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Erythrocyte Enzyme Variation in Brahmin and Rajput Populations of Himachal Pradesh. I. Shimla District

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INTRODUCTION

Because of their very diverse ethno-history, the contemporary tribal and non-tribal (caste) populations of the north-west Indian hill state of Himachal Pradesh have long interested physical anthropologists and these have been studied for various genetical, morphological and behavioural traits (Bhasin et al., 1992).

As for the biochemical genetics data on people of Himachal Pradesh, the earliest report was by Papiha et al. (1980), rapidly followed by studies of Singh et al. (1982), Chahal et al. (1982), Papiha et al. (1982), Bhasin et al. (1983) and Papiha et al, (1984). In recent years, Chahal et al. (1991), Walter et al. (1993) and Papiha et al. (1996) have provided additional such data, mostly on some non-tribal populations of the state. It is interesting to note that in the initial investigations of the early 1980's the emphasis had been on tribal populations, viz., the Gaddi, Pangwala, Kanet, Swangla and Bodh of Chamba, Kangra, Kinnaur and Lahaul-Spiti districts situated mostly in highlands of northern Himachal Pradesh. On the other hand, such studies on the majority caste populations, especially from southern lowlands of the state are almost negligible.

It is therefore, that as a part of a larger study plan on genetic variation in people of Himachal Pradesh, the distribution of various erythrocyte (red cell) enzyme polymorphisms was investigated in the Brahmin and Rajput, two predominant caste populations of the state, inhabiting seven different southern districts viz., Una, Hamirpur, Mandi, Bilaspur, Solan, Shimla and Sirmaur. Results from Shimla district are presented here, in the first instance.

SUBJECTS, MATERIAL AND METHODS

For the present biochemical study, the subjects were selected at random from among local boys and girls born and brought up in the Shimla district of Himachal Pradesh and attending various educational institutes near Shimla town. A total of 302 finger-prick blood samples (about 1 ml each) from the Brahmin (n=111) and Rajput (n=191) castes was collected in vials containing EDTA.K_2. No closely related subject was considered. The samples were temporarily stored in a refrigerator during few days of field collections and thereafter immediately transported in an ice-cooled thermocol container to Patiala (Punjab) for analysis.

On their arrival in the laboratory at Patiala, whole blood samples were transferred into glass tubes and centrifuged to remove plasma. The red cells were washed thrice with normal saline and converted into haemolysates by the freezing and thawing method (Bhasin and Chahal, 1996). All 302 haemolysates were stored at -20°C in a freezer and all of them could be typed within few weeks for phenotypes of seven different polymorphic enzyme systems, following standard electrophoretic techniques.

In this study isozymes of adenosine deaminase (ADA) and adenylate kinase (AK1) were simultaneously separated and stained on the same agarose gel as described by Murch et al. (1986). Similarly, haemolysates were electrophoresed for red cell enzymes esterase D (ESD) and phosphoglucomutase (PGM1) together on one agarose gel and stained sequentially as detailed in Wraxall and Stolorow (1986). Acid phosphatase (ACP1) was typed following Wraxall and Emes (1976), with a modification in the technique i.e. a low EEO agarose (Litex HSC) replaced starch as a medium of separation. Mixed agarose/ starch gel electrophoretic technique of Scott and Fowler (1982) was used for glyoxalase (GLO1) typing. Glucosephosphate isomerase (GPI) variants were characterized by thick starch gel electrophoresis (Detter et al., 1968), albeit following staining procedure of Papiha and Chahal (1984), which essentially

is a modification of the original method.

From phenotype data thus obtained for different polymorphic enzyme systems, allele frequencies were calculated by the gene counting method (Mourant et al., 1976). Using the Chi-square (χ^2) test, deviations from expected Hardy-Weinberg equilibrium proportions in each system as well as differences in various inter-population comparisons were checked for statistical significance.

