutination indicates a negative result and, therefore, the absence of the corresponding antigen. Anti-AB serum agglutinates erythrocytes containing A and/or B antigens. Group O human red blood cells will not react with this reagent.

Variables	Sub variable	Indicators	Values
ABO system	A	Presence Absence	---------
	B	Presence Absence	---------
	AB	Presence Absence	---------
	O	Presence Absence	---------
Rhesus system (D)	Rh positive Rh negative	Presence of Ag (D) Absence of Ag (D)	---------
Distribution of ABO blood groups and Rhesus (D)	Gender	Male Female	--------
Distribution of ABO blood groups and Rhesus (D)	Age	---------	0-10 years 11-20 years 21-30 years 31-40 years 41-50 years 51-60 years 61-70 years 71-80 years 81-90 años
ABO frequency and Rhesus	O+ O- A+ A- B+ B- AB+ AB-	Presence Absence	--------
Results obtained	Tube technique	Presence Absence	--------
	Gel technique	Presence Absence	--------

Table 1: Study variables.

Figure 1: Anti-A, Anti-B, Anti-AB and Anti-D serums for determining ABO and Rh blood group.

Basis of the technique: The procedures used with the Anti-A, Anti-B, Anti-AB, and Anti-D reagents (Figure 1) are based on the principle of agglutination. Normal human blood cells possess antigens that they will agglutinate in the presence of antibodies specific to those antigens.

Procedure

Label a test tube containing a 2% to 3% red blood cell suspension using 0.9% saline. Using a micropipette, add one drop of the suspension (100 µL) to each of the four test tubes. Label the four test tubes 1 Anti-A, 2 Anti-B, 3 Anti-AB, 4 Anti-D, and add one drop of ommercial serum to the respective labeled tube. Mix and centrifuge at 3400 rpm/min. Read the tube with gentle agitation. Observe for agglutination.

Gel technique

The ABO system was the first human blood group system discovered by Landsteiner in 1900, and remains the most important in transfusion practice. The ABO system is defined by the presence or absence of the A and/or B antigens on red blood cells and by the presence of antibodies in plasma or serum corresponding to the antigen(s) missing from the red blood cells. In the field of transfusion medicine, after the A and B antigens, the most important blood group antigen is the Rh D antigen.

Rh (D) typing is defined by the presence or absence of the D antigen (RH1) in red blood cells. Anti-A, Anti-B, Anti-AB, Anti-DVI-, and Anti-DVI+ reagents are used to perform ABO and Rh (D) typing, complemented by the reverse technique (ABO reverse typing).

Principle of the technique: The principle of the method is based on the gel technique described by Yves Lapierre in 1990 for the detection of red blood cell agglutination reactions. DG Gel cards consist of eight microtubes. Each microtube consists of a chamber, also known as an incubation chamber, in the upper chamber of a long, narrow microtube, known as a column. The plastic card microtube has been pre-filled with a buffered gel solution containing specific monoclonal antibodies (Anti-A, Anti-B, Anti-AB, or Anti-D). Agglutination occurs when the antigens on the red blood cells react with the corresponding antibodies present in the gel solution or in the serum or plasma sample (in the case of the reversed group technique). The gel column acts as a filter, trapping agglutinated red blood cells as they pass through the gel column during centrifugation of the card. The gel column separates the agglutinated red blood cells from the non-agglutinated red blood cells based on their size [8]. Agglutinated red blood cells are trapped at the top or along the length of the gel column, while the non-agglutinated red blood cells settle to the bottom of the microtube, forming a sediment. The Blood Bank System, WADiana® is a fully automated blood bank analyzer designed for pretransfusion compatibility testing, specifically using DG Gel cards (Figure 2).

Figure 2: WADiana® equipment used to run blood types on gel cards.

ABO PHENOTYPE DETERMINATION PROCEDURE

Blood type and Rh determination (Gel Method)

The principle of this technique is based on the technique described by Lapierre et al. (1990) for the detection of agglutination reactions in red blood cells [8]. Agglutination occurs when the red blood cell antigens come into contact with the corresponding antibodies present in the reagent or in the serum or plasma sample. The DG-Gel card is a plastic support consisting of 8 microtubes. Each microtube consists of a column and a dispensing/incubation chamber.

