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A study of A1A2BO blood group and rho (D) status among bhils and rajputs of Sirohi district, Mt. Abu, Rajasthan

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Abstract

The ABO and Rh blood group system plays a very important role in blood transfusion and organ transplants and are useful in population genetic studies, studying population migration patterns and resolving certain legal issues such as disputed parentage. It is therefore important to study patterns of distribution of these blood groups in every population. In the present study an attempt has been made to study the ABO & Rh Blood Groups systems among the BHILS & RAJPUTS of Sirohi District, Mt. Abu, Rajasthan and to study variation & distribution of allele frequencies of present population group. It also describes the population variation among humans living in a limited area & time frame of an ecological zone. By using the various formulas given by Bernstein allele frequencies & expected frequencies were calculated. The chi-square value of about 4.167 among BHILS and 1.8456 among Rajputs with the high probability ($0.5 > p > 0.70$) for 2 degrees of freedom reflects the internal consistency of the data i.e. observed values are in close agreement with the expected values indicating that the population is in genetic equilibrium. The most predominant type of blood group is O both among BHILS & RAJPUTS. Blood group B has been found less predominant as compared to O in both the populations followed by A₁ & A₁B. Frequency of A₂ & A₂B is same in RAJPUTS while it's higher for A₂ than A₂B among BHILS. The frequency of Rh⁺ allele has been noted to be reportedly high among both the populations as compared to Rh⁻ allele. The Chi-square values for each blood group system in both the populations depict the genetic equilibrium of the population. All the evolutionary forces are acting on the population in a balanced manner with normal random mating breeding patterns.

Keywords: ABO, Rh, Bhils, Rajputs, Rajasthan, allele, frequencies

Introduction

Human blood is a readily available tissue in human body which carries several polymorphic, serological and biochemical characters. A number of genetic studies are based upon such serological markers. ABO and Rh blood group systems are one of those widely used serological markers whose precise mode of inheritance is known and has been found to get least affected by external environmental factors. However, evolutionary forces like natural selection, genetic drift, mutation and consanguinity affect inheritance patterns of these markers as a well established fact applicable to every Mendelian population. Thus, population genetic studies based upon dealing with interaction between inheritance and genetic variations are best studied using ABO and Rh blood group systems.

ABO blood group system, comprising A, B & O groups was discovered by Landsteiner (1900, 1901). The fourth group, AB was discovered by Decastello & Sturli (1902). Later studies subdivided A group into A₁ & A₂ (Dungern & Herszfeld 1911). Rh blood group system was discovered in 1940, when Landsteiner & Weiner immunised the blood of a rabbit & guinea pig with the blood of rhesus monkeys & obtained a serum which at that time agglutinated the blood of certain individuals & while not of others and called it as Rh factor. The Rh status was proved to be a dominant character by Landsteiner & Weiner (1941) after working on 60 families. Since then, a no. of Rh variants of antigen D, C & E have been reported having their recessive forms as d, c & e respectively.

The ABO blood typing is based upon antigen-antibody reaction. The blood group antigens contain glycoproteins and glycolipids covered with protective carbohydrate chains. It is the type of sugar the carbohydrate is made up of which determines ABO blood type. In people having O blood group the carbohydrate consists of a core of 5-13 sugars called H-antigen.

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In people with blood group A an additional sugar called N-Acetylgalactosamine is present and in people having blood group B another smaller sugar galactose is present. When a person having blood group A is transfused with B blood group, the carbohydrate present in blood group B is seen as a foreign substance by antibodies present in the blood of former and vice versa and agglutination takes place as a result of antigen-antibody reaction. However, when blood group O blood is transfused to either A or B blood group person no such reaction takes place as O lacks both antigens but it posses both antibodies due to which it can only receive the blood of O type.

Blood group determination involves certain theoretical & technical difficulties as discussed below:

Rouleaux formation: Red blood cells have tendency to show apparent agglutination by arranging themselves in the form of pile of coins.

Hubener thomson phenomenon: Sometimes due to contamination of the blood sample by certain bacteria agglutination is observed by all types of human serums and the sample acts as being an AB blood type.

Contaminated normal saline solution: Sometime distilled water used in preparation of normal saline solution is contaminated with metals which weaken or destroy the blood cell receptors. To avoid the case, redistilled water is used to prepare normal saline solution.

