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Evaluation of Tribal Diversity of Pashtuns of Bajaur Agency, North-West Pakistan, on the Basis of Allelic Polymorphisms at *ABO* and *Rh* Loci

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ABSTRACT

The present study was aimed at evaluating the tribal diversity of Pashtuns currently residing in Bajaur Agency, North-West Pakistan. The immunogenetic markers of *ABO* and *Rh* loci were phenotyped in 1200 individuals selected randomly from Bajaur. In these data, Tarkani and Uthman Khel emerged as the major tribes, while Safi and Shinwari were in minor representation. Additionally, there were at least 25 sub-tribes. Wide-variation was evident at the *ABO* and *Rh* allelic systems in the Tarkanis compared to the Uthman Khels. The heterozygosity at both loci was higher in the samples ascertained from Tarkanis in comparison to Uthman Khels. The estimation of Nei's genetic distances (DA) revealed close affinities between Umer Khel and Sarkani Khel tribes of Uthman Khels (DA=0.003), and between Salarzi and Miangan tribes of Tarkanis (DA=0.002). Moreover, the coefficient of gene differentiation (G_{ST}) and absolute gene diversity (D_{ST}) were higher in Tarkanis compared to those recorded for the Uthman Khels. Collectively, these data illustrated high variability in the Pashtun tribes of Bajaur in terms of phenotypic proportions, allelic frequencies and heterozygosites at the studied loci. Further studies employing highly polymorphic genetic markers are required to fully appreciate the diversification of these tribes and to understand the micro-evolutionary processes taking place therein.

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Authors' Contributions
SM conceived the study. AUR performed filed study and colleted data. SM analyzed the data. SM and AUR wrote the manuscript.

Key words
Pashtun tribes, Bajaur population, genetic distance matrix, ABO, Rh locus, blood groups, gene diversity, allelic polymorphism, polymorphic genetic markers.

INTRODUCTION

ABO and Rh blood group markers have been extensively characterized in human populations across the globe. Due to their polymorphic nature, they have been used to appreciate the affinities among tribes and sub-populations (Shami and Rasmuson, 1994). Easy typing, inexpensive nature and the acquisition of a large amount of data make these polymorphisms highly practical to use in population studies. Due to their low resolution, however, they have been used in combination with other polymorphic markers (Goicoechea *et al.*, 2000).

The *ABO* and *Rh* allelic systems have been studied in different populations of Pakistan (Shami and Rasmuson, 1994; Malik and Amin-ud-Din, 2013). From the Khyber Pakhtunkhwa (KPK) province, blood group polymorphisms have been reported for the population of Abbottabad (Khaliq *et al.*, 1984), Nowshera (Babar *et al.*, 1999), Swat (Khattak *et al.*, 2008), and Mohmand Agency (Rehman *et al.*, 2015b). Most of these studies report phenotypic or/and allelic polymorphisms for the whole sample without further elucidating the tribal/ethnic diversity of the population under study.

The Pashtun tribes inhabiting the Federally Administered Tribal Areas (FATA) of Pakistan are highly diverse on the ethnic grounds. The Bajaur population, like several other Pashtun offshoots, claim to be of Afghan origin (Afzaul, 2006). Traditionally, they prefer marital unions within the tribe (Khan and Samina, 2011). A recent population-based study in Bajaur Agency (BA) has shown that the rate of consanguinity was 22.34% resulting in an inbreeding coefficient F=0.0134 (Ahmad *et al.*, 2016). Due to certain unique circumstances and poor law-and-order conditions in the last decade, several Pashtun populations including the population of BA have undergone internal displacements and fragmentations (Ahmad *et al.*, 2016; Rehman *et al.*, 2014, 2015a). This situation may have resulted strong demographic changes in BA which deserves scientific investigations. Little is known about the tribal assemblage and genetic diversity of the Pashtuns currently residing in this region. To this end, we have carried out a preliminary study in BA and have witnessed the tribal diversity of Pashtuns residing therein.

SUBJECTS AND METHODS

A cross-sectional study was carried out from January 2010 to January 2011 in BA. A total of 1,200 healthy male volunteers were recruited from seven towns

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with the help of local resource persons, government officials and teachers. The serological phenotyping was performed by the procedure of forward typing and the test was repeated in case of ambiguity (Rehman *et al.*, 2014). The study was approved by the Ethical Review Committee of Quaid-i-Azam University, Islamabad.