RESULTS AND DISCUSSION

The observed and expected phenotype numbers together with the Chi-square values for the seven investigated erythrocyte enzyme systems in the Brahmin and Rajput populations of Shimla district are given in table 1; the corresponding allele frequencies are presented in table 2. In either caste sample there was no significant departure from the genetic equilibrium in any of the seven systems considered. In fact, barring ESD (χ^2=9.1866, d.f. 2, $0.02 > p > 0.01$), the distribution of the remaining six enzyme systems was found to be homogeneous in the Brahmin and Rajput populations of Shimla district.

For comparison, data from Himachal Pradesh are available for 5 Brahmin and as many as 10 Rajput population samples, studied from Kangra, Chamba and Kulu districts (Singh et al., 1982;

Table 1: Distribution of erythrocyte enzyme phenotypes among the Brahmin and Rajput of Shimla district

System	*Phenotype*	*Brahmin*		*Rajput*	
		Observed Number	*Expected Number*	*Observed Number*	*Expected Number*
ADA	1	86	86.52	162	162.18
	1,2	24	22.95	28	27.64
	2	1	1.52	1	1.18
	Total	111	111.00	191	191.00
	χ^2		0.23		0.03
AK1	1	95	95.58	160	159.43
	1,2	16	14.85	29	30.15
	2	0	0.58	2	1.43
	Total	111	111.00	191	191.01
	χ^2		0.67		0.28
ESD	1	75	74.60	98	94.71
	1,2	32	32.80	73	79.59
	2	4	3.60	20	16.71
	Total	111	111.00	191	191.01
	χ^2		0.06		1.30
PGM1	1	48	51.35	109	109.32
	1,2	55	48.29	70	69.60
	2	8	11.35	11	11.08
	1,7	0	0.00	1	0.76
	Total	111	111.00	191	190.76
	χ^2		2.14		0.08
ACP1	A	6	6.81	12	11.32
	A,B	42	41.13	69	70.12
	B	62	62.06	109	108.57
	A,C	1	0.25	0	0.24
	B,C	0	0.75	1	0.75
	Total	111	111.00	191	191.00
	χ^2		2.40		0.14
GLO1	1	7	4.56	3	4.71
	1,2	31	35.88	54	50.58
	2	73	70.56	134	135.71
	Total	111	111.00	191	191.00
	χ^2		2.05		0.87
GPI	1	110	110.00	190	190.00
	1,3	1	1.00	1	1.00
	Total	111	111.00	191	191.00
	χ^2		0.00		0.00

Table 2: Allele frequencies of different polymorphic erythrocyte enzymes among the Brahmin and Rajput of Shimla district

System	*Allele*	*Brahmin*	*Rajput*
ADA	*ADA*1*	0.8829	0.9215
	*ADA*2*	0.1171	0.0785
AK1	*AK1*1*	0.9279	0.9136
	*AK1*2*	0.0721	0.0864
ESD	*ESD*1*	0.8198	0.7042
	*ESD*2*	0.1802	0.2958
PGM1	*PGM1*1*	0.6802	0.7565
	*PGM1*2*	0.3198	0.2408
	*PGM1*7*	-	0.0026
ACP1	*ACP1*A*	0.2477	0.2435
	*ACP1*B*	0.7477	0.7539
	*ACP1*C*	0.0045	0.0026
GLO1	*GLO1*1*	0.2027	0.1571
	*GLO1*2*	0.7973	0.8429
GPI	*GPI*1*	0.9955	0.9974
	*GPI*3*	0.0045	0.0026

Chahal et al., 1982; Bhasin et al., 1983; Papiha and Chahal, 1984; Chahal et al., 1991; Papiha et al., 1982, 1996).

