

ABO AND RH (D) BLOOD GROUPS DISTRIBUTION IN PAKISTAN: A SYSTEMATIC REVIEW

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ABSTRACT

The ABO blood group system was the first discovered human blood type in 1901 by Land Steiner. The ABO and Rh system is a clinically significant blood group system and extensively recognized in medical and anthropological studies, among 29 human blood group systems. The study aims to determine the frequency distribution of ABO and Rhesus (Rh) blood group system in all-region and provinces of Pakistan. According to this study, the sequence distribution of the ABO blood groups in the Pakistani population is B with frequency (33.37%), followed by O (33.14%), then A (33.99%), and AB (9.74%). The phenotypic frequency observed was 0.2399, 0.3337, 0.0974, and 0.3314 for blood groups A, B, AB, and O, respectively. While for Rh (D) positive and negative, it was 0.9063 and 0.0937, respectively. The study concluded that blood group B is most prevalent, while group AB is the least prevalent. Rh-positive is commonest while Rh-negative is the rarest blood group in Pakistan.

Keywords: ABO; ABO blood group system; RhD

INTRODUCTION

The blood group system of ABO was first discovered mechanism in human blood type in 1901 by Land Steiner (Garratty et al., 2000). Later, Landsteiner and Wiener found Rhesus (Rh) blood types in 1939 (Garratty et al., 2000; Mollison, 1994). The ABO system classifies blood into A, B, AB, and O. The blood group system for Rhesus (Rh) was established in 1941, with Rh-positive and Rh-negative streams on the surface of the red blood cells (RBCs) dependent on the frequency and absence of inherited antigenic substances such as proteins, carbs, glycoproteins and Glycolipids (Kondam et al., 2012). The ABO and Rh system is a clinically significant blood group-system among 29 human blood group systems, despite the discovery of numerous other markers such as microsatellites or groups (Amin-ud-Din et al., 2004; Armstrong et al., 2008; Begum et al., 2011; Hosoi, 2008). These are extensively recognized in medical and anthropological literature (A.u. Rehman et al., 2014). For safe blood transfusion and organ transplantation, ABO and Rh blood group systems are critical. Moreover, both systems are well known in population genetic research, population migration processes, investigation of decision-making forensic cases, and disputed paternity matters (Mondal et al., 2012; Pennap et al., 2011). Besides, blood groups are thought to be associated with different disorders such as salivary gland tumors, carcinoma, stomach carcinoma, thyroid disorders, small cell lung cancer, and cardiovascular disease (Cerny et al., 1992; Pinkston et al., 1996). The types of blood groups are hereditary. ABO blood groups are regulated by a single gene

having three alleles found on chromosome 9. Rh blood group system inheritance is dependent on the existence of either R or r alleles (Shaheen et al., 2014). All human species have the same blood grouping system, with significant variations between frequencies' different incidence. The prevalence and frequency distribution of these blood groups among various cultures and races need to be understood. The occurrence of ABO and Rh blood groups among the Pakistani population indicates a marked variance, suggesting racial variations, ethnic as well as genetic differences of the Pakistani community (Hemalatha et al., 2015; Ilyas et al., 2013; Khattak et al., 2008).

The distribution of blood types across various ethnic groups and races varies worldwide (Sidhu et al., 1980). It may vary in different populations and from one region to another in the same country. The world population is split into several ethnic groups. It is considerable to which these groups vary in terms of solely hereditary characteristics. Blood groups may be used as an easy and effective technique for this purpose. The relative prevalence of blood groups O, A, B, and AB in Western Europe is 46%, 42%, 9%, and 3%, respectively (Guyton et al., 2000). The distribution of blood groups O, A, B, and AB in the United States is 45%, 41%, 10, and 4%, respectively (Hassan et al., 2010). Eastern Europe shows a higher blood group B percentage. Pure American Indians belong to group O, almost exclusively (Begum et al., 2011). Blood group B frequency in Central Asia is the highest (Hassan, 2010). In Britain, Rh-negative is most frequent (17%), followed by Caucasoid (15%) less common

in American Blacks (5%), in African Blacks the ratio is (0%) and very rare in Asians (1%) (Mahmood et al., 2004).