The ABO system derives its significance from the known fact that A and B are strongly antigenic and persons carry anti A and anti B naturally in the plasma while lacking the corresponding antigen. These antibodies can produce haemolysis *in vivo*. The second most significant system from transfusion point of view is Rh blood group system. The knowledge of the distribution of ABO and Rh blood groups in various populations is therefore essential for effective management of blood banks inventory, studying population migration and settlement of legal issues of crime and parentage. During organ transplants the organs that match according to recipient's ABO antigen system are preferred by the surgeons. Frequencies of various blood group alleles among various human races are helpful in studying genetic constituency, history and migration.

The objective of the present study was to study distribution of ABO blood groups & Rh blood group among Rajputs and Bhils of Sirohi district, Mt. Abu, Rajasthan. Variation & distribution of allele frequencies of present population group with the population groups of area of study was also studied. Sirohi District is located in the southern part of Rajasthan. According to the 2011 census Sirohi district has a population of 1,037,185. The district has a population density of 202 inhabitants per square kilometre. Its population growth rate over the decade 2001-2011 was 21.86%. Sirohi has a sex ratio of 938 females for every 1000 males, and a literacy rate of 56.02%.

Bhils

They derive their name from the Dravidian word: *vil*, meaning a bow or arrowman. They are the ancient inhabitants of the Aravallis, where they are largely distributed even today (Tod 1881). The Bhils had been rulers in certain parts of Rajasthan, Gujarat, and Madhya Pradesh and were dislodged by the incoming Rajputs. At present they live in scattered hutments separated widely or

parched on hill tops, depending upon the topography. Women have an important role in the Bhil economy, including collecting firewood, raising livestock, fishing, planting and harvesting, and other small micro-enterprise. The Bhil have been significantly influenced by Hinduism and worship Shiva as their supreme deity. However, in Sirohi district they also worship other gods like Lord Krishna, Lord Rama, Goddesses like Lakshmi, Durga, Kali etc. They also believe in their local gods. For e.g in a household there was placed a piece of stone at a worship place. It was symbolic of God & was presented with a part of maize crop each year. BHILS practice polygamy. A Bhil woman is free to marry a man of her choice. There is the tradition of paternal family system. Joint family is considered as a major force of life. Love as well as arranged marriages both are accepted with equal love & respect. Dowry system is there but bride's family is not forced to give the bride wealth beyond their capability. Widow remarriage is there & also a divorcee can marry to a man of her choice again. Bhil girls & boys are not accepted by higher class & castes for marriages. Though with increasing education & exposure the traditions seem to change a bit.

Rajputs

Rajput populations are found in Rajasthan, Gujarat, Uttar Pradesh, Himachal Pradesh, Haryana, Jammu, Punjab, Sindh, Uttarakhand, Madhya Pradesh and Bihar. The four prominent clans in the post-Gupta period - *Chauhans*, *Paramaras*, *Pratiharas* and *Solankis*-all claimed their mythological origin to have been from a sacrificial fire at Mount Abu. Most Rajputs are Hindu. They were known for protecting Hinduism against Buddhism and Islam. Rajputs marry outside their clan. They also try to marry their daughters into clans of higher rank than their own, while accepting daughters-in-law from clans of lower rank. Love marriages are not accepted at any cost by Rajputs though it's common in some families too. Rajputs don't marry their sons & daughters in other castes & especially lower castes & tribes like those of Bhils are considered as untouchable by Rajputs. Most Rajputs are Hindu. They worship all major Hindu deities. Most Rajputs are devotees of the god Shiva. Many also worship Surya (the Sun God), and Durga as Mother Goddess. In addition, nearly every Rajput clan has its own patron god to whom it turns for protection.

The significance of the study lies in the fact that it describes the population variation among humans living in a limited area & time frame of an ecological zone. Also, as observed it has been found from literary sources that no study of this kind has been conducted in the area of interest in past however studies has been conducted in the state of Rajasthan. It is hoped that once the variation & their patterns are recognised, these can further help in explaining the present day genetic structure of this population. It may also help in isolating micro-evolutionary mechanisms operating at breeding population level within an ecosystem, assuming of course, that observations are result of genetic & environmental interaction. This study will also provide new data for the population concerned in deciphering their recent genetic makeup & thus can provide a basic background for further studies in this direction.

