ISO-ELECTRIC FOCUSING STUDY OF SERUM PROTEINS (GC, TF, PI AND ORM) IN FOUR ENDOGAMOUS GROUPS OF MAHARASHTRA, WESTERN INDIA: APPLICATION IN PATERNITY TESTING

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INTRODUCTION

Iso-electric focusing (IEF) has become an important and powerful technique to discriminate genetic variability for several single gene systems. For example, Transferrin system was found to be monomorphic in most populations with starch gel electrophoresis, but analysis with IEF have clearly shown further microheterogeneity in this system, which makes TF a useful marker for population genetics and paternity determination. Several other serum protein systems HP, PI, GC and BF have shown similar microheterogeneity.

Considerable genetic diversity which exists in human populations has its origins due to different ecological environments, settlements, mating and migration patterns. So it has become increasingly important to collect genetic data on various population groups of the world for its possible use in studies in human biology. The essential knowledge about the variation of IEF polymorphisms in different populations of the third world is still limited. In this investigation we have analysed four serum proteins (GC, PI, TF and ORM) by IEF, in four endogamous groups from the state of Maharashtra, Western India. Caste is an important element which influences the structure of Indian populations, it is therefore important to investigate various genetic polymorphisms in different caste groups to study genetic differentiation in the populations of the Indian subcontinent. Several population groups from India have migrated to the West and are now permanently settled in Europe, United States and Canada. More and more cases are being encountered in which the immigrant individuals are involved in paternity disputes. The data on highly polymorphic genetic systems in immigrant groups is warranted to provide an accurate probability of exclusion.

MATERIALS AND METHODS

Four endogamous groups (BRAHMIN, MARATHA, GUJURATI & PARSEE) from Maharashtra were sampled in Bombay, India. These groups form the major part of population of Maharashtra. A battery of genetic markers have been analysed for these groups and the results will be discussed elsewhere. In this presentation, we report results of GC, TF and PI subtypes and ORM1 and ORM2 phenotypes analysed by IEF technique. Standard methods for IEF were followed as described by Papiha et al (1987); Papiha et al (1989); Constans et al (1980) and Yuasa et al (1986), with minor modifications for better resolution. The allele frequencies were calculated using Maximum Likelihood methods ad population affinities were evaluated by R Matrix (Harpending and Jenkins, 1973). Heterozygosity was estimated from allele frequencies. The theoretical average Probability of Exclusion (PE) was calculated as given by Garber and Morris (1983).

RESULTS AND DISCUSSION

The allele frequencies of various systems are given in Table 1. For all systems, populations were in Hardy Weinberg Equilibrium. ORM2 locus was monomorphic in all populations. Pair-wise comparisons between different populations showed marked allele frequency differences, however, the allele frequencies are well within the gene frequency range already reported from the Indian region. The overall heterogeneity amongst four populations for several alleles is also statistically significant (Table 1). These marked differences are likely to be due to their population structure. R (Kinship) matrix calculated from gene frequency data suggest the least relationship of Parsees (negative values) with any of the other populations studies (Table 2). Two dimensional dendrogram representation of the four populations show distinct position of Parsee and Gujurati while the two caste groups Brahmin and Maratha cluster together (Fig. 1).

Heterozygosity estimated from gene frequencies shows a marked increase in GC, PI, and TF systems when analysed with IEF technique. Mean IEF heterozygosity ($\overline{H}$) is more than doubled (range 40-48%) compared to mean heterozygosity estimate calculated by using conventional technique (range 19-20).

The PE value for each system calculated from the gene frequencies using conventional electrophoresis and IEF are given in Table 3. IEF technique provides better PE values for most of the systems. These PE estimates are

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approximately one to twenty times higher than the conventional electrophoresis values. There is a marked difference in PE estimates in different populations indicating that it would be wrong to use the gene frequency data on any one or pooled Indian population for calculation of Paternity Index. Chakraborty and Roychoudhry (1975) reported exclusion levels for a number of genetic systems on the basis of 4 regional populations of India, but they failed to take into account the variation within the sub-populations of the Indian subcontinent. The Indian populations also differ from English population with respect to gene frequencies and PE estimates, though differences are statistically non significant.