Pakistan, officially the Islamic Republic of Pakistan, located in South Asia, is the world's fifth-most populous country with a population exceeding 220 million. It has the world's second-largest Muslim population. Pakistani community comprises 97% Muslims while small non-Muslims religious groups like Christians, Hindus, Sikhs, Qadianis, Parsis, and others 3%. Pakistan is an ethnically diverse population; among them, the largest ethnic group is Punjabi 44.15%, followed by Pakhtun or Pashtun 15.42%, Sindhi 14.1%, a transitional ethnic group between Punjabi and Sindhi making 10.53%, the Muhajir 7.51%, Balochi 3.57%, and the other groups of northern areas make up roughly 4.66% of the total population. (Haruechaiyasak & Damrongrat, 2008). Previous research studies on the distribution of ABO blood groups studied only small regions or with few sample sizes. However, as to our knowledge, no study is made to analyze the distribution of ABO blood group system in all the areas of Pakistan. Therefore, this systematic analysis is the first attempt to examine the frequency distribution of Blood groups over 21 years span between 1999 to 2020. We attempted to quantify the frequency distribution and allelic frequency of ABO and Rh(D) blood groups in Pakistan from reported studies.

MATERIALS AND METHODS

The literature search and research strategy was developed for the assessment of the ABO system distribution in Pakistan.

An online search for literature

Various search engines were used for this systematic review; this includes Google Scholar, PubMed, Science Direct, SciHub, Medscape, Web of Sciences, etc. The term and keywords searched to obtain the desired literature were Blood Group, Prevalence/Distribution/Frequency of the ABO blood group, Rhesus blood group system, and various Pakistan regions. The publication year for the literature search was restricted from January 1999 to September 2020. We expected the lack of apparent accuracy of the information, so no form of publication was prohibited. Conditions for the inclusion of studies are that:

- 1) Article must be written and published in the English language
- 2) Studied population must be Pakistani.
- 3) The published paper must be between 2001 to 2018.
- 4) Calculation of frequency distribution of ABO and rhesus blood group should be the core primary findings of the study.
- 5) Research article, review papers letter to the editor, and abstracts relevant to the distribution of ABO should be included.

Assessment of article and classification

We divided the state of Pakistan into six regions, which include four provinces of Khyber Pakhtunkhwa, Punjab, Sindh, Balochistan, and two autonomous territories of Azad

Jammu and Kashmir and Gilgit Baltistan. Details are given in Table 1. For the analysis of national statistics, the number of individuals from various areas of the same blood groups was analyzed. The number of individuals from different regions with the same blood groups was added to explore the national data with the percentage frequency distribution of ABO blood group system in Pakistan.

Table 1: Six regions of Pakistan with number of districts and populations.

S.No.	Region	No of Area/ District	Population (million)
1	Gilgit (Administrative Territory)	10	02.00
2	Azad Kashmir (Administrative Territory)	10	04.45
3	Khyber Pakhtunkhwa	35	35.53
4	Punjab	36	110.0
5	Sindh	30	47.89
6	Balochistan	33	12.34

Allele frequency

For the allelic frequency estimation, Hardy Weinberg quantitative genetics equilibrium was used (Dabholkar, 1999). The overall likelihood ratio was also estimated by this approach, supposing that the ABO system is represented by a single gene having three alleles named as A, B, and O; both A and B are autosomal-dominant over O, and both A and B are codominant. The expected phenotypic frequency was estimated based on the blood allelic frequency results. We conducted the Chi-square test to determine the genotype frequencies the independence goodness of fit for gene (Gupta, Gupta, & Sons, 2009).