Materials and Methods

The present study was carried out among Bhils & Rajputs of Sirohi District, Mt. Abu, Rajasthan. For the present study

door to door & school based samples of blood were taken from various villages namely Gova Gao, Oriya Gaon, Javai Gaon, Delwara, Bhilwara, Kumarwada, Aarna Gaon, Ser Gaon, Machgaon, Sani Gaon. A total of 150 samples were collected using finger prick method & grouped for ABO & Rh genotypes. The subjects included unrelated individuals of both the sexes & different age groups. The preparations included various off field & on field materials & methods as follows:

Off field preparations: Followed materials were arranged & packed to be taken to the field. These include various apparatus & chemicals.

Apparatus: Eppendorf (containing EDTA for collection of blood samples), Eppendorf stand, Ice packs, Ice box, Fresh and sterile lancets, Markers, cotton, Disposable gloves, Sticker tapes, Beakers, Porcelain Tiles, Forceps, Slides, coverslips, Duster, Brush, Detergent

Chemicals: Normal saline solution, EDTA (ethylene diamine tetra acetic acid), Absolute ethanol, Distilled water, Anti sera

On field technique

Taking ethical clearance and consent

All the required information about the subject (like name, age, sex, gotra/clan etc.) were recorded before carrying out the blood collection procedure & a consent form was counter signed by the respective subject

Collection of blood samples

1. Disposable gloves were worn and the subject was asked to stand relaxed.
2. Alcohol was applied on the finger tip of the ring finger and allowed to air dry.
3. Skin was punctured with one quick, continuous and deliberate stroke, to achieve a good flow of blood and to prevent the need to repeat the puncture.
4. The first drop of the blood was wiped away because it might be contaminated with tissue fluid debris.
5. Blood was collected in EDTA coated Eppendorf.
6. Each and every time after collecting few drops of blood in the Eppendorf, it was tapped vigorously to allow the mixing of blood with EDTA, thus to avoid clotting of blood.
7. When the blood collection procedure was complete, the punctured site was cleaned with the cotton swab.
8. All the equipment used in the procedure were collected to avoid contamination and accidents, and ensured that nothing is left behind, especially the used lancets.
9. At last, the samples were kept in ice box containing frozen ice packs, on the field.
10. The samples thus collected were analysed the very same day & results were interpreted & recorded.

Procedure for abo subgrouping

1. The blood sample was taken & a 5% red cell suspension was made.
2. Clean porcelain tiles were taken & their cavities were marked with the help of a marker.
3. A drop of each Anti-A serum was put in each cavity of the first row of the pair & a drop of Anti-B was put in each cavity of second row of pair.

4. The 5% red cell suspension was put respectively in the two cavities bearing the same serial no. as that of the subject.
5. The tiles were gently rotated after 15 minutes & agglutination reactions were noted.
6. If the sample showed a positive reaction with Anti-a, it was assigned group A. Similarly if it showed a positive agglutination with Anti-B, it was assigned group B. If the sample showed a positive reaction with both Anti-A & Anti-B it was assigned group AB & if it showed a negative reaction with both Anti-A & Anti-B it was assigned the group O. The group O was further confirmed by testing the sample with a drop of Anti-H.
7. Further subgrouping of blood groups was performed with the help of Anti-A₁ to assign the groups A & AB as A₁ & A₁B in case of positive agglutination or A₂ & A₂B in case of a negative agglutination reaction with Anti-A₁.

Procedure for RH subgrouping

1. Test tubes were taken & a drop of Anti-D was added in each of them. After that the tubes were marked according to the serial no. of the subjects.
2. After that a drop of 5% red cell suspension prepared earlier from blood samples was added in each test tube.
3. The test tubes were incubated for 5 minutes at 37°C & then centrifuged for 2 minutes at 2000 rpm.
4. The tubes were then gently tapped & observed for agglutination.
5. If agglutination was observed it was assigned the subtype D otherwise it was assigned to be of d type in case of no agglutination.