In the adenosine deaminase (ADA) system, the *ADA*2* allele showed a frequency of 0.1171 in the Brahmin and a comparatively low value (0.0785) in the Rajput. In other Brahmin samples reported from Himachal Pradesh the range of this allele is from as low as 0.049 in the Brahmin of Kulu district to 0.081 in the Brahmin of Kangra district. As for the Rajput, the range is from 0.032 reported in the Gaddi Rajput to 0.149 in the Rajput, both of Chuwari in Chamba district. Thus, while the frequency of the *ADA*2* allele recorded here in the Rajput of Shimla district falls well within the already reported range for this caste of Himachal Pradesh, the present sample of the Brahmin clearly showed a frequency highest so far reported among the Brahmin of the state. Compared with other Rajput subpopulations studied from Himachal Pradesh, the Rajput of Shimla district were significantly different only from their counterparts inhabiting Chuwari in Chamba district (χ^2=8.8328, d.f. 2, $0.02 > p > 0.01$). On the other hand, no such differences were found between the present Brahmin subpopulation and those reported earlier for this system.

The frequency of the *AK1*2* allele of the adenylate kinase (AK1) system was recorded 0.0721 in the Brahmin and 0.0864 in the Rajput of Shimla district. The available data for this polymorphic enzyme system from Himachal Pradesh show that this allele ranges from 0.075 (Gaddi Brahmin of Bharmour, Chamba district) to 0.132 (Brahmin of Chamba town, Chamba district) for various Brahmin subpopulations and from 0.055 (Gaddi Rajput of Palampur, Kangra district) to 0.113 (Rajput of Churah, Chamba district) for various Rajput subpopulations. While the *AK1*2* value for the present Rajput sample falls well within the range reported for this caste of Himachal Pradesh, that for the present Brahmin was the lowest so for reported among the Brahmin samples of the state. No inter-group χ^2 comparison, either among the Brahmin or among the Rajput subpopulations of Himachal Pradesh was statistically significant, suggesting homogeneity among various regional groups of the Brahmin on one hand and the Rajput on the other for the AK1 system in the state.

In the esterase D (ESD) system, the Brahmin of Shimla district showed a frequency of 0.1802 and the Rajput 0.2958 for the *ESD*2* allele. In Himachal Pradesh, the allele ranges from 0.179 (Gaddi Brahmin of Bharmour, Chamba district) to 0.262 (Brahmin, Kangra district) in the Brahmin and from a low of 0.124 (Gaddi Rajput of Bharmour, Chamba district) to 0.325 (Gaddi Rajput of Chuwari, Chamba district) among the Rajput subpopulations. Thus the ESD allele frequencies observed in the present study in both the Brahmin and Rajput samples from Shimla district fit well within the respective ranges for these two caste populations reported earlier from the state. Compared with other Brahmin subpopulations of Himachal Pradesh, the present Brahmin sample investigated from Shimla district showed statistically significant differences only with the Brahmin of Kangra district (χ^2= 7.331, d.f. 2, $0.05 > p > 0.02$). The present Rajput showed such differences with the Gaddi Rajput of Bharmour in Chamba district (χ^2=22.379, d.f. 2, $p < 0.001$), the Rajput of Chamba town in Chamba district (χ^2=6.1688, d.f. 2, $0.05 > p > 0.02$) and with the Gaddi Rajput of Kangra district (χ^2=6.7927, d.f. 2, $0.05 > p > 0.02$).

As for the phosphoglucomutase (PGM1) system, in addition to the three common phenotypes, an example of a rare variant PGM1 1,7 was found in the Rajput sample of this study. For the

*PGM1*2* allele, the present material from Shimla district showed a value of 0.3198 in the Brahmin and 0.2408 in the Rajput. Elsewhere in Himachal Pradesh, the frequency of this allele has been reported between 0.229 among the Gaddi Brahmin of Bharmour in Chamba district and 0.314 among the Brahmin of Kangra district. Among the Rajput samples of the state it varies from 0.207 in the Gaddi Rajput of Bharmour in Chamba district to 0.290 in the Gaddi Rajput of Kangra district. Thus, it is clear that the frequency of the *PGM1*2* observed in the Brahmin of Shimla district was the highest so far recorded in this caste of Himachal Pradesh, while that of the Rajput fits well within the range recorded in the Rajput samples of the state. Like in the AK1 system, in the PGM1 system also, statistical comparisons with other subpopulations of the Brahmin or Rajput reported earlier from different other districts of Himachal Pradesh revealed non-significant differences respectively with the present Brahmin and Rajput samples from Shimla district.