RESULTS

Search outcomes

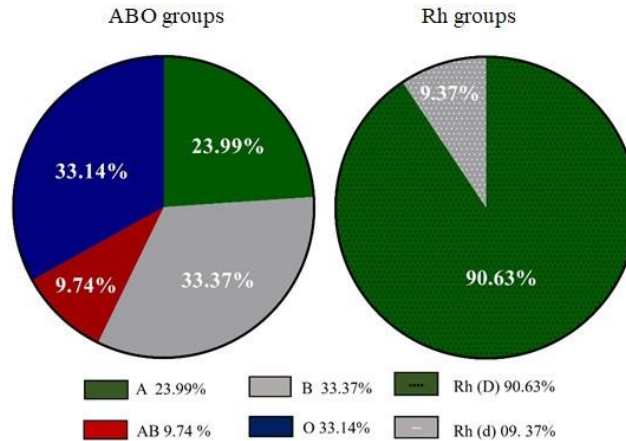
We found the research studies from all the country regions in our literature review; from all this search, 37 studies have been valid for inclusion in our research. Of the 37 eligible research studies published from all the regions, 34 were full manuscript, and 3 were abstracts.

Outcomes of the studies

Of all these 37 studies from all the six regions of Pakistan (Table 1), to assess the distribution and allelic frequency of each blood type total of 161893 individuals were used. The maximum number of studies from the most populated province/region of Pakistan Punjab has been published out of the total chosen studies, covering most of the population and the least number of studies published from Balochistan province (Table 2). Our investigation found that the overall frequency of Pakistan A, B, AB, and O blood groups was 23.99%, 33.37%, 33.14%, and 9.74%, respectively. The most common blood type in the Pakistani population is B with frequency (33.37%), followed by O (33.14%), then A (23.99%), and AB (9.74%). Among the total population, 90.63% were Rh (D) positive, while the rest of (9.37%) were Rh (D) negative (Figure 1).

Table 2: Observed frequencies of ABO and Rh (D) Blood groups from six regions of Pakistan.