Precautions taken

1. Glassware to be used was scrupulously cleaned.
2. All reagents & saline used were uncontaminated & pure.
3. Cell suspension was well mixed to ensure uniform distribution of red cells per unit volume.
4. The tile cavities & tubes were marked correctly.
5. The serum was tested with known positive & negative controls before carrying out the experiment.
6. Sterile & fresh lancets were used every time the new subject was fingerpicked.
7. All the disposable materials were collected & carried back in special bio hazard bags.

Statistical Analysis

The data collected from the field was observed & sorted categorically. The following observation table was formed separately for Bhils & Rajputs:

Table 1: A₁A₂BO blood groups among Bhils and Rajputs

S. no	Blood group type	Observed number	
		Bhils	Rajputs
1.	O	30	22
2.	A ₁	18	16
3.	A ₂	03	01
4.	B	22	20
5.	A ₁ B	09	06
6.	A ₂ B	02	01
7.	TOTAL	84	66

Table 2: Rh_o (D) Status of Bhils and Rajputs

S. no	Rh status	Observed number	
		Bhils	Rajputs
1.	Rh ⁺	76	61
2.	Rh ⁻	08	05
3.	Total	84	66

After sorting & observing the data, analysis was made using statistical tools. Chi square analysis was performed & results were presented in tabular & graphical form.

Results

Distribution of the ABO and Rh(D) blood groups among BHILS and RAJPUTS & their allele frequencies is presented in the form of tables as follows :

For BHILS:

A₁A₂BO blood group

Table 3: Observed frequency of A₁A₂BO blood groups among Bhils

Phenotypes	Observed no.	Observed Frequency
O	30	0.3571
A ₁	18	0.2143
A ₂	03	0.0357
B	22	0.2619
A ₁ B	09	0.1071
A ₂ B	02	0.0238
TOTAL	84	1.0007

It is observed that in the present population of BHILS the phenotype O is the most prevalent one followed by B, A₁, A₁B, A₂ & A₂B. The order of prevalence of various blood groups has been recorded as to be O>B>A₁>A₁B>A₂>A₂B. The same observation has been presented in the form of graphical representation in fig: 1

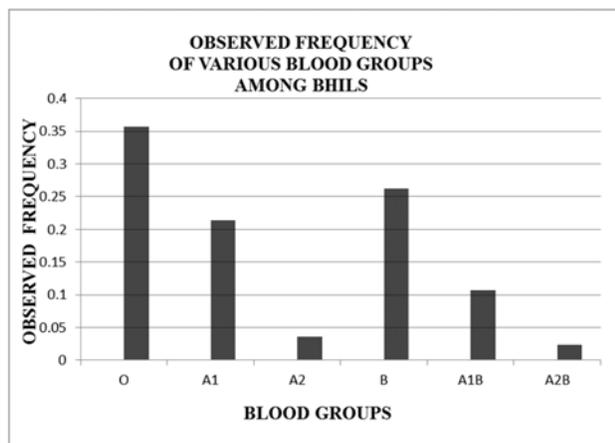


Fig.1: Graphical representation of observed frequencies of various blood groups among Bhils

Table below shows the chi-square value of about 4.167 with the high probability (0.5>p>0.70) for 2 degrees of freedom which reflects the internal consistency of the data for A₁A₂BO blood group distribution among BHILS of Sirohi district, Mt. Abu, Rajasthan, i.e. observed values are in close agreement with the expected values indicating that the population is in genetic equilibrium.

Table 4: Expected frequencies of A₁A₂BO among Bhils

Phenotypes	Genotypes	Expected Frequencies
O	OO=(r) ²	0.3805
A ₁	A ₁ A ₁ =(p ₁) ²	0.0248
	A ₁ A ₂ =2(p ₁ p ₂)	0.0095
	A ₁ O=2(p ₁ r)	0.1942
	Total	0.2285
A ₂	A ₂ A ₂ =(p ₂) ²	0.0009
	A ₂ O=2(p ₂ r)	0.0371
	Total	0.038
B	BB=(q) ²	0.0382
	BO=2(qr)	0.2412
	Total	0.2794
A ₁ B	A ₁ B=2(p ₁ q)	0.0615
A ₂ B	A ₂ B=2(p ₂ q)	0.0118
Total		0.9997

