

Biochemical Polymorphisms in Reddy Population of Visakhapatnam District in South India

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Abstract

Introduction: The investigation of human blood genetic markers is a convenient tool to study genetic variation within and between human populations. The present investigation reports the variation in the distribution of some genetic markers (blood groups, plasma proteins and red cell enzymes) in Reddy, an endogamous population from State of Andhra Pradesh, India.

Methods: Eighty venous blood samples collected from health and unrelated individuals living in Visakhapatnam city were used for typing eight genetic markers- two blood group systems namely ABO and Rb (D), four serum proteins which include Albumin (ALB), Haptoglobin (HP), Caeruloplasmin (CP), Group Specific Component (GC), and two red cell enzymes Esterase D (ESD), Superoxide Dismutase (SOD). The gene frequencies were estimated and goodness of fit between the observed and expected phenotype frequencies was also tested.

Results: The frequency of 'A' group is found to be predominant recording a frequency of 50% which was followed by 'AB' group with a frequency of 26.25. 'O' group shows the least frequency with 5%. The homogeneity test for goodness of fit between observed and expected phenotypes is statistically significant ($\chi^2 = 9.2037$; d.f = 1; $0.01 > p > 0.001$) in Reddy's with respect to ESD system and HP system ($\chi^2 = 20.2780$; d.f = 1; $0.001 > p$) and non-significant with respect to GC system. The Caeruloplasmin (CP) and Albumin (ALB) locus were monomorphic in the present study.

Conclusions: The results of these biochemical genetic markers were found to be in accordance with other populations of Andhra Pradesh.

Keywords: Polymorphism. Blood groups. Red cell enzymes. Serum proteins. Caste population. Andhra Pradesh.

Introduction

Human population genetics deals with the extent of genetic variation within and among populations and the processes that influence this variation. The change in the genetic makeup of a population over time, usually measured in terms of allele frequencies, is equivalent to evolutionary change. For this reason, population genetics provides the groundwork for scientists understanding of evolution, in particular microevolution, or changes

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within one or several populations over a time span. Existence of sufficient variation even at a small number of loci is amenable to study population variations. The study of distribution of a large number of polymorphic alleles is useful to understand the various aspects of population dynamics.

The people of India are estimated to consist of over 40,000 Mendelian populations. As estimated 37,000 endogamous groups are structured in a system commonly referred to as the Hindu caste system. There are some 3000 Mendelian populations that are strictly speaking outside the caste system. These populations include tribal autochthonous groups and religious communities (Malhotra 1984). Andhra Pradesh, one of the twenty-eight states, harbors about 200 Hindu castes, thirty-three tribes and a few religious minority communities (Babu et al. 2001). Genetic differentiation is expected among these populations due to their ethnic and linguistic differences and the practice of endogamy (Babu et al. 2001). Thus, these endogamous populations offer good research material for the study of genetic differentiation human microevolution. Until recent past these studies have been confined to morphological, physiological and the common blood group investigations. Although a large number of populations were studied for varied sets of genetic markers, they constituted only a small fraction of the estimated number of Indian populations.

Reddy is an endogamous caste population mainly inhabited in the state of Andhra Pradesh. Reddy is a dominant caste in all parts of the state. A considerable number of Reddy's settled in the neighbouring states of Karnataka and Tamil Nadu. Reddy are Telugu-speaking farming community. However, several members of the community are very wealthy landowners and businessmen. Some of their members are also involved in other professions including service in government and private-run organisations. The present investigation reports on the distribution of ABO and Rh (D) blood groups, some red cell enzymes (ESD and SOD) and plasma proteins (HP, GC, CP, ALB) markers in Reddy caste population of Andhra Pradesh.

Material and methods

For this work, venous blood samples from a total of 80 healthy and unrelated individuals of both sexes of the Reddy caste were collected. The samples were collected from Visakhapatnam, a district headquarters city in the state of Andhra Pradesh. The unrelatedness of the individual is assessed by drawing the pedigree of the families in the study area. Before, drawing the blood samples, the participants were informed the purpose of the study and their consent was obtained.