Region	Area	A	B	AB	O	Comments	Rh+ve	Rh-ve	Total	Reference
Gilgit	Gilgit	24.2	40	10	25.8	B>O>A>AB	89.8	10.2	150	(Islam & Robert, 2010)
	Skardu	30.62	26.8	15.98	26.6	A>B=O>AB	94.83	5.17	1045	(Alam, 2005)
Azad Kashmir	Kashmir	25.93	32.59	17.26	24.2	B>A>O>AB	83.6	16.4	2300	(Chishti et al., 2012)
	Poonch	21.4	36.6	7	35	B>O>A>AB	89.5	10.5	3328	(M. Khan et al.,2009)
	Mirpur	26.38	32.5	9.47	31.65	B>O>A>AB	91.04	8.96	2937	(M Khalid et al.,2006)
Khyber Pakhtunkhwa	Karak	21.86	32.09	9.76	24.65	B>O>A>AB	88.37	11.63	645	(Abbas et al., 2020)
	Hazara	24	32	11	33	O>B>A>AB	85.8	14.2	2300	(Ullah & Ahmad, 2015)
	Swabi	27.6	34.4	8.8	32.2	B>O>A>AB	94.3	5.7	2500	(Ullah & Ahmad, 2015)
	Peshawar	28	34	7	31	B>O>A>AB	91.87	8.13	429	(Parveen et al., 2016)
	Bannu	31.03	36.23	7.67	25.07	B>A>O>AB	89.37	10.63	2581	(M. S. Khan et al., 2004)
	Swat	27.92	32.28	10.57	29.1	B>O>A>AB	90.13	9.87	22897	(Khattak et al., 2008)
	Bajaur Agency	29.42	30	10.5	30.08	O>B>A>AB	91.43	8.57	1200	(A. u. Rehman et al., 2014)
	Nowshetra	27.12	32.04	11.04	29.8	B>O>A>AB	92.88	7.12	4510	(Babar et al.,1999)
	Dir Lower	31.94	27.99	27.99	28.66	A>O>B>AB	92.45	7.55	13758	(Ullah & Ahmad, 2015)
	Mardan	24.75	27.97	19.36	27.93	B>O>A>AB	94.3	5.7	2893	(MUHAMMAD et al.,2013)
Punjab	Faisalabad	23.8	38	10	28.2	B>O>A>AB	89.1	10.9	200	(Hammed et al.,2002)
	Lahore	20.38	40.45	8.09	31.08	B>O>A>AB	89.48	10.52	618	(Kanwal et al.,2016)
	Liaquatpur	20.88	35.54	2.02	44.56	O>B>A>AB	90.35	9.65	1389	(A. REHMAN et al., 2005)
	Mandi Bahauddin	15.83	28.32	4.48	55.22	O>B>A>AB	91.4	8.6	2524	(Anees et al.,2007)
	Multan	26.57	34.15	9.61	29.67	B>O>A>AB	90.72	9.28	937	(F. U. Rehman et al., 2015)
	Multan	21.39	37.81	6.97	33.83	B>O>A>AB	92	8	900	(Ullah & Ahmad, 2015)
	Gujrat	17.4	22.29	4.35	55.96	O>B>A>AB	79.58	20.42	2647	(Anees & Mirza, 2005)
	Bahawalpur	21	36	6	37	O>B>A>AB	95	5	29659	(Ullah & Ahmad, 2015)
	Punjab	22.6	32.4	8.6	30.5	B>O>A>AB	93.9	6.1	5000	(Rahman & Lodhi, 2004)
	Rawalpindi/ Islamabad	25.53	33.33	10.04	31.1	B>O>A>AB	92.45	7.55	2518	(M. S. Khan et al., 2006)
	Sahiwal	22	36.9	9.9	31.3	B>O>A>AB	87.1	12.9	20010	(M. I. Khan et al., 2009)
	Gujranwala	22.91	35.36	9.32	32.41	B>O>A>AB	92.03	7.97	4754	(Ilyas et al., 2013)
	Multan	21.92	36.95	7.33	33.8	B>O>A>AB	92.17	7.83	3000	(Alam, 2005)
	Lahore	19.03	38.36	10.62	31.99	B>O>A>AB	93.99	6.01	2900	(Siddiqui et al.,2011)
	Rawalpindi/ Islamabad	24.2	34.3	10.1	31.3	B>O>A>AB	91	9	4642	(Shakir et al.,2012)
	Wah Cantt	18	24	5	53	O>B>A>AB	73.9	26.1	4462	(Iqbal, 2009)
Lahore	24.2	37.8	9.1	28.8	B>O>A>AB	93	7	3000	(UMER et al., 2014)	
Islamabad	24.64	34.72	9.28	31.36	B>O>A>AB	92	8	625	(Jabin et al., 2018)	
Lahore	20.12	37.45	10.57	32.11	B>O>A>AB	92.97	7.03	514	(Butt et al.,2016)	
Sindh	Karachi	24.1	33.1	7.2	35.6	O>B>A>AB	91.1	8.9	3521	(Ahmed et al.,2019)
	Sindh	25.83	28.17	8.3	37.78	O>B>A>AB	95.76	4.24	3000	(Ullah & Ahmad, 2015)
Balochistan	Balochistan	23.2	31.7	10.1	35	O>B>A>AB	94.75	5.25	1600	(Hussain et al., 2001)
Total	Observed Frequency	23.99	33.37	9.74	33.14	B>O>A>AB	90.63	9.37	161893	



Figures 1. Pie charts summarize ABO and Rh (D) distribution in Pakistan. A total of 161893 samples. The national average of blood group: 90.63% for Rh (D) (green color) and 9.37% for Rh (d) (Gray color). Involving 23.99% for A (Green color), 33.37% for B (gray color), 09.74% for AB (Red color) and 33.14% for O (blue color).

Allelic frequency distribution of blood groups ABO and Rh (D) in Pakistan

According to the quadratic equation and Hardy Weinberg law of equilibrium calculated results, the frequencies for IA(p), IB(q), and i(r) were 0.2435, 0.1809, and 0.5756 according to those data. Similarly, the Rh (D) positive and Rh (d) negative allelic frequencies for ID(v) and Id(u) were 0.6939 and 0.3061, respectively (Tables 3 and 4).

Table 3: Expected frequencies of ABO and Rh (D) System.