In the polymorphic enzyme system acid phosphatase (ACP1), for the *ACP1*A* allele the Brahmin of Shimla district showed a value of 0.2477 and the Rajput 0.2435. Among various Brahmin subpopulations of Himachal Pradesh, the allele has been reported in the range of 0.225 to 0.308 (recorded in two different samples of the Gaddi Brahmin of Bharmour, Chamba district). In the Rajput subpopulations of the state, it varies from 0.215 (Gaddi Rajput of Kangra district) to 0.342 (Rajput of Churah, Chamba district). The *ACP1*A* values of this study clearly fit into respective caste ranges of the state. Like in the AK1 and PGM1 enzyme systems, in the ACP1 system also no inter-group comparison of the present caste groups with other Brahmin or Rajput regional groups of the state was found to be statistically appreciable.

As for the glyoxalase (GLO1) system, comparative data from Himachal Pradesh are available only from the Chamba district of the state. The *GLO1*1* allele frequency among the present Brahmin subpopulation studied from Shimla district was 0.2027 while in the Rajput it was 0.1571. The allele frequency in the only Brahmin sample reported earlier from Himachal Pradesh in Chamba town was 0.167 while in the Rajput samples it varied from 0.232 (Rajput of Chamba town) to 0.265 (Rajput of Churah). Thus the present Brahmin showed a higher value and the Rajput a much lower value of the *GLO1*1* allele in respective subpopulations of these two castes in the state. Inter-population Chi-square comparisons revealed highly significant differences between the present Rajput of Shimla district and the Gaddi Rajput of Chuwari (χ^2=9.572, d.f. 2, $0.01 > p > 0.001$), the Rajput of Chuwari (χ^2=11.244, d.f. 2, $0.01 > p > 0.001$) and the Rajput of Churah (χ^2=11.273, d.f. 2, $0.01 > p > 0.001$).

In the glucosephosphate isomerase (GPI) system, in addition to the commonly occurring phenotype GPI 1, an example of a rare variant GPI 1,3 was found each in the Brahmin and Rajput samples tested here from Shimla district. Among ten Brahmin and Rajput subpopulations earlier studied for this enzyme system from Himachal Pradesh, presence of this variant has been reported in 2 out of 3 samples of the former caste and 5 out of 7 of the latter. Thus, the detection of two cases of GPI 1, 3 in the present caste material from Shimla district is in conformity with earlier observations and confirms widespread distribution of this rare GPI variant in caste populations of the state.

CONCLUSION

In conclusion, the present biochemical investigation among the Brahmin and Rajput of Shimla district demonstrates that their genetic constitution is very similar to each other. Furthermore, comparison with other such samples of these two predominant caste populations of Himachal Pradesh inhabiting different regions (districts) revealed that there is genetic homogeneity among various Brahmin subpopulations on one hand and the Rajput subpopulations on the other in this hill state of north India.

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KEY WORDS Erythrocyte Enzyme Polymorphisms. Caste Populations. Himachal Pradesh.

ABSTRACT As part of a larger study plan among people inhabiting southern districts of Himachal Pradesh in north-west India, data are presented on the distribution of various erythrocyte enzyme polymorphisms in the Brahmin and Rajput populations of Shimla district, in the first instance. The present biochemical data revealed that the genetic make up of these two castes was very similar. Comparison with such other regional data indicated almost homogeneous distribution of the studied enzyme systems in subpopulations of the respective caste populations of this hill state.

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