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Enzyme Variation Among the Muslim Community of Punjab

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INTRODUCTION

Genetic polymorphisms provide us with pointers to the variation of specific chromosome segments. In this sense the polymorphisms are genetic markers and thus our door to the understanding and measurement of genetic variation. They can be studied from any cell or biological fluid but blood is by far the favourite material because it is most easily obtained and gives the greatest opportunities for detecting them.

Proteins active as specific catalysts in biochemical reactions i.e. enzymes are usually present in the blood in small concentrations. When it was learned how to uncover them through very sensitive and specific staining reactions (Hunter and Markert, 1957), enzymes provided the first statistical evidence that polymorphisms are much more common than earlier believed. Both protein and enzyme polymorphism data in human populations have been used to characterize them in terms of allele frequencies (Mourant et al., 1976; Bhasin et al., 1992a). Furthermore, such data are also useful in studying the history and geography of human genes (Cavalli-Sforza et al., 1994).

A review of literature reveals that only a handful Muslim populations have been investigated in India for genetic markers, including the polymorphisms of the red cell enzymes. These include those inhabiting Pulwama (Chahal et al., 1989) and Srinagar (Bhasin et al., 1992b) districts of north Indian state of Jammu and Kashmir, Lucknow in Uttar Pradesh (Lanchbury et al., 1992), Delhi (Papiha et al., 1976; Ghosh, 1977), Surat in Gujarat (Papiha et al., 1981), Indore in Madhya Pradesh (Roberts et al., 1974), Hyderabad in Andhra Pradesh (Roberts et al., 1980; Kumar and Rao, 1982), North Kanara district in Karnataka (Vijayakumar et al., 1987), Calicut in Kerala (Saha et al., 1976), Calcutta in West Bengal (Mukherjee et al., 1974) and Guwahati in Assam (Mukherjee et al., 1989). However, in view of large number of the Muslims in India, constituting about 12% of the country's population (Census, 1991), these studies are clearly still inadequate to characterize this religious minority community fully and accurately.

As for the people of the North-West Indian state of Punjab, the bulk of its population falls into two major religious communities viz., the Hindus and Sikhs; the Muslims and Christians are in minority. From this border state, data pertaining to the distribution of biochemical genetic markers are essentially limited to various caste populations of the former two (Bhasin et al., 1992a) and except for a solitary G-6-PD deficiency report on the Muslims (Bansal et al., 1976) the minorities have been totally neglected.

It is therefore that this detailed genetic investigation based on red cell enzyme markers was planned from Malerkotla town of Sangrur district where nearly 80% of the population is comprised by the Muslims, with a larger number of Sunnis than Shias. The main aim is to provide genetic profile and baseline date for this minority community of Punjab using biochemical information from as many as seven different red cell enzyme polymorphisms.

MATERIAL AND METHODS

For this work blood samples (about 1 ml each) from a total of 992 apparently healthy not closely related Muslim subjects of the two endogamous sects of this religious community viz., the Sunni (803) and Shia (189) were collected in batches. The sampling was done from the Government College, Malerkotla; Islamia Senior Secondary School, Malerkotla; Islamia Girls High School, Malerkotla; City School, Azimpura as well as Khojia and Azimpura Mohallas of the Malerkotla town. The samples were transported on the same day to Patiala personally for laboratory analyses.

Haemolysates, prepared by freezing and thawing technique, were electrophoresed for studying variabilities of different red cell enzymes in the present Muslim material. Barring Glyoxalase locus 1, for which mixed agarose-starch gel electrophoresis was performed (Scott and Fowler, 1982), typings of other six red cell enzyme systems included in this study were done by

horizontal starch gel electrophoresis following the original techniques of Hopkinson et al. (1963) for Acid Phosphatase locus 1 (ACP1), Spencer et al. (1964) for Phosphoglucomutase locus 1 (PGM1), Fildes and Harris (1966) for Adenylate Kinase locus 1 (AK1), Spencer et al. (1968) for Adenosine Deaminase (ADA), Detter et al. (1968) for Phosphohexose Isomerase (PHI) and Hopkinson et al. (1973) for Esterase D (ESD).

From phenotype data allele frequencies were calculated using the gene counting method (Mourant et al., 1976). Chi-square tests were applied both to check deviations from the Hardy-Weinberg equilibrium and to seek heterogeneity between the two studied Muslim samples for the enzyme marker distributions. Genic differentiation was studied following Nei (1973) and Wahlund (1928), and genetic relationships following Nei (1972).