The relative usefulness of a genetic marker in paternity dispute analysis depends upon the level of its polymorphism. These four serum proteins can be used in routine paternity work as their genetics have been formally worked out and they provide better cumulative probability of exclusion (CPE), which averages around 60%. The individual PE values reported here are better than many blood groups and serum proteins listed by Chakraborty et al (1974).

In conclusion, serum protein analysis by IEF showed high level of heterozygosity and are very informative to study the genetic differentiation in various sub-populations It is imperative that genetic information should be collected from a number of populations, especially from the third world countries as well as the immigrant populations in the West to provide better understanding of gene frequency variation and their usefulness in paternity and forensic work.

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Table 1. Subtype Allele frequencies in four endogamous groups

SYSTEM/ ALLELE		BRAHMIN	MARATHA	GUJURATI	PARSEE	HETERO-GENEITY
		119	147	84	53	χ^2
GC	1S	0.680±0.031	0.615±0.029	0.556±0.039	0.686±0.046	8.20*
	1F	0.092±0.019	0.108±0.018	0.130±0.036	0.078±0.027	2.27
	2	0.228±0.028	0.227±0.026	0.314±0.036	0.236±0.042	4.43
PI						
	M1	0.640±0.032	0.741±0.026	0.671±0.037	0.651±0.046	7.06
	M2	0.259±0.029	0.143±0.021	0.183±0.030	0.236±0.041	11.83*
	M3	0.101±0.020	0.115±0.019	0.146±0.028	0.113±0.031	2.00
TF	C1	0.789±0.027	0.874±0.020	0.825±0.031	0.710±0.058	12.09*
	C2	0.158±0.024	0.085±0.017	0.110±0.025	0.258±0.056	15.98*
	C3	0.039±0.013	0.041±0.012	0.052±0.018	0.016±0.016	1.42
	D	0.014±0.008	----	0.013±0.009	0.016±0.016	3.64
ORM1						
	1	0.681±0.031	0.715±0.027	0.804±0.031	0.696±0.045	4.62
	2	0.310±0.030	0.275±0.010	0.196±0.031	0.304±0.045	4.62
	3	0.009±0.006	0.010±0.006	----	----	

*Significant at 5% level.

Table 2. R matrix of 4 Maharashtrian populations

1.	BRAHMIN	0.006			
2.	MARATHA	0.001	0.007		
3.	GUJARATI	-0.002	0.001	0.008	
4.	PARSEE	-0.005	-0.008	-0.007	0.019
		1	2	3	4

Fig. 1. Dendogram of four populations

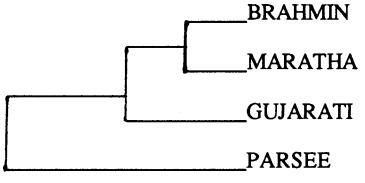

Table 3. Average Probability of Exclusion (PE)

SYSTEM	BRAHMIN		MARATHA		GUJURATI		PARSEE		ENGLISH	
	CON.	IEF	CON.	IEF	CON.	IEF	CON.	IEF	CON.	IEF
GC	0.145	0.243	0.160	0.273	0.169	0.299	0.147	0.234	0.162	0.303
PI	NI	0.261	NI	0.256	NI	0.266	NI	0.263	0.058	0.279
TF	0.012	0.176	NI	0.156	0.012	0.159	0.016	0.196	0.003	0.173
ORM	NI	0.170	NI	0.162	NI	0.133	NI	0.170	NT	NT
Cumulative PE	0.155	0.612	0.160	0.599	0.179	0.623	0.161	0.623	0.212	0.584

CON. - CONVENTIONAL NI - NOT INFORMATIVE NT - NOT TESTED