Phenotype	Observed frequency	Genotype	Expected frequency
A	0.2399	AA AO/OA	$p^2+2pr=0.3396$
B	0.3337	BB BO/OB	$q^2+2qr=0.2409$
O	0.3314	OO	$r^2=0.3314$
AB	0.0974	AB	$2pq=0.0881$
D	0.9063	DD/Dd	$V^2=0.4814,$ $v^2+2uv=0.9063,$ $2uv=0.4248$
d	0.0937	dd	$U^2=0.0937$

Allelic frequency of O

r^2 =Phenotypic frequency of O

$$r = \sqrt{r^2}$$

$$r = \sqrt{0.3314}$$

$r=0.5756.$

*Observed frequency of O=0.3314

Allele frequency of A

$WA=AA+AO$

$$=P^2 + 2pr$$

$$=P^2 + 2p (0.5756)$$

$$=P^2 + 1.152p=0.3399$$

$$=P^2 + 1.152p-0.3399=0$$

According to quadratic equation

$$\frac{x = -b \pm \sqrt{b^2 - 4ac}}{2a}$$

$a=1$

$b=1.152$

$p=0.2435$

$c=-0.3399$

*Observed frequency of A=0.2399

Allelic frequency of B

According to Hardy Weinberg equation

$$P + q + r=1$$

$$0.2435+q+0.5756=1$$

$q=0.1809$

*Observed frequency of B=0.3337

Allele frequency of d

U^2 =Frequency of d Phenotype

$$U^2=0.0937$$

$$U = \sqrt{0.0937}$$

$U=0.3061$

*Observed frequency of d=0.0937

Allele frequency of D

V =frequency of D phenotype

$$U + V=1$$

$V=1-0.3061$

$V=0.6939$

*Observed frequency of $D=0.9063$

According to the Chi-square test for ABO and Rh (D), the goodness of fit was statistically not significant ($p \geq 0.5$) (Table 3)

ABO and Rh (D) groups distribution with their allelic frequencies on a regional basis in Pakistan

Our study divided the state of Pakistan into six regions, including four provinces of Khyber Pakhtunkhwa, Sindh, Punjab, Balochistan, and two autonomous territories of Gilgit Baltistan and Azad Kashmir; details of the regions and its population are given in Table.1. According to our calculated data, blood group B is more prevalent in Punjab (34.41%), followed by Gilgit (33.40%), then Kashmir (32.50%), and Khyber Pakhtunkhwa (31.65%). In comparison, the O blood group is most common in Sindh (36.68%) and Balochistan regions (35%), as described in figure no 2. Individuals with Rh-positive blood groups were dominant in Balochistan (94.75%) compare to the rest of the regions, (Figure 2).

This review also calculated the gene frequency for the ABO and Rh (D) genes using the Hardy Weinberg equilibrium.

According to that the allelic frequency of $O=i(r)$, $A=IA(p)$, $B=IB(q)$, $D=ID(v)$ and $d=Id(u)$ were 0.5756, 0.2435, 0.1809, 0.3061 and 0.6939 respectively details are given in Table .3. According to our studies the observed phenotypic frequency of A, B, AB, O and Rh (D) positive and negative blood groups were 0.2399, 0.3337, 0.0974, 0.3314, 0.9063 and 0.0937 whereas expected phenotypic frequency were 0.3396, 0.2409, 0.0881, 0.3314, 0.9063 and 0.0937 respectively (Table 4). Chi-square test shows the two values of observed and expected frequencies of ABO and Rh blood system were not statistically significant ($p \geq 0.05$) (Table 4).