RESULTS AND DISCUSSION

Table 1 shows phenotype distribution of red cell enzymes ACP1, PGM1, AK1, ADA, PHI, ESD and GLOI in the Sunni and Shia Muslims of Punjab, and Table 2 their allele frequencies. Goodness of fit Chi-square test revealed that barring one instance (ACP1 in the Sunnis), the distribution of phenotypes in each of the enzyme systems typed was in genetic equilibrium in both the Muslim sects studied.

Table 1: Phenotype distribution of various red cell enzyme systems in the Muslims of Punjab

System	*Phenotype*	*Sunni Muslims (n = 803)*	*Shia Muslims (n = 189)*
ACP1			
	ACP1 A	111	22
	ACP1 A,B	327	79
	ACP1 B	362	83
	ACP1 A,C	2	1
	ACP1 B,C	1	4
	χ^2	6.37*	0.01
PGM1			
	PGM1 1	432	93
	PGM1 1,2	301	80
	PGM1 2	69	15
	PGM1 1,3	-	1
	χ^2	2.51	0.21
AK1			
	AK1 1	626	153
	AK1 1,2	165	34
	AK1 2	12	2
	χ^2	0.09	0.01
ADA			
	ADA 1	656	160
	ADA 1,2	143	29
	ADA 2	4	-
	χ^2	1.65	1.31
PHI			
	PHI 1	800	188
	PHI 1,3	2	1
	PHI 1,5	1	-
	χ^2	0.00	0.00
ESD			
	ESD 1	450	128
	ESD 1,2	301	50
	ESD 2	52	11
	χ^2	0.03	3.82
GLO1			
	GLO1 1	65	8
	GLO1 1,2	302	68
	GLO1 2	436	113
	χ^2	1.53	0.31

* d.f. = 1, 0.05>p>0.025

Acid Phosphatase Locus 1 (ACP1)

In the two sects of the present Muslim material from Punjab, the frequencies of three common alleles of the system viz. *ACP1*A, ACP1*B* and *ACP1*C* were found to be almost identical (Table 2). In fact, the Chi-square test revealed that both these sects are genetically close (χ^2 = 1.00, d.f. 2, 0.70>p>0.50). The incidence of *ACP1*A* in the Sunni Muslims of the present study (0.3431) is one of the highest reported among the Indian Muslim populations.

For comparison, data are available from 11 Muslim samples from as many states of India, providing a range of 0.206 (Muslims of Kerala; Saha et al., 1976) to 0.328 (Muslims of Madhya Pradesh; Roberts *et al*., 1974). The *ACP1*A* frequencies observed in the present Sunni (0.3431) and Shia (0.3280) Muslims are very similar to the Sunni Muslims of Jammu and Kashmir (0.320; Chahal et al., 1989) and Muslims of Madhya Pradesh (0.328) as well as Andhra Pradesh (0.320; Roberts et al., 1980).

Phosphoglucomutase Locus 1 (PGM1)

The frequency of the *PGM1*2* allele was found to be 0.2737 in the Sunni and 0.2910 in the Shia Muslims of Punjab (Table 2). In addition in the latter sample a rare allele of the system viz., *PGM1*3* was also encountered. Like the ACP1 system, the typing of PGM1 system in the present Muslim sects demonstrate that they are genetically homogeneous (χ^2 = 1.83, d.f. 2, 0.50>p>0.30).

Table 2: Allele frequencies of enzyme systems investigated in the Muslims of Punjab

System	*Allele*	*Sunni Muslims*	*Shia Muslims*
ACP1			
	*ACP1*A*	0.3431	0.3280
	*ACP1*B*	0.6550	0.6587
	*ACP1*C*	0.0019	0.0132
PGM1			
	*PGM1*1*	0.7263	0.7064
	*PGM1*2*	0.2737	0.2910
	*PGM1*3*	-	0.0026
AK1			
	*AK1*1*	0.8823	0.8995
	*AK1*2*	0.1177	0.1005
ADA			
	*ADA*1*	0.9060	0.9233
	*ADA*2*	0.0940	0.0767
PHI			
	*PHI*1*	0.9981	0.9974
	*PHI*3*	0.0013	0.0026
	*PHI*5*	0.0006	-
ESD			
	*ESD*1*	0.7478	0.8095
	*ESD*2*	0.2522	0.1905
GLO1			
	*GLO1*1*	0.2690	0.2222
	*GLO1*2*	0.7310	0.7778

In different Muslim populations studied from India the *PGM1*2* allele varies from 0.252 in Delhi (Papiha et al., 1976) to 0.363 in Jammu and Kashmir (Chahal et al., 1989). The present values fall well within this range and in particular show similarities with the Muslims of Jammu and Kashmir (0.275; Bhasin *et al*., 1992b), Uttar Pradesh (0.273; Lanchbury et al., 1996) and Madhya Pradesh (0.274; Roberts *et al*., 1974).