Table 5: Chi-Square values for each blood group

Phenotypes	Observed		Expected		Chi-square Value
	Number	Frequency	Number	Frequency	
O	30	0.3571	31.96	0.3805	0.120
A ₁	18	0.2143	19.19	0.2285	0.073
A ₂	03	0.0357	3.19	0.038	0.011
B	22	0.2619	23.47	0.2794	0.092
A ₁ B	09	0.1071	5.166	0.0614	2.845
A ₂ B	02	0.0238	0.9912	0.0118	1.026
Total	84	1.0007	83.98	0.9997	4.167

Table 6: Observed frequency & allele frequencies of Rh_o (D) blood types among Bhils

Phenotypes	Observed Number	Observed Frequency	Allele Frequency	
			D	d
Rh ⁺	76	0.904	0.692	0.308
Rh ⁻	08	0.095		
Total	84	0.99	1	

As far as the Rh blood group is considered, in the present population of BHILS the Rh⁺ individuals outnumber the Rh⁻ ones. The proportion of homozygotes & heterozygotes of Rh blood group system shows DD has a percentile frequency of 0.4789, Dd of 0.4263 & dd being the least as 0.0949.

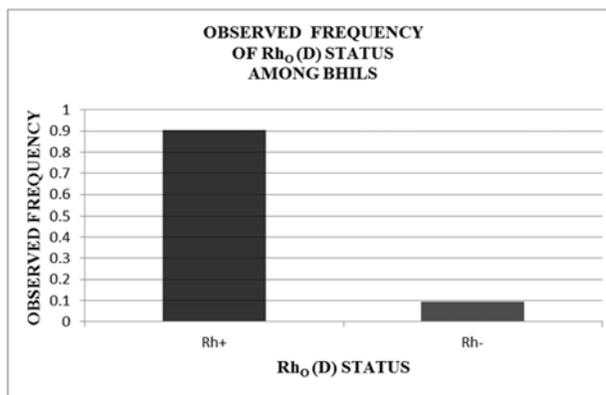


Fig 2: Graphical representation of Rh blood group among Bhils

The chi-square value is 0.0001 for 1 degree of freedom which corresponds to a probability of .50>p>.99 which is not statistically significant & reflects the internal consistency of data.

Table 7: Showing Chi-Square values for Rh blood group among Bhils for Rajputs

Phenotypes	Observed		Expected		Chi-square
	Number	Percentile	Number	Percentile	
Rh ⁺	76	0.904	76.04	0.9052	0.0000
Rh ⁻	08	0.095	7.97	0.0949	0.0001
TOTAL	84	0.99	84.01	1.0001	0.0001

a1a2bo blood group

It is observed that in the present population of RAJPUTS the phenotype O is the most prevalent one followed by B, A₁ & A₁B while A₂ & A₂B being equally prevalent. The order of prevalence of various blood groups has been recorded as to be O>B>A₁>A₁B>A₂=A₂B. The same observation has been presented in the form of graphical representation in fig: 3

Table 8: Observed frequencies of various blood groups among Rajputs

Phenotypes	Observed no.	Observed Frequency
O	22	0.3333
A ₁	16	0.2424
A ₂	01	0.0151
B	20	0.3030
A ₁ B	06	0.0909
A ₂ B	01	0.0151
TOTAL	66	0.9998

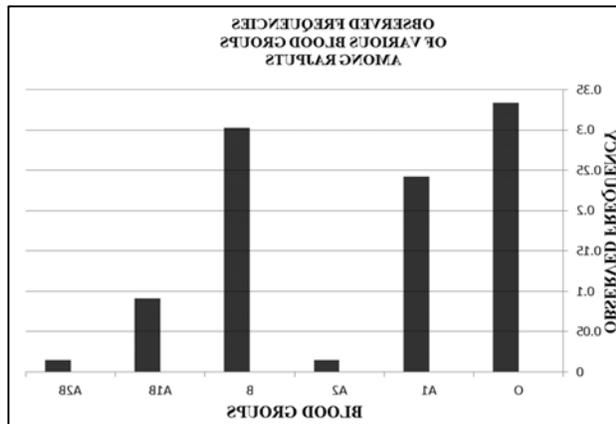


Fig.3: Graphical representation of observed frequencies of various blood groups among Rajputs