Each column contains polymerized dextran microspheres in a buffered medium that act as a filter. The dextrans are mixed with a reagent containing specific antibodies or a buffer. The microtubes containing the specific antibodies incorporated into the gel solution act as a reaction medium, and the red blood cells agglutinate upon contact with the antibodies. Microtubes without antibodies are used in techniques in which antibodies react directly with red blood cells in the incubation chamber and for controls.

During centrifugation, agglutinated red blood cells are trapped by size, either on the surface or along the gel column. Non-agglutinated red blood cells sink to the bottom of the microtube. To perform blood group determination, perform the following procedure: Centrifuge the new cards for 20 min at 1110 rpm. Identify the card. Dilute the red blood cell sample to 5% with DG-Gel Sol. Dispense 10 µl of the red blood cell dilution into microtubes 1-6. Dispense 50 µl of Serigrup-Diana into microtubes 7-8. Centrifuge for 9 min at 1110 rpm. Read the results.

Quality control for AB0/Rh cards

Quality control of the cards is carried out according to the manufacturer's specifications (GRIFOLS 2015) in order to check their good condition, as well as to evaluate their specificity and sensitivity, for which the following procedure is performed: Prepare a 5% red blood cell suspension of A, B and O cells. Dispense 50 µl of the previous red blood cell suspensions into the wells of each card marked as A, B, AB, D' cH

- Card 1: Group A cells
- Card 2: Group B cells
- Card 3: Group 0 cells

Dispense 50 µl of A cells (serigrup) to the wells marked N/A1 and 10 µl of B cells (serigrup) to the wells marked N/B. Add 50 µl of serum or plasma to the latter two wells.

- Card 1: Group A serum
- Card 2: Group B serum
- Card 3: Group 0 serum

Centrifuge for 9 minutes at 1100 rpm. Read and record the result.

Statistical analysis

Statistical analysis was applied to determine the correlation between the Tube Technique and the Gel Microtyping Technique through concordance measures, considering the proportion of coincidences compared to the total number of subjects (a+d)/n (Table 2). Qualitative variables were dichotomous (two possibilities), and two classification methods or two clinical scales were compared, represented by a frequency table [9].

		Gel method		
		Positive	Negative	
Tube method	Positive	a	b	f1
	Negative	c	d	f2
		c1	c2	n

Table 2: Measures of agreement.

Results

Based on the results obtained, we can confirm that, out of a total of 110 patients in the study, the predominant sex was female, with 60%, equivalent to 66 patients, while the other 40% corresponds to the male sex, equivalent to 44 patients (Figure 3).

When classifying the blood groups of the patients according to sex, the following results were obtained: With respect to the total number of male patients in the study (44 patients), the most frequent blood group was group O positive with 56.8%, followed by A

