

## Distribution of *ABO* and *Rh(D)* Allelic Polymorphisms North Waziristan Agency, (Federally Administered Tribal Areas), Pakistan

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**ABSTRACT** This population based-study was conducted in the North Waziristan Agency (NWA) along the Pakistan-Afghan border, in order to determine the frequencies of ABO and Rh(D) blood groups. A total of 1026 subjects belonging to four tehsils (Doss Ali, Mir Ali, Miran Shah and Spin Wam) of NWA were recruited and phenotyped. The blood groups 'A', 'B', 'AB' and 'O' were found to be 27.4, 36.7, 10.9 and 25.0 percent, respectively. Similarly, Rh (+) and Rh (-) blood types were observed to be 90.6 percent and 9.4 percent, respectively. At the *ABO* locus, alleles *A*[*p*], *B*[*q*] and *O*[*r*] showed frequencies 0.216, 0.277 and 0.507, respectively. At *Rh(D)* locus, frequencies for alleles *D* and *d* were 0.693 and 0.307, respectively. Distribution of blood group frequencies at both *ABO* and *Rh(D)* loci were observed to vary significantly across the tehsil-wise samples ( $p < 0.05$ ). In the same way, blood group frequencies also showed significant variation at *ABO* locus but not at *Rh(D)* locus when the data were analyzed with respect to the major ethnicities of NWA. Nei's genetic distances analyses revealed closed affinities between the sample drawn from NWA and the neighbouring populations of Hangu and Kohat districts.

### INTRODUCTION

Historically, a turbulent and war affected territory, North Waziristan Agency (NWA) is located along the Durand line (Pak-Afghan border), and is adjoined by Kurram Agency and Kohat in the North, Bannu in the East, South Waziristan Agency in South and Afghanistan in the West. NWA is the second largest agency in Federally Administered Tribal Areas (FATA) of Pakistan and has an area of 4,707 sq. km. Topographically, NWA is a difficult terrain with rugged mountains covering most of the landscape. Lack of proper infrastructure like roads, irrigation system, health-care facilities and educational institutions further increase the miseries of local tribal groups (FATA 2015).

NWA has a population of 361 million inhabitants with an annual growth rate of 2.46 percent. NWA has a population density of 77 persons per sq.km. and has a gender ratio of 114 males for

every 100 females (PBS, 1998). The majority of the masses (that is,  $\geq 98\%$ ) have rural dwellings. The main tribes of the agency are Dawars, Wazir and Tori Khel. Administratively, NWA is divided into three sub-divisions, that is, Miran Shah, Mir Ali, and Razmak with the Agency Headquarter based at Miran Shah. The Political Agent (PA) is the Chief Administrator of the agency who runs the agency with the help of Assistant Political Agents, *Tehsildars* and Tribal elders/*Maliks* (FATA 2015).

The ABO and Rh(D) blood group antigens are the most widely studied genetic markers in the human populations and their distributions have been established for many countries/towns. It has been observed that heterogeneity is present not only between continents and regions within them, but also among adjoining countries and cities (Bhasin 2009; Malik and Amin-ud-Din 2013; Ali and Malik 2014). Information regarding the blood group markers is helpful in medical and anthropological studies, and to describe the pattern of genetic variation within populations (Bhasin 2009 Sachdev 2012; Malik and Amin-ud-Din 2013; Reddy et al. 2013). From a bio-demographic viewpoint, NWA is largely an unexplored area due to its remote geographic location, difficult terrain, war situations, as well as

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social and political reasons. There is no data available on the genetic structure of this population. Thus, this pilot study documents the frequencies of *ABO* and *Rh(D)* blood group genes in the population of NWA.

### MATERIAL AND METHODS

A cross-sectional epidemiological study was conducted in the NWA during March-August, 2014. The study proposal was approved by the ethical review committee of the Hazara University, Mansehra. First-hand data on two serological markers (*ABO* and *Rh*) was randomly acquired from male subjects who belonged to Doss Ali, Mir Ali, Miran Shah and Spin Wam tehsils of NWA. Informed verbal consent was also obtained from each participant during the study. Sampling was made through door-to-door visits and by surveying educational institutions located in relatively safe territories of the agency. The local resource person always accompanied the research team during field visits.

