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Genetic Diversity at ABO and Rh (D) Loci in the Tribal Groups of Mohmand Agency (Federally Administered Tribal Areas), Pakistan

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ABSTRACT This study was aimed at determining the frequencies of ABO and Rh blood groups in the population of Mohmand Agency, which is a severely war-affected territory in the North-West Pakistan. A total of 1,019 unrelated subjects (963 males, 56 females) consented to participate in this study. Frequencies of blood types 'A', 'B', 'AB' and 'O' were observed to be 31.9 percent, 27.9 percent, 11.0 percent and 29.2 percent, respectively, while 87.6 percent subjects were found to be Rh-positive. At the ABO locus, allele *O* showed the highest frequency (0.538), while alleles *A* and *B* were 0.244 and 0.218, respectively. At the Rh (D) locus, alleles *D* and *d* showed frequencies of 0.648 and 0.352, respectively. The researchers also established the distribution of blood types across the tehsils and Pashtun tribes of Mohmand Agency. Further, the Nei's genetic distances were measured between the sample obtained from Mohmand Agency and the populations of adjoining districts.

INTRODUCTION

Mohmand Agency (MA) in Federally Administered Tribal Areas (FATA) at the Pakistan-Afghanistan border. The infrastructure of health-services has been severely marred; basic health units are either deficient or unable to adequately fulfill the requirements of routine and emergency medical situations. An orderly blood-banking facility is lacking which could be of vital importance in the prevention and alleviation of injuries and mortalities, irrespective whether these are caused by natural, accidental or war situations (Begovic et al. 1994).

ABO and Rh blood groups are two basic immuno-genetic systems, and are of great significance in blood transfusions and organ transplantation. These blood types are the most frequently reported genetic markers in different populations and reveal significant variation across geographic locations reflecting the underlying genetic and ethnic diversity in human populations (Bhasin 2009; Reddy et al. 2013; Ali and Malik 2014; Dewan 2015).

The safe and timely provision of blood is crucial in the mitigation of morbidity/mortality due to trauma (Begovic et al. 1994). Further, the documentation and screening of blood is pertinent to reduce the risk of transfusion-transmitted infections (Nigam et al. 2014). The blood transfusion particularly from the point of view of a war-time emergency service is an ignored subject in Pakistan. There is no systematic collection of blood transfusion/grouping data with respect to the bio-demographic, geographic and ethnic attributes of Pakistani sub-populations. Hence, the information on blood type frequencies of a particular community can be of great help for the patients, blood-banks, health planning, and as a reference. Among the seven Agencies of FATA, ABO and Rh blood types of only Bajaur Agency are reported (Rehman et al. 2014). Towards this end, the researchers intend to contribute to the elucidation of blood group types in the population of MA, which is an assemblage of Pashtun tribes.

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MATERIAL AND METHODS

This study was conducted in three *tehsils* of Mohmand Agency (MA) namely Lakarai, Ghalanai and Ekka Ghund. Travelling and data col-

lection was possible with the help of local resource persons and after the permission by the tribal heads/*Maliks*. A total of 1,019 subjects (963 Male, 56 Female) ranging in age from 8-50 years, were recruited. Only one individual was ascertained from a particular household. Due to the strict cultural limitations of the local Pashtun tribes, only a small number of females could be enrolled in the sample.

Serological phenotyping was performed by the procedure of forward typing (Plasmatec, Kent, United Kingdom). For each subject a clean glass slide was divided into three spots. The ring finger of each subject was pricked with a sterile blood lancet and one blood droplet was put on each spot of slide. Antisera anti-A, anti-B and anti-D were added onto the blood droplets and were mixed thoroughly. Agglutination in the spots was considered as the presence of the respective blood types. Each test was repeated in the case of ambiguity. Phenotypic frequencies were expressed in percentages while allelic frequencies at the *ABO* locus were calculated using standard methods (Mather 1964; Silva 2002). Homogeneity was tested between samples. Z-test was employed to estimate the heterogeneity of blood group proportions (Neel and Schull 1954). Nei's genetic distances (DA) were calculated through the allelic frequencies (Nei and Roychoudhury 1982).