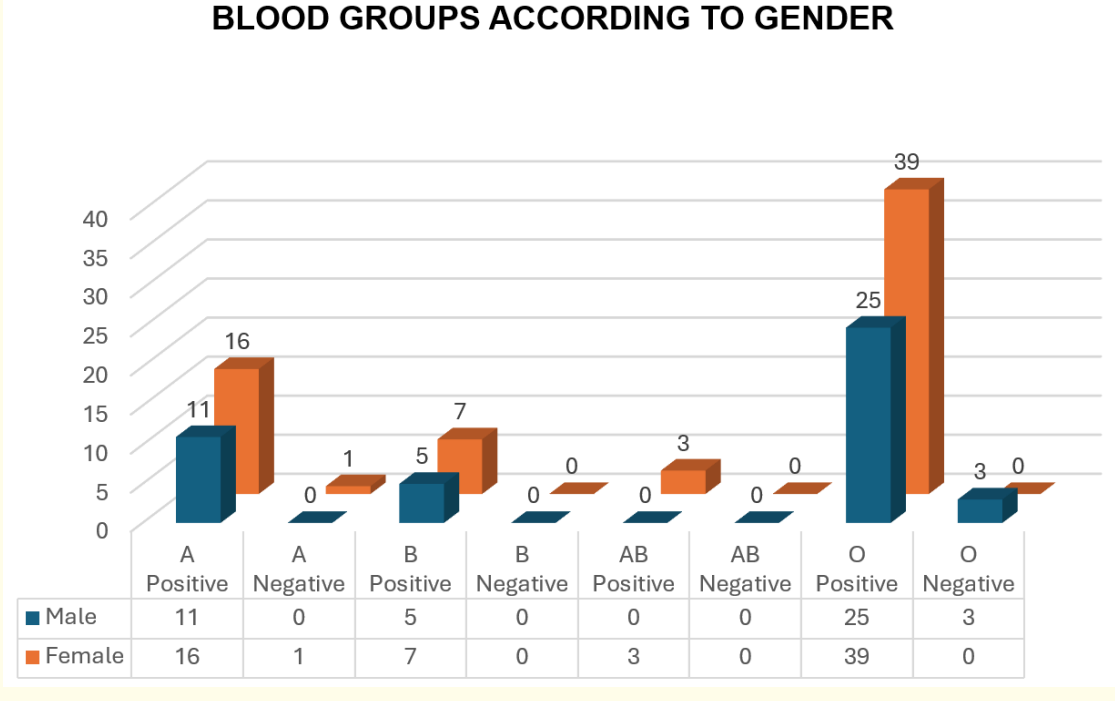

Figure 3: Result of the distribution of ABO and Rh blood groups according to the gender of the patients.

positive with 25%, B positive 11.3%, O negative 6.8% and A negative, B negative, AB positive, AB negative, the last four with 0%.

With similar results regarding the total number of female patients in the study (66 patients), the most frequent blood group was group O positive with 59%, followed by A positive with 24.2%, B positive 10.6%, AB positive 4.5%, A negative 1.5% and B negative, AB negative and O negative, the last three with 0%.

Regarding the data on the relationship between age and sex recorded, the results were different between both genders: In the total age ranges in the male gender (44 patients), a greater number of patients were admitted in the ages of 71-80 years with 25% and 51-60 years with 22.7% while the lowest recurrence was in the range of 81-90 years with 2.2% and 0-10 years with 4.5%.

On the other hand, in the total age ranges in the female gender (66 patients), a greater number of patients were admitted between the ages of 21-30 years with 21.2% and 31-40 years with 18.1%, while the lowest recurrence rates were in the range of 0-10 years with 0% and 81-90 years with 4.5% (Figure 4).

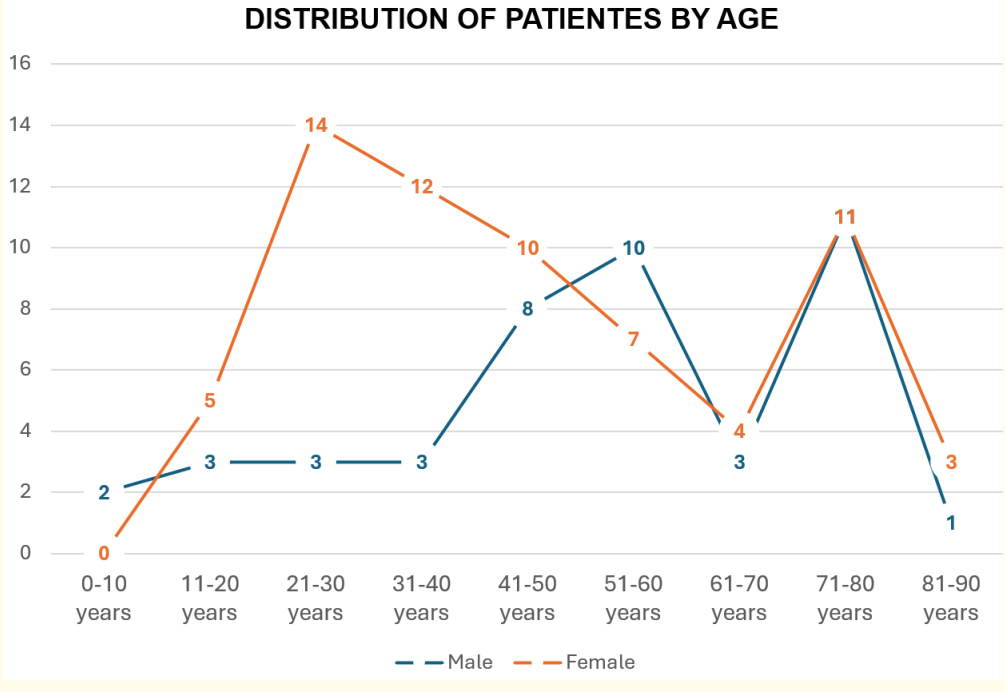

Figure 4: Results of the age-gender relationship of patients.

The results corresponding to the frequency of ABO and Rh blood groups were as follows: The blood group with the highest frequency was group O positive with 64 individuals representing 58.18% of the total subjects in the study, followed by group A positive with 27 individuals representing 24.54%, B positive with 12 individuals equivalent to 10.90%, AB positive with 3 individuals equivalent to 2.72%, O negative with 3 individuals equivalent to 2.72%, finally the groups with zero frequency were groups B negative and AB negative with 0 individuals and representing 0% (Figure 5).