Table 9: Expected frequencies of A₁A₂BO among Rajputs

Phenotypes	Genotypes	Expected Frequencies
O	OO=(r) ²	0.3407
A ₁	A ₁ A ₁ =(p ₁) ²	0.0325
	A ₁ A ₂ =2(p ₁ p ₂)	0.0047
	A ₁ O=2(p ₁ r)	0.2106
	Total	0.2476
A ₂	A ₂ A ₂ =(p ₂) ²	0.0002
	A ₂ O=2(p ₂ r)	0.0152
	Total	0.0154
B	BB=(q) ²	0.0497
	BO=2(qr)	0.2602
	Total	0.3099
A ₁ B	A ₁ B=2(p ₁ q)	0.0804
A ₂ B	A ₂ B=2(p ₂ q)	0.0058
Total		0.9998

Table below shows the chi-square value of about 1.8456 with the high probability (0.5>p>0.70) for 2 degrees of freedom which reflects the internal consistency of the data

for A₁A₂BO blood group distribution among RAJPUTS of Sirohi district, Mt. Abu, Rajasthan, i.e. observed values are in close agreement with the expected values indicating that the population is in genetic equilibrium.

Table 10: Showing Chi-Square values for Rh blood group among Rajputs

Phenotypes	Observed		Expected		Chi-square Value
	Number	Frequency	Number	Frequency	
O	22	0.3333	22.49	0.3407	0.0218
A ₁	16	0.2424	16.34	0.2476	0.0208
A ₂	01	0.0151	01.02	0.0154	0.0196
B	20	0.3030	20.45	0.3099	0.0220
A ₁ B	06	0.0909	5.31	0.0804	0.1299
A ₂ B	01	0.0151	0.38	0.0058	1.6315
TOTAL	66	0.9998	65.99	0.9998	1.8456

As far as the Rh blood group is considered, in the present population of RAJPUTS the Rh⁺ individuals outnumber the Rh⁻ ones. The proportion of homozygotes & heterozygotes of Rh blood group system shows DD has a percentile frequency of 0.5275, Dd of 0.3976 & dd being the least as 0.0749.

Table 11: Observed frequency & allele frequencies of Rh₀ (D) blood types among Rajputs

phenotypes	observed number	percentile frequency	Allele Frequency	
			D	d
Rh ⁺	61	0.9242	0.7263	0.2737
Rh ⁻	05	0.0758		
Total	66	1	1	

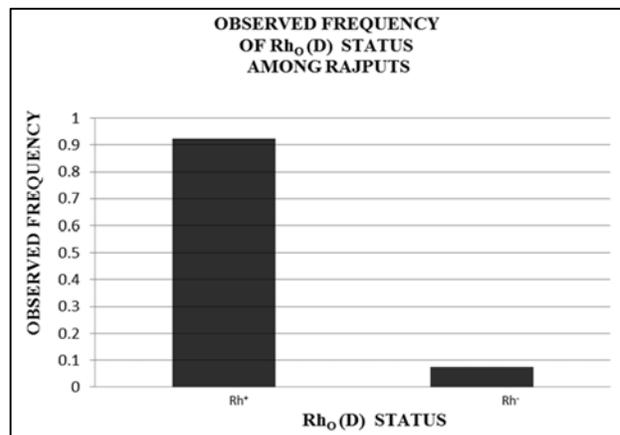


Fig. 2: Graphical representation of Rh blood group among Rajputs

The chi-square value is 0.0007 for 1 degree of freedom which corresponds to a probability of .50>p>.99 which is not statistically significant & reflects the internal consistency of data.

Table 12: Showing Chi-Square values for Rh blood group among Rajputs

Phenotypes	Observed		Expected		Chi-square
	Number	Frequency	Number	Frequency	
Rh ⁺	61	0.9242	61.05	0.9251	0.0000
Rh ⁻	05	0.0758	04.94	0.0749	0.0007
TOTAL	66	1	65.99	1	0.0007

Table 13: Contingency table Of Abo blood groups for Rajputs & Bhils

	O	A	B	AB	Total
Bhils	30	21	22	11	84
Rajputs	22	17	20	07	66
Total	52	38	42	18	150

Chi-square value comes out to be 0.4829 which is not statistically significant according to probability value 0.9226 at 0.05 level of significance. This shows the inter population variation among Bhils and Rajputs. The two populations don't intermarry among themselves and mating populations of both is different. There exists reproductive isolation among them. Both the populations are in genetic equilibrium according to Hardy-Weinberg law.