In this study eight genetic markers- two blood group systems namely ABO and Rh (D), four serum proteins which include Albumin (ALB), Haptoglobin (HP), Caeruloplasmin (CP), Group Specific Component (GC), and two red cell enzymes - Esterase D (ESD) and Superoxide Dismutase (SOD) were studied. 5ml of venous blood was collected into sterilized test tubes containing EDTA as anti coagulant. The samples were brought to the laboratory in a thermos flask containing ice, within few hours of sample collection. The plasma was separated. Few red cells were used for blood grouping (ABO and Rh) by the antigen-antibody agglutination test. And with remaining red cells, haemolysates were prepared and stored at -20°C until use. The plasma proteins were typed by acrylamide gel electrophoresis. Group Specific Component (GC) and Albumin (ALB), Haptoglobin (HP) and

Caeruloplasmin (CP) were typed as described by Bhasin and Chahal (1996). Esterase-D (ESD) and Superoxide dismutase (SOD) were typed by agarose gel electrophoresis described by Wraxall & Stolorow (1986). The gene frequencies were estimated by using maximum likelihood methods of Balakrishnan (1988) and goodness of fit between the observed and expected phenotype frequencies were also tested.

Results

Distribution of phenotypes and allele frequencies of genetic markers are shown in Table -1. The present population exhibited the phenotypes O, B, A, and AB in the decreasing order of predominance $A > AB > B > O$ respectively. The frequency of 'A' group is found to be predominant recording a frequency of 50% which was followed by 'AB' group with a frequency of 26.25. 'O' group shows the least frequency with 5%. The frequencies of A, B and O alleles is 0.1275, 0.1685 and 0.7040, respectively, which are within the range of distribution reported earlier for the populations of Andhra Pradesh. The chi-square test for goodness of fit between observed and expected phenotypes is not significant ($\chi^2 = 0.1245$; d.f = 1; $0.80 > p > 0.70$) for Reddy population indicating genetic equilibrium for ABO system. Regarding Rh system, all the individuals are showing Rh positive phenotype. No Rh negative phenotype was reported in this population.

Three commonly occurring phenotypes identified in the present study and were designated as ESD 1-1, ESD 2-1 and ESD 2-2. These are determined by two alleles ESD*1 and ESD*2 at an autosomal locus. The 1-1 phenotype is predominant with 71.25% followed by 2-1 phenotype which recorded 20%. The ESD 2-2 phenotype recorded the lowest percent, with 8.75%. The frequency of ESD*1 and ESD*2 alleles among Reddy are 0.8100 and 0.1900, respectively. The homogeneity test for goodness of fit between observed and expected phenotypes is statistically significant ($\chi^2 = 9.2037$; d.f = 1; $0.01 > p > 0.001$) with respect to ESD system. Considering HP system, it is polymorphic with 2 classes of alleles HP*1 and HP*2 yielding 3 phenotypes: Hp1-1, Hp2-2, and Hp2-1. The study population showed the predominant occurrence of Haptoglobin 2-1 phenotype (67.5%). The frequency of HP*1 and HP*2 alleles are 0.3375 and 0.6625, respectively. The homogeneity test is statistically significant ($\chi^2 = 20.2780$; d.f = 1; $0.001 > p$). Considering the GC system, all the three phenotypes (1-1, 2-1, & 2-2) were observed in the present study. These are determined by two alleles GC*1 and GC*2. The 1-1 phenotypes are found to be the highest, recording 63.75%. These were followed by 2-1 phenotypes with 32.5%. The 2-2 phenotype recorded the least with 3.75%. Considering gene frequencies, the GC*1 allele records higher value (0.8000) compared to GC*2 allele (0.2000). The chi-square test for homogeneity was found to be non-significant ($\chi^2 = 0.0138$; d.f = 1; $0.95 > p > 0.90$) in Reddy with respect to GC system.

The Caeruloplasmin (CP) locus was monomorphic in the Reddy population showing only the BB phenotype. The ALB locus was also found to be monomorphic with the common allele, ALB*N in the present study as is the case with most of the populations from Andhra Pradesh. On the other hand the electrophoretic separation for red cell superoxide dismutase among Reddy reveals the presence of normal SOD 1-1 phenotype.