The data were recorded in MS-Excel and analyses were carried out through MS-Excel and Graphpad Prism (Garstman, 2008). At the *ABO* locus, allele frequencies were estimated through the maximum likelihood method and the concordance with Hardy-Weinberg Equilibrium (HWE) was checked (Strickberger, 2005; Silva, 2002). Allelic frequencies at the *Rh* locus were calculated from the recessive phenotypes (Strickberger, 2005). Heterozygosities and their respective variances at both *ABO* and *Rh* loci were estimated as per Nei (1978). Z-test was employed to check the heterogeneity of blood group proportions among the investigated populations (Pagano and Gauvreau, 2000). G-test statistics was employed to assess the independence between categorical variables (Eyduran, 2008). Homogeneity was also assessed by the *ABO* and *Rh* gene frequencies (Neel and Schull, 1954). The sub-tribes with sample size ≥30 were grouped separately and were further compared with respect to the allelic variation and the differentiation of *ABO* and *Rh* loci (Nei, 1978). Genetic distances between the tribes/sub-tribes were estimated through the Nei's measure of D implemented in DISPAN (Nei and Roychoudhury, 1982; Ota, 1993). The degree of differentiation (G_{ST}) and absolute gene diversity (D_{ST}) at the studied loci were calculated (Nei, 1975, 1978). In order to appreciate the affinities among the tribal-systems, a genetic distance matrix was generated and the results were displayed through principal component analyses (PCA) (Peakall and Smouse, 2012).

RESULTS

Distribution of blood types

In the Bajaur sample, four major tribal groups were determined (Table I). Tarkanis and Uthman Khels had the largest representation comprising 62.5% (n=775) and 31.7% (n=380) of the total sample, respectively, but Safi and Mohmand appeared in marginal proportions. In the four major tribes, the phenotypic frequencies of blood types A, B, AB and O ranged in percentages from 27.3-38.5, 28.7-30.8, 9.1-15.4, and 15.4-34.7, respectively, while Rh– blood varied between 4.6-9.7.

More detailed analyses have been carried out on samples from the Tarkani and Uthman Khel tribes. In Tarkanis, the most prevalent blood group at the ABO system was type A (30.7%), followed by types 'B' (30.2%), O (27.7%), and AB (11.4%) (Table I). In

Table I.- Distribution of phenotypic proportions, allelic frequencies and heterozygosities at *ABO* and *Rh loci* in the major Pashtun tribes of BA

Major tribe	No.	ABO locus							HWE at ABO*	Rh locus				Heterozygosity		
		Phenotype proportions				Allele frequencies				Phenotype proportions		Allele frequencies				
		A	B	AB	O	p[A]	q[B]	r[O]		Rh+	Rh–	[D]	[d]	ABO	Rh	Pooled
Tarkani	775	30.7	30.2	11.4	27.7	0.239	0.235	0.526	0.017	91.10	8.90	0.702	0.298	0.611	0.419	0.516
Uthman Khel	380	27.4	28.7	9.2	34.7	0.203	0.211	0.586	0.273	90.26	9.74	0.688	0.312	0.572	0.430	0.502
Safi	22	27.3	36.4	9.1	27.3	0.204	0.263	0.533	0.094	95.45	4.55	0.787	0.213	0.619	0.343	0.493
Mohmand	13	38.5	30.8	15.4	15.4	0.324	0.269	0.407	0.063	92.31	7.69	0.723	0.277	0.683	0.417	0.573
Others	10	20.0	30.0	10.0	40.0	0.162	0.223	0.615	0.166	100.00	0.00	1.000	0.000	0.574	0.000	0.303
All	**1,200**	**29.4**	**30.0**	**10.5**	**30.1**	**0.225**	**0.231**	**0.544**	**0.203**	**91.43**	**8.57**	**0.707**	**0.293**	**0.598**	**0.420**	**0.509**

*all samples were in conformity with HWE

Uthman Khels, the predominant phenotype was determined as O (34.7%), followed by B (28.7%), A (27.4%), and AB (9.2%). At the Rh system, Rh-type was observed in 8.9% and 9.7% individuals, respectively, in the Tarkanis and Uthman Khels.