Table 3 shows the results of the comparison between the tube agglutination technique and the gel microagglutination technique, where each of the 110 patients studied had their blood type typed using both techniques. The results were as follows: Groups B negative and AB negative did not have a representative sample. Groups A negative, B positive, AB positive, O positive and O negative showed identical results in both techniques in each of the patient samples. On the other hand, in group A positive, 26 of the 27 patients obtained identical results in both techniques, the remaining

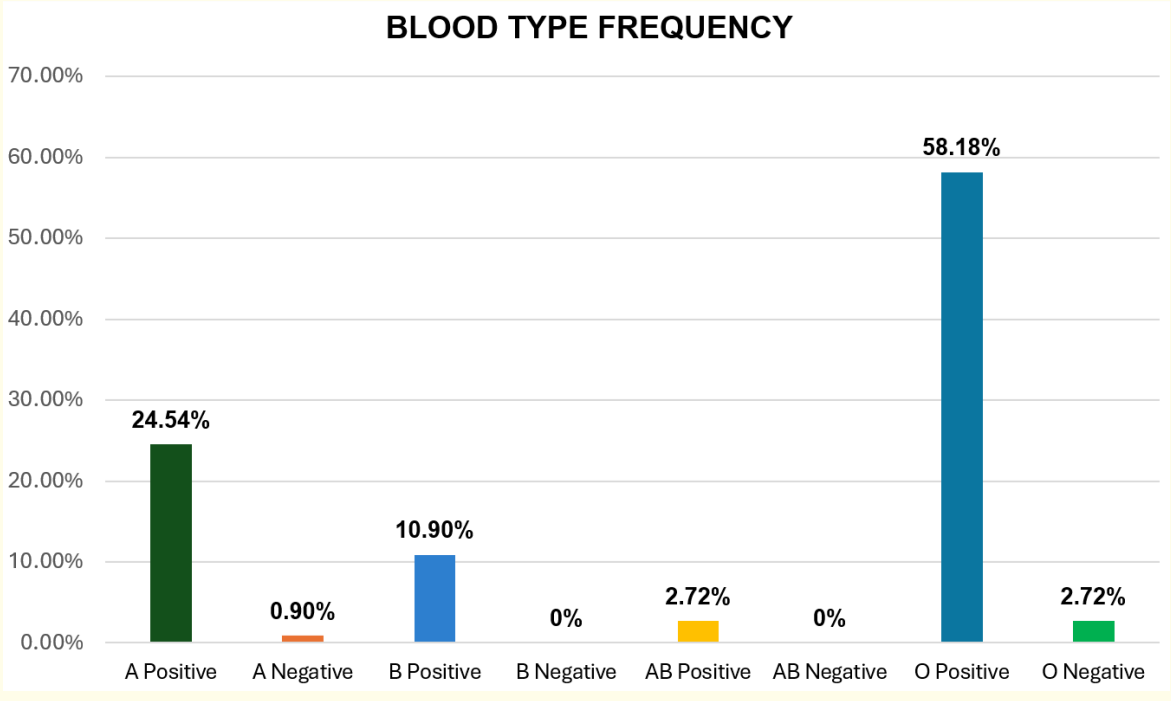

Figure 5: Results of ABO and Rh blood group frequencies.

Blood group	Tube technique	Gel technique	Discrepancy
A Positive	27	26	1*
A Negative	1	1	There is not
B Positive	12	12	There is not
B Negative	0	0	There is not
AB Positive	3	3a	There is not
AB Negative	0	0	There is not
O Positive	64	64	There is not
O Negative	3	3	There is not

Table 3: Comparison of the tube agglutination technique with the gel microagglutination technique for blood group determination.

* Although the patient's blood type is A positive by tube technique, the gel technique showed, in addition to being A positive, a small population of O positive erythrocytes.

patient was A positive through the tube technique, while, in the gel technique, although the patient also tested A positive, a small population of O positive erythrocytes could also be seen.

As shown in Table 3, the correlation between the results of both techniques with respect to blood types is excellent; both results were the same. Regarding the statistical calculation, when applying Molinero's (2001) concordance formula, the results corresponded to one. Table 3 presents the results obtained in the Tube Technique and the Gel Technique, for the classification of the Rhesus (D) group, which indicate a greater predominance of the Rh (D) positive group with a total of 106 cases corresponding to 96% and, to a lesser extent, the Rh (D) negative group with 4 cases corresponding to 4%.