Table 1: Distribution of phenotype and allele frequencies of red cell enzymes and plasma protein systems in Reddy caste population

System	Phenotype	Observed	Expected	Allele	Frequency	χ^2
ABO	A	40	39.66	ABO A B O	0.1275±0.0097 0.1685±0.0097 0.7040±0.0097	0.1245
	B	15	15.66			
	AB	21	21.44			
	O	4	3.44			
	Total	80	80.00			
Rh (D)	+ ve	80	--	D	1.0000±0.0000	
	Total	80	80.00			
ESD	1-1	57	52.48	ESD 1 2	0.8100±0.0310 0.1900±0.0310	9.2037
	2-1	16	24.62			
	2-2	7	2.9			
	Total	80	80.00			
HP	1-1	0	9.11	HP 1 2	0.3375±0.0375 0.6625±0.0375	20.2780
	2-1	54	35.78			
	2-2	26	35.11			
	Total	80	80.00			
GC	1-1	51	51.2	GC 1 2	0.8000±0.0310 0.2000±0.0310	0.0138
	2-1	26	25.6			
	2-2	3	3.2			
	Total	80	80.00			
ALB	Normal	80	--	ALB*N	1.0000±0.0000	
	Total	80	80.00			
CP	Normal	80	--	CP*B	1.0000±0.0000	
	Total	80	80.00			
SOD	1-1	80	--	A*1	1.0000±0.0000	
	Total	80	80.00			

Discussion

The present study results are compared with other caste populations of Andhra Pradesh, and the allele frequencies of the present study genetic markers among available caste population are compiled. Table 2 shows the data on allele frequencies of ABO blood groups. The lowest frequency of 'O' allele is found in Madiga II - 0.5720 (Char et al. 1989) and the highest is found in Kalinga Vysya - 0.8216 (Lakshmi 1994). The 'B' allele frequency is within the range of other caste populations of Andhra Pradesh. The highest is reported in Mala-I - 0.3050 (Reddy & Mukerjee 1982) and the least is found in Brahmin-III - 0.1158 (Prasad & Busi 1988). With regards to A, the allele frequency variation in other castes of Andhra Pradesh ranged from 0.0000 in Thrivarnika

(Lakshmi 1994) to 0.1509 in Brahmin-I (Naidu & Veeraju 1982). Interestingly complete absence of an 'A' blood group was observed in Thrivarnika, an endogamous sub population of vysya community. This condition may be due to founder effect followed by genetic drift (Lakshmi et al. 2000). The Allele frequencies of ABO blood groups of the present study Reddy population are within the range of Andhra caste populations. Regarding Rh system, all the individuals showed Rh positive condition. No Rh negative were reported in this population. The present data is compared with that of the castes of Andhra Pradesh (Table 3). The d allele frequency ranges from 0% in Yadava (Lakshmi 1986) and Brahmin II (Reddy et al., 1980) to 35.23% in Kamma I (Reddy et al. 1980).

Esterase D enzymes in human red cells exhibit genetic polymorphism with three common phenotypes-EsD 1-1, 2-1 and 2-2, which are determined by two autosomal codominant alleles ESD*1 and ES*2. The present data is compared with that of the castes of Andhra Pradesh (Table 4). Among Andhra caste populations, the least frequency (0.2190) recorded for ESD*2 allele was in Kamma (Lakshmi 1986), and the highest (0.5161) was in Arya Vysya (Lakshmi 1994). The present study population values falls within the range for other caste populations of Andhra Pradesh. A review of the distribution of ESD system among Andhra Pradesh populations reveals that only two alleles ESD*1 and ESD*2 are present. Rare variants at ESD such as 3-1 (ESD*3) among Konda Kapu (Veerraju 1988) have been reported for tribal populations of Andhra Pradesh. In addition, the occurrence of a silent or null allele ESD*O has also been reported in an African population (Marks et al. 1977).