Homogeneity between samples

The Tarkani and Uthman Khel tribes were largely concordant at the phenotypic proportions of blood group systems. G-test of Goodness of fit employed at the ABO and Rh blood types revealed no statistical differences in their distributions in both major tribes (6.35; p=0.096 and 0.211, p=0.646, respectively).

Differences between the Tarkanis and Uthman Khels were evident at the allele frequency estimates at the studied loci. The results demonstrated that the frequencies of the alleles p[A] and q[B] were higher in Tarkanis whereas the alleles r[O] and Rh[d] were higher in Uthman Khels (Table I). Samples from the major tribes were in agreement with HWE expectations at the *ABO* locus. Combined estimates of heterozygosity were slightly higher in Tarkanis when compared to Uthman Khels (0.516 vs. 0.502). The overall heterozygosities at *ABO*, *Rh* and both loci were found to be 0.598, 0.420, and 0.509, respectively.

Sub-tribes in Bajaur Agency

The detailed data regarding 25 sub-tribes in the sample are presented in Table II. Tarkanis and Uthman Khels comprised fifteen and six sub-tribes, respectively. Among the sub-tribes, the frequencies of alleles p[A], q[B], r[O], and Rh[d] ranged from 0.061–0.349, 0.100–0.255, 0.328–0.382, and 0.0–0.447, respectively.

Several unusual observations were made in the sub-tribes. For instance, the lowest frequencies of alleles p[A], q[B], r[O], and Rh[d] were observed to be 0.061, 0.100, 0.328 and 0.169, in the Deganan (Tarkani), Ayengar (Tarkani), Sadat (Tarkani), and Others (Uthman Khel) tribes, respectively (excluding the null alleles). Additionally, the highest frequencies of these alleles were 0.410, 0.356, 0.710, and 0.447 observed in Sadat (Tarkani), Sayeds (Tarkani), Sarni Khel (Uthman Khel), and Gud Khel (Tarkani) tribes, respectively. This wide variation could be partially attributed to a relatively small sample size for several tribal systems. There were only two instances of deviation from HWE at the *ABO* locus, *i.e.*, Gud Khel and Sarni Khel. The deviation in Gud Khels could be due to small sample size (*i.e.*, n=20), very low representation of O blood type (*i.e.*, 20%), and the absence of blood type AB. In Uthman Khels, the deviation from HWE could be attributed to an unusually high representation of O blood type (*i.e.*, 60%).

Gene diversity analysis

There were five sub-tribes in both Tarkanis and Uthman Khels with sample size ≥30, and differentiation of *ABO* and *Rh* loci was assessed in both major tribes separately. Results showed that Tarkanis were more diversified and had higher estimates of heterozygosities than Uthman Khels (Table III). Coefficient of inter-population gene differentiation (G_{ST}) was higher in Tarkanis compared to Uthman Khels (0.02 vs. 0.019). Absolute gene diversity (D_{ST}) was also higher in Tarkanis compared to Uthman Khels (0.011 vs. 0.009). For the total samples, absolute gene diversity was 0.016 and gene differentiation was 0.032. Genetic diversity across all samples (H_T) was estimated to be 0.506, while diversity within the populations (H_S) was calculated to be 0.49.

Genetic distance matrix

Nei's genetic distances (DA) were calculated between the tribes, DA matrix was generated, and the results were displayed through PCA (Fig. 1). The highest affinities were evident between the samples of Umer Khel and Sarkani Khel tribes (D=0.0003). There were also close affinities between Salarzi and Miangan, and between Umar Khel and Miangan (D=0.0007 each). On the other hand, marked heterogeneity was evident between the samples ascertained from Ayengar and Sayeds (D=0.0395), and between Ayengar and Moliyan (D=0.0275).

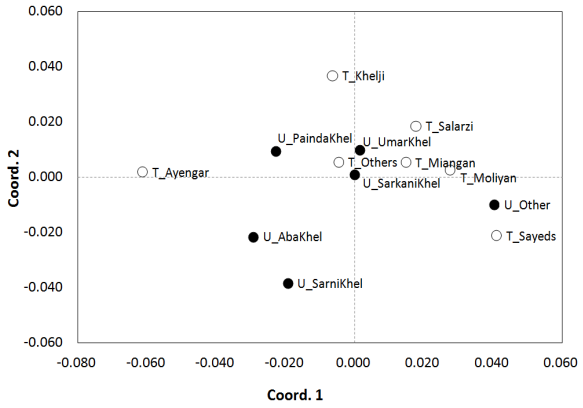

Fig. 1. Scatter plot depicting the output of Principal Component Analyses generated through DA matrix. Black dots represent sub-tribes of Uthman Khels and open dots show sub-tribes of Tarkanis.