Immuno-serological phenotyping was performed by the procedure of the forward typing (Plasmatec, Kent, United Kingdom). In this procedure, ring finger tip of each participant was pricked with the help of a sterilized lancet and three blood droplets were taken onto a clean glass slide which was already marked as A, B and D. It was followed by the addition of a single drop of anti-A, anti-B and anti-D antisera onto three blood spots, correspondingly and were mixed thoroughly for agglutination reaction. The test was repeated in case of any ambiguity. Data were entered into Microsoft Excel (2007). Phenotypic frequencies of both blood group markers were

expressed in percentages, and allele frequency estimates at the *ABO* locus were obtained by the maximum likelihood method (Strickberger 2005). Chi-square test was applied using GraphPad Prism software in order to check any difference in the distribution of blood group frequencies observed in tehsil-wise and caste-wise/ethnic data.

### RESULTS

A total of 1026 unrelated male subjects ranging in age from 12 to 83 years were recruited. These subjects originated from four tehsils and 20 villages/sampling sites of NWA. Collectively, blood group 'B' was most frequent (36.7%) in the sample, followed by blood groups 'A', 'O' and 'AB' with their frequencies of 27.4 percent, 25.0 percent and 10.9 percent, respectively (Table 1). At the *ABO* locus, frequencies for alleles A, B and O were observed to be 0.216, 0.277 and 0.507, respectively. Further, 90.6 percent subjects were found to be positive for the Rh factor whereas only 9.4 percent were Rh-negative. At the *Rh(D)* locus, alleles D and d were calculated to be 0.693 and 0.307, respectively (Table 2).

#### Tehsil-wise Distribution

The data were further analyzed with respect to four tehsils of NWA, and the phenotypic frequencies at both *ABO* and *Rh* systems were observed to vary across geographic locations (Table 1). For instance, type 'B' was emerged as the most common blood group across four tehsils, that is, Doss Ali (38.5%), Mir Ali (37.6%), Miran Shah (33.7%) and Spin Wam (38.4%), whereas

**Table 1: Distribution of phenotypic and allelic frequencies at *ABO* locus in North Waziristan Agency**

	Phenotypes, No. (%)					Allelic frequencies		
	Sample	A	B	AB	O	A	B	O
<i>Tehsil*</i>								
Doss Ali	57	12 (21.1)	22 (38.5)	11 (19.3)	12 (21.1)	0.223	0.344	0.432
Mir Ali	553	145 (26.2)	208 (37.6)	43 (7.8)	157 (28.4)	0.189	0.263	0.547
Miran Shah	291	90 (30.9)	98 (33.7)	34 (11.7)	69 (23.7)	0.244	0.262	0.494
Spin Wam	125	34 (27.2)	48 (38.4)	24 (19.2)	19 (15.2)	0.267	0.348	0.385
<i>Tribe**</i>								
Dawar	737	207 (28.1)	252 (34.2)	74 (10.0)	204 (27.7)	0.214	0.254	0.532
Wazir	289	74 (25.6)	124 (42.9)	38 (13.2)	53 (18.3)	0.219	0.340	0.441
Total	1026	281 (27.4)	376 (36.7)	112 (10.9)	257 (25.0)	0.216	0.277	0.507

\*  $\chi^2=28.01$ ;  $df=9$ ;  $p<0.001$

\*\*  $\chi^2=13.84$ ;  $df=3$ ;  $p=0.003$

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

	Phenotypes, no. (%)			Allelic frequencies	
	Sample	Rh+	Rh-	D	d
<i>Tehsil*</i>					
Doss Ali	57	53 (93.0)	4 (7.0)	0.735	0.265
Mir Ali	553	488 (88.3)	65 (11.7)	0.657	0.343
Miran Shah	291	267 (91.8)	24 (8.2)	0.713	0.287
Spin Wam	125	121 (96.8)	4 (3.2)	0.821	0.179
<i>Tribe**</i>					
Dawar	737	666 (90.4)	71 (9.6)	0.690	0.310
Wazir	289	263 (91.0)	26 (9.0)	0.700	0.300
Total	1026	929 (90.6)	97 (9.4)	0.693	0.307