Although earlier rare alleles of this enzyme system such as *PGM1*5* (Papiha et al., 1976; Roberts et al., 1980) and *PGM1*7* (Papiha et al., 1976, 1981; Chahal et al., 1989; Bhasin et al., 1992b) have been encountered in the Muslims of India, this is the first report on the presence of the rare allele *PGM1*3* in them.

Adenylate Kinase Locus 1 (AK1)

In the present Sunni and Shia Muslim samples from Punjab *AK1*2* frequencies were observed 0.1177 and 0.1005, respectively (Table 2). Like in the previous two enzyme markers, the comparison of phenotypic distribution of AK1 phenotypes in these two sects did not reveal any significant genetic differences ($\chi^2 = 0.88$, d.f. 2, $0.70>p>0.60$).

In earlier studies from different other states the incidence of *AK1*2* has been reported from a low of 0.062 in the Sunni Muslims of Jammu and Kashmir (Chahal et al., 1989) to 0.120 in Muslims of Kerala (Saha et al., 1976). The *AK1*2* values observed in the present Muslim samples from Punjab are clearly on the higher side of this range.

Adenosine Deaminase (ADA)

The *ADA*2* frequency was recorded 0.0940 in the Sunni and 0.0767 in Shia Muslims of Punjab (Table 2). When compared statistically, these two sects showed non-significant differences in this enzyme system also ($\chi^2 = 1.64$, d.f. 2, $0.50>p>0.40$).

The *ADA*2* allele has shown a wide range of distribution in different Muslim populations of India, varying from 0.091 in Uttar Pradesh (Lanchbury et al., 1996) to a surprisingly high value of 0.500 in Assam (Mukherjee et al., 1989). Thus, the incidence of this allele recorded in the present Shia Muslims (0.0767) is the lowest so far reported in this largest religious minority community of India.

Phosphohexose Isomerase (PHI)

In addition to the common phenotype PHI 1, rare variant phenotype 1,3 of this enzyme was encountered in both the sects of the present Muslim material from Punjab, while an another rare variant 1,5 was found only in the Sunni sample (Table 1). In this enzyme system also the two Muslim sects of Punjab failed to reveal any statistically significant differences ($\chi^2 = 0.092$, d.f. 1, $0.80>p>0.70$).

The distribution of PHI demonstrates that the Muslims of Jammu and Kashmir (Bhasin et al., 1992b), Gujarat (Papiha et al., 1981) and Andhra Pradesh (Roberts et al., 1980) lacked any variant, while in each of the remaining 5 samples reported, the rare phenotype of the enzyme 1,3 was present. The detection of this variant in both the Muslim sects of Punjab was therefore in agreement with earlier observations among the Muslims of India. Another variant PHI 1,9 was found in the Muslims of Delhi by Papiha et al. (1976). It is therefore interesting to note that an example of PHI 1,5 variant observed in the present material is the first report of this rare phenotype in the Muslims of

India as well as in people of Punjab.

Esterase D (ESD)

The frequency of the *ESD*2* allele was observed 0.2522 in the Sunni and 0.1905 in the Shia Muslims of Punjab (Table 2). This is the only enzyme system of the seven investigated in which statistical treatment demonstrated significant differences between the two endogamous Muslim sects of the present study ($\chi^2 = 8.95$, d.f. 2, $0.02>p>0.01$).

In Muslim populations inhabiting different states of India, the *ESD*2* frequency has been reported in a range of 0.185 (Muslims of Gujarat; Papiha et al., 1981) to 0.293 (Muslims of Assam; Mukherjee et al., 1989). The values of the allele observed in the Muslim samples of Punjab in this study are thus well within this range.

Glyoxalase Locus 1 (GLO1)

Allele frequency data of red cell enzyme GLO1 in this study reveal that the Sunni sect has a higher incidence of the *GLO1*1* (0.2690) than the Shia sect (0.2222). However, once again inter-group Chi-square comparison between these two endogamous Muslim sects of Punjab was not found to be statistically significant ($\chi^2 = 4.04$, d.f. 2, $0.20>p>0.10$).

Like other Indian populations, GLO1 system has been sparingly studied among the Muslims of India and only four reports are available. From Jammu and Kashmir, in the Sunni Muslims (Ahmidiyya) of Pulwama district, Chahal et al. (1989) reported a frequency of 0.297 for the *GLO1*1* allele and in the Muslims of Srinagar district it was found comparatively low (0.205; Bhasin et al., 1992b). In a small sample of Delhi Muslims, Ghosh (1977) estimated the frequency of this allele 0.303, while in a representative sample from Hyderabad (Andhra Pradesh) it was reported still higher (0.327; Kumar and Rao, 1982). The values of the *GLO1*1* found in the two sects from Punjab in this study fit well into the range provided by these few studies available on the Muslims of India for GLO1 polymorphism.