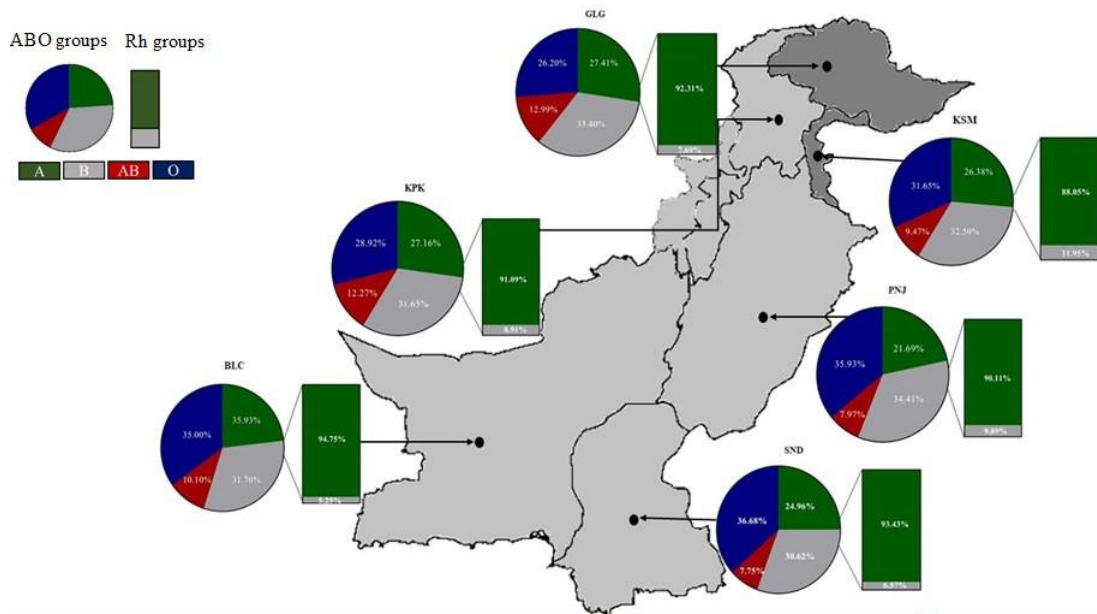
DISCUSSION

Pakistan is a country with a 220 million population, according to the 2017 consensus of the Government of Pakistan. It is a residence for many ethnic and religious communities.

No significant data is available in the Pakistani population concerning the distribution of blood groupings of ABO and Rh (D) and their allelic frequencies so far. Still, there are several regional studies available that analyze the distribution in their specific regions. A multicentric analysis by (Ullah & Ahmad, 2015) presented most of the ABO and Rh (D) blood groups. However, the analysis was not focused on the

Table 4: Table Comparison of Observed and Expected frequencies of ABO and Rh (D) Blood Group System.

Existing Allelic Frequency					Existing Genotype Frequency of ABO and Rh System						Expected Genotype Frequency of ABO and Rh System					
O $I_A=p$	A $I_B=q$	B $i=r$	C $ID=v$	D $Id=u$	O	A	B	AB	Rh+	Rh-	O r^2	A p^2+2pr	B q^2+2qr	AB $2pq$	Rh+ v^2+2uv	Rh- $U2$
0.2435	0.1809	0.5756	0.6939	0.3061	0.3314	0.2399	0.3337	0.0974	0.9063	0.0937	0.3314	0.3396	0.2409	0.0881	0.9063	0.0937
Total=1		Tota=1			Total=1			Total=1		Total=1			Total=1			



Figures 2. Map showing the frequency (%) of ABO and Rhesus blood groups in different regions/provinces of Pakistan. Pie chats represent per state average proportions of ABO; and bars show the proportion of Rh (D) groups. GLG=Gilgit; KSM=Kashmir; KPK=Khyber Pakhtunkhwa; PNJ=Punjab; SND=Sindh; BLC=Balochistan.

frequency and distribution necessary to reflect all populations' overall image. We analyzed six regions of Pakistan, including four provinces and two autonomous territories of the state, which include 0.15 million of the population in our review. We tried to include the reported studies from January 1999 to September 2020 to cover all the population of Pakistan.