\* $\chi^2=10.02$ ;  $df=3$ ;  $p=0.018$

\*\* $\chi^2=0.10$ ;  $df=1$ ;  $p=0.754$

'AB' was noticed as the least common blood type in three tehsils, that is, Doss Ali (19.3%), Mir Ali (7.8%) and Miran Shah (11.7%). Interestingly however, blood type 'O' was witnessed to be the least predominant blood type in tehsil Spin Wam (15.20%). Similarly, Rh(+) blood type had the highest frequency in Spin Wam (96.80%) whereas Rh(-) blood type had the highest representation in Mir Ali (11.75%) (Table 1). The blood group frequencies at the ABO locus were observed to show statistically significant differences in both tehsil-wise as well as caste-wise data ( $p<0.05$ ). Coefficient of variance (CoV) at the phenotypic proportions was calculated across the tehsil-wise samples, and blood type 'B' was observed to be the least variable (CoV=0.06), followed by 'A' (CoV=0.15), 'O' (CoV=0.25), and 'AB' (CoV=0.40). Likewise, blood group frequencies at Rh(D) locus showed statistically significant differences when the data were analyzed in tehsil-wise samples but not among the ethnic groups (Table 2).

The allelic frequencies at ABO locus showed similar trends across the four tehsils. For example, allele O was highly represented in all tehsils and was followed by alleles B and A. Individually, however, allele O showed the highest frequency in Mir Ali tehsil (0.547), allele B in Spin Wam (0.348) and allele A also in Spin Wam tehsil (0.267) (Table 1). The allele frequency distributions in three tehsils were observed to be consistent with Hardy-Weinberg expectations (HWE); however, the sample ascertained from Mir Ali was not concordant with HWE (Chi-test= 4.49;  $p<0.05$ ). This deviation could be attributed to a very high representation of O allele and blood type 'O', and very low representation of A allele and 'AB' blood

group in tehsil Mir Ali compared to all other three tehsils. At the Rh locus, allele D showed the highest frequency in Spin Wam (0.821) whereas allele d was the most conspicuous in Mir Ali (0.343) (Table 2).

#### Caste-wise/Ethnic Distribution

The present study also presents blood group data obtained from the two major ethnic strata, that is, Dawars and Wazirs, of NWA. Dawars showed highest representation (72%) in the present sample as compared to the Wazirs (28%). Phenotypic frequencies were observed to be 34.2 percent, 28.1 percent, 27.7 percent and 10.0 percent in Dawars whereas 42.9 percent, 25.6 percent, 18.3 percent and 13.2 percent in Wazirs for blood types 'B', 'A', 'O' and 'AB', respectively (Table 1). Likewise, the frequencies for Rh(+) and Rh(-) blood groups were found to be 90.4 percent and 9.6 percent in Dawars whereas 91 percent and 9 percent in Wazirs, respectively (Table 2). At the ABO locus, alleles A, B and O were found to be 0.214, 0.254 and 0.532 in Dawars, whereas 0.219, 0.340 and 0.441 in Wazirs, respectively, and were concordant with Hardy-Weinberg expectations. At the Rh(D) locus, alleles D and d were calculated to be 0.690 and 0.310 in Dawars, whereas these estimates were 0.700 and 0.300 in Wazirs, respectively (Tables 1 and 2).

#### Affinities between the NWA and Adjoining Populations

In order to establish genetic affinities between the NWA and adjoining populations, Nei's genetic distances (DA) matrix was generated

which was based on the allelic frequencies at the studied blood group polymorphisms. Blood group data of six neighbouring populations was extracted from the published literature (PMRC 1982; Guloon 1997; Baber et al. 1999; Khattak 2004; Shoaib et al. 2004; Ali 2010; Ali and Malik 2015). These populations included Kohat, Karak, Hangu, Bannu, Peshawar and Nowshera. All these populations are politically included in the Khyber Pakhtunkhwa province of Pakistan. The NWA population was observed to have the highest affinities with Hangu and Kohat populations (DA=0.001 and 0.001, respectively), while it depicted marked difference with the population of Karak district (DA=0.037) (Table 3). Among the other analyzed populations, the greatest heterogeneity was observed between Karak and Peshawar populations (DA=0.074), and between Karak and Nowshera (DA=0.056).