Discussion

There is an immense amount of work done to tell about how common each of the ABO blood types is around the world & it is quite clear that the distribution patterns are complex. From fig. (7) It can be shown that allele B is highest in Central Asia and lowest among the indigenous people of the America and Australia. However, there are relatively high frequency pockets in Africa as well. Overall in the world, B is the rarest ABO blood allele. Only 16% of humanity has it. The A blood allele is somewhat more common around the world than B. About 21% of all people share the A allele. The highest frequencies of A are found in small, unrelated populations, especially the Blackfoot Indians of Montana (30-35%), the Australian Aborigines (many groups are 40-53%), and the Lapps, or Saami people, of Northern Scandinavia (50-90%). The A allele apparently was absent among Central and South American Indians. The O blood type is very common around the world. About 63% of humans share it. Type O is particularly high in frequency among the indigenous populations of Central and South America, where it approaches 100%. It also is relatively high among Australian Aborigines and in Western Europe (especially in populations with Celtic ancestors). The lowest frequency of O is found in Eastern Europe and Central Asia, where B is common.

The majority of the people in the world have the Rh+ blood type. However, it is more common in some regions. Native Americans and Australian Aborigines were very likely 99-100% Rh+ before they began interbreeding with people from other parts of the world. This does not imply that Native Americans and Australian Aborigines are historically closely related to each other. Most Sub-Saharan African populations are around 97-99% Rh+. East Asians are 93-99+% Rh+. Europeans have the lowest frequency of this blood type for any continent. They are 83-85% Rh+. The lowest known frequency is found among the Basques of the Pyrenees Mountains between France and Spain. They are only 65% Rh+. Generally in India, the average value of allele B is more as compared to A allele, whereas the frequency of O is about 57%. Among ethnic groups viz., castes, scheduled castes, scheduled tribes & communities of India, the value of B is high as compared to A. However the difference between A & B frequencies is less among scheduled tribes (A=21 & B=22 %) as compared to castes (A=14 & B=23%) & scheduled castes (A=20 & B=24) whereas the average frequency of O is higher & similar among castes & communities (58%) as compared to scheduled tribes (57%) scheduled castes (54%). In different

zones the frequencies of A (17%) & B (19%) are low among the various population groups of South India & high in the different groups of North India (A=20% & B=27%) whereas the frequency of O is high (63%) among the various groups of South India & it is low (54%) in North India. The A frequency is high in the population groups of East India (21%) whereas B is predominant in North India (27%).

In most of the Europe the d allele frequency is about 40%. In Africans, the allele varies around 20%. In Asia, it is difficult to see any definite trend in the frequency of d & there is a fall in the regions inhabited by people who are wholly or partly Mongoloid.

On the whole, in India the general frequency of the Rh allele (d) is observed to be 23%. With regard to different zonal regions the highest value for D is observed among the population groups of South India (29%), followed by East, West & North India (25, 24 & 20% respectively).

Table 14: A₁A₂BO & Rh(D) phenotypes & the allele frequencies among Bhils (N=84) & Rajputs (N=66) Of Sirohi District, Mt. Abu, Rajasthan.

Phenotypes	Observed Number		Observed Frequency	
	Bhils	Rajputs	Bhils	Rajputs
A ₁ A ₂ BO System				
O	30	22	0.3805	0.3407
A ₁	18	16	0.2285	0.2476
A ₂	03	01	0.038	0.0154
B	22	20	0.2794	0.3099
A ₁ B	09	06	0.0614	0.0804
A ₂ B	02	01	0.0118	0.0058
Rh System				
Rh ⁺ (D)	76	61	0.692	0.7263
Rh(d)	08	05	0.308	0.2737

In the present area of study i.e. Sirohi district, Mt. Abu, Rajasthan, the most predominant type of blood group is O both among BHILS & RAJPUTS. Blood group B has been found less predominant as compared to O in both the populations followed by A₁ & A₁B. Frequency of A₂ & A₂B is same in RAJPUTS while it's higher for A₂ than A₂B among BHILS.