RESULTS

In the overall sample of MA, blood types 'A', 'B', 'AB' and 'O' were observed to be 31.9, 27.9, 11.0 and 29.2 percent, respectively (Table 1). Additionally, Rh+ and Rh- blood types were 87.6 and 12.4 percent, respectively. The frequencies of blood types both at ABO and Rh systems were highly variable in the tehsil-wise data. At the ABO system, type 'A' was most abundant in Lakarai (33.9%), type 'B' in Ghalanai (29.9%), type 'AB' also in Lakarai (12.0%), and type 'O' in Ghalanai (30.7%). At the Rh system, Rh+ blood type was highest in Ekka Ghund (93.7%) and lowest in Lakarai (80%) (Table 2). At the *ABO* locus, allele *O* showed the highest frequency (0.538) which was followed by alleles *A* and *B* with frequencies of 0.244 and 0.218, respectively. Likewise, frequencies for alleles *D* and *d* were estimated to be 0.648 and 0.352, respectively. Among the tehsils, the allele *A* was highest in Lakarai (0.264), *B* was highest in Ghalanai (0.228) and *O* was highest in Ghalanai (0.550). At the *Rh* locus, allele *D* was estimated to be highest in Ekka Ghund (0.749) and *d* was highest in Ghalanai (0.318).

There were a number of minor tribal/ethnic groups which could be coalesced into eight major categories (Table 1). Blood group phenotypes as well as allele frequencies depicted considerable variations. For instance, blood type 'A' was highest in Safi_Masood (44.4%), 'B' in Mohmand_Others (35.0%), 'AB' in Safi_ Masood, and 'O' in Others (35.2%), while Rh+ was highest in Safi_Gurbaz (96.3%) (Table 2). The distributions of Rh blood types (but not ABO types) were statistically significant in the tehsils-wise and ethnic samples ($p<0.0001$). Variability was estimated from the proportions of blood

Table 1: Distribution of phenotypic and allelic frequencies at ABO locus in Mohmand Agency

	Phenotypes[#]					*Allelic frequencies*		
	Sample	*A*	*B*	*AB*	*O*	*A*	*B*	*O*
Tehsil[*]								
Lakarai	333	113 (33.9)	87 (26.1)	40 (12.0)	93 (27.9)	0.264	0.213	0.523
Ghalanai	385	111 (28.8)	115 (29.9)	41 (10.6)	118 (30.7)	0.222	0.228	0.550
Ekka Ghund	301	101 (33.6)	82 (27.2)	31 (10.3)	87 (28.9)	0.251	0.210	0.539
Tribe[**]								
Mohmand_Halemzai	330	95 (28.8)	102 (30.9)	31 (9.4)	102 (30.9)	0.214	0.228	0.558
Mohmand_Tarakzai	287	99 (34.5)	77 (26.8)	30 (10.5)	81 (28.2)	0.258	0.208	0.533
Mohmand_Others	40	14 (35.0)	14 (35.0)	5 (12.5)	7 (17.5)	0.280	0.280	0.441
Safi_Qandhari	93	20 (21.5)	32 (34.4)	12 (12.9)	29 (31.2)	0.188	0.271	0.541
Safi_Masood	45	20 (44.4)	7 (15.6)	8 (17.8)	10 (22.2)	0.378	0.180	0.443
Safi_Gurbaz	27	11 (40.7)	5 (18.5)	2 (7.4)	9 (33.3)	0.280	0.140	0.580
Shinwari	109	41 (37.6)	22 (20.2)	17 (15.6)	29 (26.6)	0.311	0.196	0.493
Others	88	25 (28.4)	25 (28.4)	7 (8.0)	31 (35.2)	0.203	0.203	0.595
Total	1019	325 (31.9)	284 (27.9)	112 (11.0)	298 (29.2)	0.244	0.218	0.538

[#]Numbers and percentages, [*]($\chi^2=3.74$;df=6; p=0.711), [**]($\chi^2=29.14$;df=21; p=0.111)

Table 2: Distribution of phenotypic and allelic frequencies at Rh (D) locus in Mohmand Agency

	Phenotypes[#]		*Allelic frequencies*		
	Sample	*Rh+*	*Rh-*	*D*	*d*
Tehsil[*]					
Lakarai	333	265 (79.6)	68 (20.4)	0.548	0.452
Ghalanai	385	346 (89.9)	39 (10.1)	0.682	0.318
Ekka Ghund	301	282 (93.7)	19 (6.31)	0.749	0.251
Tribe[**]					
Mohmand_Halemzai	330	298 (90.3)	32 (9.7)	0.689	0.311
Mohmand_Tarakzai	287	268 (93.4)	19 (6.6)	0.743	0.257
Mohmand_Others	40	37 (92.5)	3 (7.5)	0.726	0.274
Safi_Qandhari	93	80 (86.0)	13 (14.0)	0.626	0.374
Safi_Masood	45	39 (86.7)	6 (13.3)	0.635	0.365
Safi_Gurbaz	27	26 (96.3)	1 (3.7)	0.808	0.193
Shinwari	109	65 (59.6)	44 (40.4)	0.365	0.635
Others	88	80 (90.9)	8 (9.1)	0.699	0.302
Total	1019	893 (87.6)	126 (12.4)	0.648	0.352

[#]Numbers and percentages, [*](χ^2=31.89;df=2; p<0.0001), [**](χ^2=93.66; df=7; p<0.0001)

types. Across all ascertainment types, blood type 'O' was least variable (CoV=0.17), followed by 'A' (CoV=0.19), 'B' (CoV=0.24), and 'AB' (CoV=0.27).