DISCUSSION

Tarkani is the main tribe of FATA and BA. The Tarkanis also have a sizable population in Dir Lower, and

Table II.- **Allelic frequencies and heterozygosities at *ABO* and *Rh* loci in Pashtun sub-tribes of BA**

Castes		No.	ABO locus				Rh locus		Heterozygosity		
Major	Minor		p[A]	q[B]	r[O]	HWE Test	[D]	[d]	ABO	Rh	Average
Tarkani	Salarzi	110	0.252	0.283	0.464	0.88	0.730	0.270	0.643	0.396	0.522
Tarkani	Moliyan	79	0.208	0.283	0.509	2.60	0.775	0.225	0.621	0.351	0.489
Tarkani	Miangan	63	0.214	0.277	0.509	0.05	0.718	0.282	0.623	0.408	0.520
Tarkani	Khelji	43	0.344	0.213	0.443	1.64	0.695	0.305	0.648	0.429	0.545
Tarkani	Sayeds	32	0.114	0.356	0.530	1.34	0.750	0.250	0.588	0.381	0.493
Tarkani	Ayengar	31	0.277	0.100	0.622	1.45	0.560	0.440	0.534	0.501	0.526
Tarkani	Deganan	25	0.061	0.274	0.665	2.39	0.717	0.283	0.489	0.414	0.461
Tarkani	Mamond	22	0.262	0.174	0.564	0.00	0.631	0.369	0.597	0.477	0.549
Tarkani	Mandori	22	0.348	0.223	0.429	1.44	0.699	0.302	0.660	0.431	0.558
Tarkani	Paracha	21	0.246	0.128	0.627	0.12	0.622	0.378	0.544	0.482	0.526
Tarkani	Gud Khel	20	0.237	0.237	0.527	***3.85****	0.553	0.447	0.626	0.507	0.582
Tarkani	Yousaf Khel	20	0.190	0.253	0.558	1.01	1.000	0.000	0.604	0.000	0.310
Tarkani	Qaziyan	15	0.383	0.191	0.426	1.30	0.635	0.365	0.657	0.480	0.589
Tarkani	Sadat	15	0.410	0.262	0.328	0.43	1.000	0.000	0.678	0.000	0.351
Tarkani	Sheikhan	14	0.199	0.199	0.602	0.02	0.622	0.378	0.579	0.488	0.554
Tarkani	Others	243	0.244	0.212	0.545	0.00	0.699	0.301	0.601	0.422	0.512
Uthman Khel	Sarkani Khel	73	0.218	0.236	0.546	1.40	0.690	0.310	0.603	0.431	0.520
Uthman Khel	Painda Khel	61	0.254	0.201	0.546	0.01	0.616	0.384	0.603	0.477	0.544
Uthman Khel	Umar Khel	60	0.238	0.249	0.513	0.31	0.684	0.316	0.624	0.436	0.534
Uthman Khel	Aba Khel	54	0.185	0.151	0.664	0.50	0.640	0.360	0.507	0.465	0.491
Uthman Khel	Sarni Khel	43	0.145	0.145	0.710	***20.43****	0.695	0.305	0.459	0.429	0.449
Uthman Khel	Zargaran	19	0.173	0.141	0.686	0.01	0.676	0.324	0.493	0.450	0.485
Uthman Khel	Others	70	0.180	0.281	0.539	0.21	0.831	0.169	0.602	0.283	0.446
Mohmand	All	13	0.324	0.269	0.407	0.06	0.723	0.277	0.683	0.417	0.573
Safi	Shinwari	22	0.204	0.263	0.533	0.09	0.787	0.213	0.619	0.343	0.493
Others	Minor groups	10	0.162	0.223	0.615	0.17	1.000	0.000	0.574	0.000	0.303
Coefficient of variance (%)			**34.51**	**26.42**	**16.98**		**16.82**	**43.46**	**9.94**	**39.21**	**15.10**

* samples not in conformity with the Hardy-Weinberg equilibrium are shown in bold-face and italics.