The frequency of Rh⁺ allele has been noted to be reportedly high among both the populations as compared to Rh⁻ allele. The Chi-square values for each blood group system in both the populations depict the genetic equilibrium of the population. All the evolutionary forces are acting on the population in a balanced manner with normal random mating breeding patterns.

Summary and Conclusion

There are differences in the distribution of ABO, and Rh (D) blood groups amongst different populations. The study of blood groups plays an important role in various genetic studies, in clinical studies for reliable geographical information and in blood transfusion practice, which will help in reducing morbidity and mortality rate. Knowledge of distribution of ABO and Rhesus (Rh) blood group is also essential for effective management of blood bank inventory. Blood group is a characteristic of an individual's *red blood cells*, defined in terms of specific substances (*carbohydrates* and *proteins*) on the cell membrane while DNA fingerprinting is a very quick way to compare shorter DNA sequence of any two living organisms. More realistically,

knowledge of DNA sequences and blood groups can prove useful in identification projects. These include reuniting families torn apart by war or by the actions of repressive regimes, identifying corpses, checking paternity, and most commonly, investigating and prosecuting crimes. Forensic uses of blood groupings and DNA finger printing technology inspires great hope but arouses considerable controversy.

ABO blood groups have shown to have some association with various noninfectious and infectious diseases. In most people A and B antigens are secreted by the cells and are present in the blood circulation. It seems that nonsecretors are susceptible to a variety of infections. The possible pathogenesis for this susceptibility is that as many organisms that may bind to polysaccharide on cells and soluble blood group antigens may block this binding.

The present study was aimed to identify distribution of ABO and Rh (D) blood groups among BHILS & RAJPUTS Sirohi District, Mt. Abu, Rajasthan. For the study, the data were collected from BHILS & RAJPUTS of various villages of Sirohi District. 150 samples were collected and analyzed for ABO and Rh(D) blood group systems. All the necessary precautions were taken for random sampling. Individual data of each subject- name, age, sex, caste, sub-caste, maternal uncle's caste, place of birth etc. were also collected using a brief questionnaire. The samples were analyzed for blood groups ABO and Rh(D); the test was done using Anti-A, Anti-A₁, Anti-B, Anti-H and Anti-D. For ABO blood grouping, cell suspension technique was used. Samples were collected in normal saline solution, washed and analyzed in 3-5% suspension of red cells in saline. For Rh(D) blood grouping, test tube method was used.

A₁A₂BO blood group

The allele frequencies have been calculated using Bernstein's formula & the Chi-square test for goodness of fit was done. Among 84 BHILS tested for A₁A₂BO blood group it was observed that O has the highest percentage followed by B, A₁, A₁B, A₂ & A₂B. Among 66 RAJPUTS, the pattern was almost the same, only difference observed was equal percentage of A₂ & A₂B. The Chi-square value for BHILS came out to be 4.167 & for RAJPUTS it came out to be 1.8456. Both the values are non significant with 2 degrees of freedom & 5% probability level. Hence the result indicates a good agreement between observed & expected frequencies. Both the populations appear to be in genetic equilibrium.

Rh blood group

Out of 84 BHILS & 66 RAJPUTS it was observed in both the populations that Rh⁺ allele was present in significantly very high level as compared to Rh⁻ allele. The Chi-square value for BHILS & RAJPUTS was calculated to be 0.0001 & 0.0007 respectively for 1 degree of freedom at 5% probability level. Hence, a good agreement between observed & expected frequencies has been established showing that the populations are in genetic equilibrium w.r.t Rh blood group system.

It is interesting to know that even after being a part of northern India the present populations show significant differences from other populations of same geographical zone where B allele comes out to be the highest one opposite to that of O observed in the present study. It has been observed also that prevalence of d allele is very low as compared to that observed in earlier studies.

Both these differences can be attributed to relatively small sample size & availability of time & resource. These limitations can be taken into consideration in future studies & attempts can be made to perform the study on a large sample size for good amount of time under sufficient availability of resources. Further Rh genotyping can also be done in this area for all the three C, D & E alleles. The present area of study is a quite untouched & interesting place for human population genetics studies having scope of some more interesting findings.

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