Nei's genetic distances (DA) were established between MA and neighboring Pashtun populations of Khyber Pakhtunkhwa province (Table 3). Mohmand population was witnessed to have the highest affinities with the populations of Swat and Bajaur (DA=0.0022 and 0.0036, respectively), while there were least similarities with Swabi and Peshawar populations (DA= 0.0179 and 0.0145, respectively) (Table 3). Among the other populations, the highest heterogeneity was observed between Swat and Swabi populations (DA=0.0091).

Nei's genetic distances (DA) were also calculated between the tribal samples. Close affinities were witnessed between Mohmand_ Halemzai and Others, and between Mohmand_ Halamzai and Mohmand_Tarakzai (DA=0.0010 and 0.0043, respectively) (Table 4). In these anal-

Table 3: Genetic distance (DA) matrix showing the affinities between Mohmand population and other Pashtun populations

	Mohmand	*Bajaur*	*Dir-Lower*	*Swat*	*Buner*	*Mardan*	*Peshawar*	*Nowshera*
Bajaur	0.0036							
Dir-Lower	0.0063	0.0005						
Swat	0.0022	0.0006	0.0022					
Buner	0.0090	0.0017	0.0020	0.0023				
Mardan	0.0078	0.0010	0.0001	0.0029	0.0020			
Peshawar	0.0145	0.0037	0.0021	0.0062	0.0017	0.0014		
Nowshera	0.0078	0.0008	0.0005	0.0021	0.0006	0.0005	0.0013	
Swabi	0.0179	0.0056	0.0031	0.0091	0.0038	0.0022	0.0004	0.0028

Table 4: Genetic distance (DA) matrix showing the affinities between Pashtun tribes of Mohmand Agency

	Mohmand Halemzai	*Mohmand Tarakzai*	*Mohmand Others*	*Safi Qandhari*	*Safi Masood*	*Safi Gurbaz*	*Shinwari*
Mohmand_Tarakza	0.0043						
Mohmand_Others	0.0121	0.0071					
Safi_Qandhari	0.0053	0.0180	0.0206				
Safi_Masood	0.0251	0.0231	0.0193	0.0298			
Safi_Gurbaz	0.0172	0.0060	0.0220	0.0419	0.0393		
Shinwari	0.1255	0.1620	0.1567	0.0901	0.0877	0.2150	
Others	0.0010	0.0054	0.0185	0.0085	0.0313	0.0147	0.1340

yses, the Shinwari tribe emerged as a distinct entity depicting great heterogeneity with all other tribes.

DISCUSSION

ABO and Rh blood groups are classical markers which have been frequently reported in the medical and anthropological literature (Shami and Rasmuson 1994; Bhasin 2009). These polymorphisms have also been explored in various populations of Pakistan (Malik and Amin-ud-Din 2013), However, it is of note that there was also wide variation in the sample sizes which may partly explain the fluctuations in the phenotypic and allelic frequencies in the ascertainment categories.

Comparison of MA population with that of other Pashtun populations through Nei's genetic distance measure (DA) witnessed that the allele frequencies of both loci in the total MA sample run close to Bajaur Agency and Swat populations (Table 3). The similarity with Bajaur Agency makes sense as it is located in close proximity of MA. On the other hand, the affinities with Swat population could be partially explained by the fact that there has been migration of large number of families from MA to the Swat district. Conversely, the allele frequencies in MA sample demonstrated wide differences from Peshawar and Swabi populations. This could be explained by the multi-ethnic and cosmopolitan nature as well as distant geographic locations of the later two populations.

CONCLUSION

In conclusion, this study gives a detailed account of the distribution of phenotypic and allelic composition of ABO and Rh polymorphisms in Mohmand Agency. The data on blood type frequencies could be of great help for the patients, blood-banks, health planning, and as a reference. This study also presents the diversity of ABO and Rh loci in the Pashtun tribes of MA.

RECOMMENDATIONS

The wide variation in the distribution of blood group phenotypes reiterates the systematic establishment of blood-banks. The development of blood grouping and transfusion database would be useful in the effective delivery of health-services in a number of aspects. This study is the first record of blood types from the population of MA. It is strongly recommended to establish such database for all the populations of FATA and Khyber Pakhtunkhwa province of Pakistan.

LIMITATIONS

The present study has several limitations, like higher male representation and only a small number of females in the sample, and the employment of only two immunogenic markers which may have limited heterozygosity in populations. Hence, the findings of this study need to be iterated through a large set of polymorphic markers like microsatellites, which would be more useful in understanding the structure of this population.

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