Discussion

The objective of this study was to compare the tube agglutination technique with the gel microagglutination technique for determining ABO blood types and Rh factor. In this study, the most common blood type was type O positive, with 64 individuals representing 58.18% of the total subjects studied. The distribution of the ABO system has been widely studied worldwide, yielding a wide variety of results. However, despite this variation, blood type O has been reported as the most frequent in most of these studies, and is the most common blood type in Latino populations.

The blood group followed by the frequency of the O positive blood group was group A positive with 27 individuals representing 24.54%, B positive with 12 individuals equivalent to 10.90%, AB positive with 3 individuals equivalent to 2.72%. Data that coincides with studies previously carried out in 2010 and in 2018 where it was determined that group AB has been continuously described as the least frequent in most human populations [10,11].

In this study, a distribution in order of frequency of blood groups O > A > B was found, coinciding with the distributions reported in studies carried out in Mexico [12,13]; likewise, they coincide with the distributions reported in populations of Nigeria and Iraq [10,14], contrasting slightly with studies carried out in Latin populations of other countries where distributions A > 0 > B > AB are reported [15], this difference can be attributed to the different sample sizes used in the studies, in addition to the interbreeding typical of each country. The distribution of the four blood groups A, B, AB and O varies in different populations around the world and depends on the frequency of the three alleles of the ABO gene in the populations, with group O being the most frequent, followed by group A, group B and group AB [16].

In the research conducted by Anifowoshe et al. in 2017, the distribution and gene frequencies of ABO and Rh blood group systems were studied in major parts of Nigeria with representatives in each of the six geopolitical zones with a total of 318,940 and 280,514 individuals who were matched for ABO and Rh respectively in this study. The total data collected showed the following percentages revealing that ABO blood group frequencies were found in the order O>A>B>AB (52.93%, 22.77%, 20.64% and 3.66% respectively) while the prevalence of Rh+ was 94.90%.

However, a 2011 study by Rai and Kumar determined the frequencies of ABO and Rh blood groups in an unrelated population in the Koari, Yadav, Kurmi, and Maurya provinces of Uttar Pradesh, India. Of a total of 1,065 samples analyzed, blood group B had the highest frequency at 36.81%, followed by O at 32.68%, A at 23.66%, and AB at 6.85%. Of the 1,065 samples, 95.59% were Rh+ and 4.41% were Rh–.

Obtaining similar results Basu et al. (2018), who evaluated 1,528 individuals ranging from 18 to 65 years of age, of which 89% were men and 11% women from Kolkata in the south of the state of West Bengal, India, yielding the following ABO system frequencies, B) 33.7%, O) 32.07%, A) 25.13%, AB) 9.03%. Regarding the Rh system, they reported Rh + 96.60% and Rh – 3.40% of the participants in the study.

Data that agree with the results obtained in the present investigation in the comparison of the typing of the Rh system, the Tube Technique and the Gel Technique, for the classification of the Rhesus (D) group, which indicate a greater predominance of the Rh (D) positive group with a total of 106 cases corresponding to 96.36% and, to a lesser extent, the Rh (D) negative group with 4 cases corresponding to 3.64%.

Likewise, according to trials conducted by Musa et al. in 2015, out of a total of 1,014 volunteer blood donors included in the study in Malaysia, 68.6% were male and 31.4% female, of whom 42.8% were Chinese, 35.5% Malay, and 16.2% Indian, while the remaining 5.5% consisted of donors from other ethnic minority groups. Over 82% of donors in this study were Rh+ and 17.8% were Rh-. In addition, the largest donor category was blood type O Rh+, which consisted of 32.1%, followed by B Rh+ at 27%, A Rh+ at 23%, and AB Rh+ at 0.2%.

As part of the research by Liu et al. (2018), which included a total of 3,827,125 participants from China, of which 51.18% were women and 48.82% men. Their blood type was determined by plate

agglutination method, indicating that the number of participants with blood type A was the highest 30.54%, followed by O 30.37%, B 29.42% and AB 9.66%, only 1.02% of the participants were Rh -.