Thus the present enzyme variation study demonstrates that barring ESD system, there were no significant differences in the distribution of other enzyme polymorphisms between the Sunni and Shia endogamous sects of the Muslims inhabiting Malerkotla. Similarities in allele frequencies of the biochemical markers studied here from Punjab with other Muslim populations reported from different other states of India were also evident.

Using the present enzyme allele frequency data from two sects of the Muslims of Punjab and that available uniformly for the 7 enzymes for four other Muslim populations of other states of India, an attempt has been made in the following to study their genetic structure.

Genetic Differentiation

Genetic differentiation was estimated using G_{ST}, a measure proposed by Nei (1973), and f proposed by Wahlund (1928). The calculation of values of these two different indices incidentally yielded the same figures, both for the present two sects (0.002) as well as for various Muslim populations of India (0.021). This demonstrates that the extent of genic differentiation in two endogamous sects of the Muslims of Punjab is only about one tenth that of the different Muslim populations of India. This suggests genetic homogeneity in the Muslim groups of Punjab.

Genetic Distance

Genetic affinities among the Muslim populations inhabiting different states of India were assessed by calculating Nei's standard genetic distance (D) and constructing a dendrogram based on the D values (Fig. 1). This figure shows that the two Muslim groups of Jammu and Kashmir differentiate themselves at early stages from other Muslim populations of India. The present two Muslim sects from Punjab are placed in a single sub-cluster and the Muslims of Delhi alongwith those of Andhra Pradesh form an another sub-cluster.

Thus while on one hand genetic constitution of both the Muslim samples reported from north Indian state of Jammu and Kashmir appear to be quite different from that of other Muslim populations of India, genetic similarities between the present two endogamous Muslim sects of Punjab are also equally evident from genetic distance analysis. Furthermore, the Muslims from Punjab were found to be genetically closer to the Muslims of Delhi and Andhra Pradesh than that of Jammu and Kashmir.

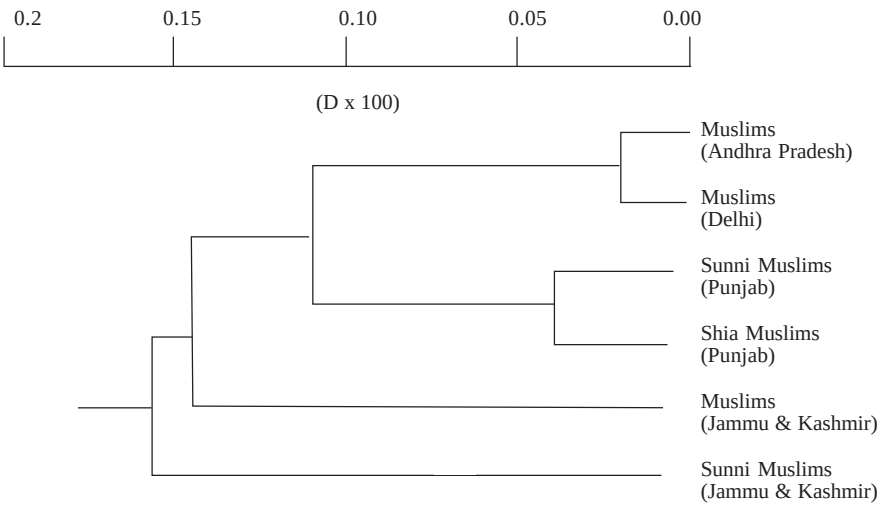

Fig.1. Dendrogram showing genetic relationships among different Muslim populations of India.

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KEYWORDS Genetic Markers. Red Cell Enzymes. Polymorphism. Community.

ABSTRACT To characterize genetically the Muslim population of Punjab, a north–west Indian state, a biochemical genetics investigation using red cell enzyme markers was planned. For this, blood samples from a total of 992 Muslim subjects, belonging to the Sunni (803) and Shia (189) endogamous sects inhabiting Malerkotla town were collected. Haemolysates were analysed for the phenotypes of seven different polymorphic red cell enzymes viz., Acid Phosphatase locus 1, Phosphoglucomutase locus 1, Adenylate Kinase locus 1, Adenosine Deaminase, Phosphohexose Isomerase, Esterase D and Glyoxalase locus 1, following standard electrophoretic techniques. The results of the present study demonstrate that there were no significant genetic differences between the Sunni and Shia Muslim populations of Malerkotla and their genetic constitutions were similar. Compared with their counterparts reported from various other states of India, the two Muslim groups studied here from Punjab were found to be genetically homogeneous with most of them .

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