### DISCUSSION

Knowledge regarding the distribution of ABO and Rh(D) blood groups in any population is vital because the frequencies of ABO and Rh (D) blood types vary greatly from region-to-region and across ethnic groups. For instance, Western Europeans have predominantly blood group 'O' (46%), in contrast to Eastern Europeans which have the highest proportion of blood type 'B' (40%). Similarly, the Americans have the highest proportion of blood group 'O' (45%), as compared to all other blood types. Besides their significance in transfusion medicine and organ transplantation, ABO and Rh(D) blood groups can be used as genetic markers to trace the evolutionary history of man, and to draw phylogenies of different ethnic and/or linguistic groups around the world (Rehman et al. 2014). Information regarding distribution and frequencies of these genetic markers is largely missing for different parts of Pakistan in general, and north-

western populations in particular due to various geopolitical factors (Zafar et al. 1997; Malik and Amin-ud-Din 2013; Rehman et al. 2014; Rehman et al. 2015a, b).

Several peculiarities were evident in the distribution of blood groups in the NWA population. Unlike previous reports on the Northern populations of Pakistan by Rehman and colleagues (2014, 2015), NWA showed the highest proportion for blood group 'B' (36.7%) followed by 'A' (27.4), 'O' (25.0%) and 'AB' (10.9%). This distribution appears to be similar to other populations adjoining the NWA, that is, districts Hangu, Kohat and Bannu. Here, it is pertinent to mention that Hangu and Bannu districts share geographic boundaries with NWA. It is quite likely that there had been frequent migrations between these districts during the geopolitical crisis. In addition, present estimates of Rh(+) and Rh(-) blood types (90.6% vs. 9.4%, respectively) are in close conformity with other populations of Khyber Pakhtunkhwa and FATA (Khaliq et al. 1984; Khattak et al. 2008; Rehman et al. 2014; Rehman et al. 2015a, b).

Genetic affinity between the NWA and adjoining populations of Khyber Pakhtunkhwa province was observed on the basis of allelic frequencies at the studied loci. Nei's genetic distances (DA) were calculated and a cross-tabulation matrix was prepared for the included population. The sample drawn from the NWA was observed to exhibit the highest affinities with populations of Hangu and Kohat districts. Hangu district shares boundary with NWA and Kohat district adjoins Hangu district. The affinities between these populations could be due to their similar compositional and demographic characteristics and geographic neighborhoods. On the other hand however, Karak district which shares boundary with district Bannu, a close neighbor of NWA, appeared highly heterogeneous with NWA. Karak population also depicted least af-

**Table 3: Genetic distance (DA) matrix showing the affinities between NWA population and the adjoining populations of Khyber Pakhtunkhwa province**

	<i>NWA</i>	<i>Kohat</i>	<i>Karak</i>	<i>Hangu</i>	<i>Bannu</i>	<i>Peshawar</i>
Kohat	0.001					
Karak	0.037	0.047				
Hangu	0.001	0.002	0.031			
Bannu	0.003	0.003	0.050	0.003		
Peshawar	0.007	0.004	0.074	0.009	0.003	
Nowshera	0.002	0.001	0.056	0.004	0.001	0.001

finities with other neighboring populations (Table 3). It is quite likely that the Pashtun tribes residing in the Karak district are distinct from the rest of the populations.

This is a pilot study on the distribution of blood group polymorphism in NWA which is a highly turbulent, remote and inaccessible part of Pakistan. Information in this study would be of great significance for the blood banks, health planners, policy makers and specially individuals in need due to war-situation. In addition, this report would provide a baseline for future studies to understand the genetic architecture of Pashtun populations. At the point of time where huge population displacements have occurred due to socio-political conflicts, war situations and natural disasters.

### CONCLUSION

This, first ever comprehensive report, focused on determination of ABO and Rh (D) blood group frequencies in a tribal population of North Waziristan Agency, in Federally Administered Tribal Areas of Pakistan. Findings of this study revealed blood group 'B' as the most common blood phenotype (36.7%) followed by blood groups A (27.4%), O (25.0%), and AB (10.9%). Of the total sample, only 90.6 percent subjects were Rh-positive. Though the data revealed significant variation when analyzed with respect to ethnic and geographic affiliations of the subjects; these findings showed close affinity between North Waziristan Agency and the neighboring populations of Khyber Pukhtunkhwa province.

### RECOMMENDATIONS

Keeping in view the unprecedented casualties in north-western Pakistan in general and FATA in special, information concerning ABO and Rh blood group markers is vital for mitigation of huge morbidities and mortalities related to blood transfusions. Therefore, development of blood banks and management of rare blood groups is highly recommended for the health planners and policy makers in the region.