In the study conducted by Jaff (2010) over a period of 5 years (2005-2009) in Krudus, a total population of 53,234 apparently healthy subjects were included; 53.30% were male and 46.70% female. Their age ranged between 18 and 46 years (mean 27.4 ± 6.2). All subjects belonged to known Kurdish ethnic tribes from different provinces of the Kurdistan Region of Iraq. Using a plate agglutination method, the blood groups of the study population were typed. The most common blood group was O 37.16%, followed by blood groups A 32.47% and B 23.84%. Blood group AB had the lowest prevalence representing only 6.53%. The prevalence of ABO phenotypes linked to the Rh phenotype was O+ (34.03%), followed by A+ (29.99%), B+ (21.69%), and AB+ (6.02%). The lowest prevalence was AB (0.51%).

In another study conducted by Garratty et al. in 2004 in the United States, the first donation from each donor recorded in a 10-year database was selected to determine each donor's race or ethnic origin and ABO/Rh phenotype. ABO and Rh typing was performed at five blood banks in the United States. The highest percentage of type O was found in Hispanic donors (56.5%), American Indian donors (54.6%), and non-Hispanic black donors (50.2%). Hispanic and non-Hispanic black donors had a much lower percentage of Rh (7.3%) and 7.1%, respectively, compared to non-Hispanic white donors (17.3%). The O, Rh– and B, Rh– types were found more frequently (8.0% and 1.8%) in non-Hispanic white donors compared with Hispanic donors (3.9% and 0.7%), non-Hispanic black donors (3.6% and 1.3%), and Asian donors (0.7% and 0.4%). These data confirmed that the highest percentages of O, Rh+; B, Rh+/AB Rh+ and Rh- are present in Hispanic, Asian, and non-Hispanic white donors, respectively.

Various population studies have been conducted to evaluate the distribution of blood types in Mexico. One of these was conducted in 2018 by Canizalez-Román et al., which evaluated 271,164 patients who attended Salud Digna para Todos clinics in 17 states of the Mexican Republic from 2014 to 2016. It was found that blood type O was the most common at 61.82%, followed by A at 27.44%, B at 8.93%, and AB at 1.81%. Rh+ was found in 95.58% of the individuals studied, and 4.42% were identified as Rh-.

According to the study conducted by Iturbe-Chiñas et al. in 2013 in the Montaña Region of Guerrero, Mexico, a total of 25,984 volunteer donors from the communities of Tlapa de Comonfort, Malinaltepec, Atlixtac, and Cualac were included. Blood types were typed using agglutination techniques. The allele distribution according to frequency was 0, at 88%, followed by A at 9% and B at 3%. AB was absent. Regarding the Rh system, the allele frequencies were 89% for Rh+ and 11% for Rh-.

Similarly, the descriptive study conducted in La Paz, Baja California Sur, Mexico, in 1998, which evaluated 1,809 blood donors who attended the General Hospital of the Mexican Social Security Institute that year, obtained data on the date, name, ABO, Rh group, gender, and place of residence of the individuals who came to donate blood in 1998. Blood typing tests on the donors were performed by Blood Bank personnel. ABO blood type was determined by tube agglutination using the direct and reverse tests. The population revealed the following frequencies: 0, 58.49%; A, 31.40%; B, 8.40%; AB, 1.71%; Rh+, 95.36%; and Rh-, 4.64% [12].

The discrepancy observed in an individual of group A Rh positive was due to the fact that although the patient is blood group A positive by tube technique, in the gel technique he showed, in addition to being A positive, a small population of O positive erythrocytes.

Conclusion

This study found a blood group distribution of 0 > A > B, with no statistically significant association between ABO blood type and the various independent variables. The tube agglutination technique was compared with the gel microagglutination technique for determining ABO and Rh blood types.

In general, the correlation between the results of both techniques with respect to the Rhesus system was excellent because the same results were obtained, one of the advantages of the gel card in relation to the tube technique is its sensitivity since it can increase up to 1000 times the affinity between antigen and antibody, because in the gel technique the saline solution of low ionic strength is used whereas in the tube technique 0.9% saline solution is used, which is a disadvantage of the method since low affinity antibodies can be lost with washing.

Optimal screening for the Rhesus (D) system is very important because the D antigen is highly immunogenic and stimulates the production of anti-D. Anti-D antibodies are of considerable importance because they can cause severe hemolytic disease of the newborn and transfunctional hemolytic reactions. The D antigen and the weak form of the D antigen (called Du) are commonly considered in the routine selection of blood for transfusion.

Conflict of Interests

The authors have not conflict of interest.

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