Table III.- Gene diversity analysis for *ABO* and *Rh* loci in Bajaur tribes (n>30).

Population	Locus	H_T	H_S	D_{ST}	G_{ST}
Tarkanis	ABO	0.616	0.603	0.013	0.021
	Rh	0.417	0.409	0.008	0.020
	Pooled	0.516	0.506	0.011	0.020
Uthman Khels	ABO	0.571	0.561	0.009	0.016
	Rh	0.426	0.416	0.009	0.022
	Pooled	0.498	0.489	0.009	0.019
All tribes	ABO	0.595	0.582	0.013	0.022
	Rh	0.416	0.398	0.019	0.045
	Pooled	0.506	0.490	0.016	0.032

Marawara and Shortan areas of Kunar, Afghanistan. Uthman Khel, the second largest tribe in BA, is also distributed in Dir Lower, Malakand Agency, Mohmand Agency and some areas of Mardan (FATA, 2010). Safi and Shinwari tribes, which have minor representation in BA, also claim their origin from the Kunar area of Afghanistan (Afzaul, 2006).

With the aim of understanding the tribal diversity of Bajaur population, we have employed *ABO* and *Rh* markers. Substantial variability was observed at the phenotypic and allelic frequencies among the Bajaur tribes. Despite the marked demographic transitions in BA in the last decade the allelic frequencies in most of the tribes/sub-tribes were in conformity with the HWE. The comparison of the Tarkani and Uthman Khel samples demonstrated that there were low heterozygosities in the Uthman Khels. One of the likely reasons for this reduced heterozygosity could be inbreeding in this population (Ahmad *et al*., 2016). Furthermore, estimates of coefficient of gene differentiation (G_{ST}) for only the Tarkani and Uthman Khel tribes were relatively low (0.02 and 0.019, respectively). However, it was observed to be elevated when all tribes were considered (*i.e*., 0.032), depicting only a minor loss of heterozygosity in sub-populations (H_T =0.506 vs. H_S =0.490). A recent study showed that coefficient of gene differentiation at *ABO* and *Rh* loci was found much lower in the populations of Upper KPK compared to BA (Ali and Malik, 2015).

The major tribes of BA were compared with neighboring Pashtun populations on the basis of allelic frequencies (Ali and Malik, 2015). The sample from Tarkanis depicted affinities with Dir-Lower and Swat populations, while Uthman Khel tribe showed affinities with the Dir-Lower population only. These similarities could be due to the migration of Tarkanis and Uthman Khels tribes from BA to Dir-Lower and Swat districts in the recent past. Nonetheless, it was anticipated that grouping among the sub-tribes of Tarkanis and Uthman Khels might be possible. However, the clustering of sub-tribes of Tarkanis and Uthman Khels based upon the genetic distance matrix gave perplexing results, *i.e.*, certain sub-tribes of Tarkanis demonstrated high affinities with sub-tribes of Uthman Khels and clusters within the main tribes could not be established (Fig. 1). It is quite likely that despite the distinct tribal identities the apparent affinities between sub-tribes are meaningful. Alternatively, more informative genetic markers would be required to further resolve the sub-tribes.

These results depicted that there was more variance at *Rh* allelic system compared with the *ABO*. A previous study demonstrated that in central and southern Pakistan, the frequency of q[B] allele remained much higher than allele p[A] (Malik and Amin-ud-Din, 2013). Further in north-western populations like BA, both these alleles were found to be almost equally prevalent (Ali and Malik, 2015). It would be very interesting to observe the clinal and geographic trends of these alleles in the Pakistani populations in a prospective study.

The present investigation has several limitations. First, only the male individuals were recruited in this study. Second, the sample size of various sub-tribes is very small, thus limiting the use of many statistical inferences. Third, the low resolution power of the investigated serological markers cannot reveal detailed micro-evolutionary events occurring in a population. However, as a reference these data could be useful in future studies. The present study witnessed substantial diversity in the Pashtun tribes of BA and gave a clue to considerable differentiation and substructuring at the sub-tribal level which could be due to population displacement. In a prospective study it would be worthwhile to observe the genetic structure of all the tribal populations of FATA through highly polymorphic markers and to compare them with adjoining Afghan populations.

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Conflict of interest statement

None declared.

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