Blood group distribution is different regionally, ethnically, and from one population to another. According to our study, the sequence of the distributions of blood groups in the Pakistani population is B with frequency (33.37%) followed by O (33.14%) then A (23.99%), and AB (9.74%) is the least frequent blood group in the overall population of Pakistan. Our analyzed data is in line with a study published by (Afzal et al., 1977). This was also in agreement with a paper reported by (Nanu & Thapliyal, 1997) in neighboring India. The results are also consistent with the study in which B is the most prevalent blood group in Africans and European populations. In contrast, blood group O and A is commoner in Australians (P. J. A. Mollison & Analgesia, 1954). Other research conducted in various populations around the world indicate that blood group O is more predominant in Saudi Arabia (52%) (Bashwari et al., 2001), US (46%) (Frances, 2002), and in the Iranian population (41.16) (Marzban et al., 1988)

In addition, our research showed that gender or position had no significant impact on the occurrence of ABO blood groups in line with a previous study conducted in Nigeria (Akhigbe et al., 2009). According to the Rh blood group findings, Rh+ was the highest in our study population (90.63%) than (9.37%) Rh-group. Our Rh-blood group population, however, is higher than those globally registered in Tanzania (3.2%) (Jahanpour et al., 2017) and 1.0% in China (Guo et al., 2012). 16% of the sample population with the Rh-blood group was higher than our studies (Saad, 2016), and 8% of the Rh-blood group in the Fezzan area were confirmed by Al Bayda city's local results in eastern Libya (Salih, Abdrhman, Irhuma, Elgadi, & Abd El Latef, 2005). Several studies have concluded that Rh+ is a dominant antigen compared to Rh- (Abbas et al., 2004).

In this review, we found that blood group B is most prevalent in Punjab (34.41%), Gilgit (33.40%), Kashmir (32.50%), and Khyber Pakhtunkhwa (31.65%) province. However, in Sindh and Balochistan, blood group O is prevalent, with 36.68% and in Balochistan 35%, respectively. The environmental influence and natural selection in the various areas of Pakistan will interpret this unequal blood distribution. Our study found that the B blood group is the most prevalent in Pakistan's population as the findings comply with another study (umer et al., 2014). *Vibrio cholera*'s deadly cholera infection is considered to become more prevalent in Pakistan's regions (Kanungo et al., 2010). This can be justified by Row et al. by their hypothesis of region-specific selection burden (Rowe et al., 2009). Therefore, it may be concluded that there is a tremendous geographical and environmental influence on blood groups. Similar findings by (Dewan, 2015) have been reported in Bangladesh, which has identified more B blood group tendency in coastal areas

to cholera prevention (Dewan, 2015).

Limitations of the study

We tried our best to determine the frequency distribution of blood group systems and their frequencies in the Pakistani population in our systematic study, but still, our study poses some limitations, which are as follows;

1) In this review, all of the representative populations are not covered entirely because some of the regions did not publish more number of studies during our inclusion period (1999 to 2020) and only a paper or two was published which studied a limited number of individuals.

2) We tried to include many published studies, but all the published articles were not full manuscripts; some of them were abstracts used in our review.

3) The chances of overlapping the populations also exist because there were multiple studies published from the same region.

4) We include only D antigen in the Rh blood group system, whereas D antigen frequency can be influenced by other antigen systems such as C, c, e, and E.

CONCLUSION

All humankind shares specific blood groups which cannot be changed throughout their life. The blood group system is variable in different ethnic groups, races, and other geographical locations. We attempted to evaluate and measure the ABO blood group system's frequency and distribution in our current research analysis nationwide. Our study in Pakistan's population presented that blood group B is most prevalent, and AB is least prevalent, and more than 90 percent of the population is Rh-positive. Our findings indicate that the allelic frequency of blood groups in Pakistan is allegedly distributed. The research we discoursed in the paper indicates that this unequal blood distribution along with genetic influence can be viewed in various regions of Pakistan as an environmental effect and natural selection. We believe that the knowledge of the frequency distribution of ABO system in the entire nation and especially in the country's specific areas, is essential for blood bank information, safe transfusion of blood, population migration, and disease trend concerning blood groups. The future national studies are planned with more sample size and other blood group systems to provide insight into the relationship of blood group antigens in relation with diseases within Pakistani populations and to lead to safe transfusion facilities in the countries.

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