### LIMITATIONS

Limitations of this study comprised small sample size, participation of only male subjects and representation of four tehsils instead of a total seven tehsils. In addition, the data represented a highly disturbed and fragmented popu-

lation that has undergone severe socio-demographic and geo-political shifts during the last few decades. Therefore, further study with an extended sample size and use of polymorphic markers would better explain the genetic structure of this population.

### REFERENCES

- Ali S, Malik S 2014. Phenotypic distribution, allelic diversity and degree of differentiation at ABO and Rh loci in the population of Haripur District, Khyber Pakhtunkhwa, Pakistan. *Pak J Zool*, 46(1): 1-7.
- Ali S, Malik S 2015. Genetic heterogeneity and gene diversity at ABO and Rh blood group polymorphisms in seven Pashtun populations of Upper Khyber Pakhtunkhwa, Pakistan. *Pak J Zool*, 47(3): 649-656.
- Baber M, Hassan HS, Habib U, Khan MH 1999. ABO and Rhesus blood group distribution in district Nowshera. *Journal of Postgraduate Medical Institute*, 3: 70-72.
- Bhasin MK 2009. Morphology to molecular anthropology: Castes and tribes of India. *Int J Humn Genet*, 9(3-4): 145-230.
- Khaliq MA, Kahan JA, Shah H, Khan SP 1984. Frequency of ABO and Rh (D) blood groups in Hazara Division Abbottabad. *Pak J Med Res*, 23(4): 102-104.
- Khattak ID, Khan TM, Khan P, Shah SMA, Khattak ST, Ali A 2008. Frequency of ABO and Rhesus blood groups in district Swat, Pakistan. *J Ayub Med Coll Abbottabad*, 20(4): 127-129.
- Khattak MA 2004. *Blood Grouping and Monogenic Traits in Khattak Sub-tribe of Karak District (N.W.F.P)*. MSc Thesis. Department of Zoology. Peshawar: University of Peshawar.
- Malik S, Amin-ud-Din M 2013. Genetic heterogeneity and gene diversity at ABO and Rh loci in the human population of Southern Punjab, Pakistan. *Pak J Zool*, 45(5): 1185-1190.
- Official Home Page of Federally Administered Tribal Areas 2015. North Waziristan Agency at a Glance. From <<http://www.fata.gov.pk>> (Retrieved on 12 June 2015).
- Official Home Page of Pakistan Bureau of Statistics, Government of Pakistan 1998: Area & Population of Administrative Units: 1998 Census. From <<http://www.pbs.gov.pk>> (Retrieved on 15 June 2015).
- Pakistan Medical Research Council, KMC Peshawar 1976-1982. *Some Normal Parameters of Pakistanis (Peshawar Area)*. Pakistan, pp. 25-26.
- Reddy KK, Reddy KSN, Basha DA, Naik JL 2013. Distribution of ABO and RH (D) blood groups in Motati Reddi of Kadapa District, Andhra Pradesh. *Anthropologist*, 15(2): 239-240.
- Rehman AU, Ahmad I, Zaman M, Malik S 2015a. Transition in consanguinity in Dir Lower district, a victim of war, natural disaster and Population displacement, in north-west Pakistan – a response to Sthanadar et al. (2015). *J Biosoc Sci*, page 1 of 6 © Cambridge University Press, doi:10.1017/S0021932015000152.
- Rehman AU, Rashid A, Malik S 2015b. Genetic diversity at ABO and Rh (D) loci in the tribal groups of

- Mohmand Agency (Federally Administered Tribal Areas), Pakistan. *Anthropologist*, 19(3): 679-683.
- Rehman AU, Wahab ZU, Khattak MNK, Malik S 2014. ABO and Rh (D) blood groups polymorphism in four tehsils of Bajaur Agency (Federally Administered Tribal Areas), Pakistan. *Anthropologist*, 18(1): 259-261.
- Sachdev B 2012. Incidence of ABO and Rh (D) blood groups among Banjara, a nomadic group of Rajasthan, India. *Anthropologist*, 14(1): 87-88.
- Strickberger MW 2005. *Genetics*. 3<sup>rd</sup> Edition. New York: The Macmillan Company, pp. 669-687.
- Zafar NJ, Hasan K, Bukhari K 1997. Prevalence of ABO and Rh blood group amongst voluntary blood donors. *J Rawal Med Coll*, 1: 78-80.

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