The three phenotypes SOD 1-1, 2-1 and 2-2 are controlled by 2 alleles SODA* 1 and SODA*2. The frequency of SODA*2 allele is low in most of the populations. In India many populations from nearly all regions of the country have been typed for the SOD system. The corresponding data have been compiled by Roychoudhury and Nei (1988) and Bhasin et al. (1992). With the exception of one small group (Vania Soni, Surat, Gujarat, with a sample of 82), in which one variant has been observed, all the others showed the phenotype SOD A1-phenotype, so that one can say, that the SOD A*1 allele is also typical for the populations of India, irrespective of their ethnic or regional origin (Bhasin and Walter 2001), which is in agreement with the present study. The present data is compared with that of the castes of Andhra Pradesh (Table 5). Interestingly, extremely rare variant of superoxide dismutase (SOD A*2) homozygous phenotype was observed (Khaja et al. 1996) from Shia Muslim population of Vizianagaram district in Andhra Pradesh.

The HP system is polymorphic. Three patterns, namely HP1-1, 2-1 and 2-2 are present which are controlled by a pair of codominant alleles, HP*1 and HP*2. The highest frequency of HP*2 is found among Asians (Approximately 0.75) while Europeans show frequencies of about 0.60 and the Negroid population in Africa show frequencies of about 0.30 to 0.40. In India, hypohaptoglobinaemia (HP O) phenotype has been detected in almost all the population groups studied and high frequency of this is observed from South India -Meplahs Muslims (0.131). It is suggested that this phenotype has some selective advantage in malaria since it is observed in high frequency (0.30 to 0.40) among some population groups from West and Central Africa (Bhasin and Walter 2007). However, it is rather difficult to evaluate HP O in relation to malaria in India since it has been

found in almost all the population groups from India, albeit in low frequency (Bhasin, Walter and Danker-Hopfe 1994; Bhasin and Walter 2001). No HP*O phenotype has been found in the present study population. HP*2 allele in Andhra caste populations (Table 6) ranges from 74.75% in Kalinga Vysya (Lakshmi 1994) to 92.4% in Kamma III (Lakshmi 1986). It is to note that the HP*2 allele in Reddy population falls within the range for other caste populations of Andhra Pradesh. The distribution of HP among various populations of Andhra Pradesh reveals that only HP*1 and HP*2 are present. Surprisingly, although the frequency of the HP*1 allele is found to be 0.3375, the Haptoglobin1-1 phenotype could not be traced in this population. The Kapu (Rao & Ramaswamy 1974) and Viswa Brahmin (Rao 1996) populations of Andhra Pradesh also showed the absence of 1-1 phenotype and a relatively lower frequency of HP*1 allele (Bhasin et al. 1994).

Family studies by Hirschfeld et al (1960) showed that the GC types are determined by a pair of autosomal co-dominant alleles called GC*1 and GC*2, with three phenotypes-GC 1-1, 2-1 and 2-2. Besides the two common variants, there are number of other types which are mostly rare. They are Gc-X and GC-Y (Hirschfeld 1962), GC-Chip and GC-Ab (Cleve et al. 1963), Gc-Negro (Parker et al., 1963), GC-Caucasian (Parker et al. 1963), Gc-2 (Henning and Hoppe 1965), GC-Norwegian (Reinskou 1965) and GC-Bangkok (Rucknagel et al. 1968). The existence of a rare GC*0 silent allele was suggested by Henningsen (1966). In nearly all the populations studied so far, the GC*2 gene has lower frequency than GC*1. The single exception is a figure of 0.69 for the GC*2 gene frequency among Xavante Indian tribes of Brazil and of 0.56 in another Brazilian tribe, the Caingang (Bhasin and Walter 2007). Among Europeans, the GC*2 gene frequency is fairly constant, averaging about 0.26. African Negroes generally have low GC*2 frequencies and in populations of Asia are also similar to or lower than those of Europe with the exception of the Israeli Ashkenazi jews (0.34) and the Kurumbas of India (0.35). Very few studies were reported on GC system among castes of Andhra Pradesh (Table 7). Among the castes shown in the table, the GC*2 allele ranges between 0.1800 in Paidi (Ramesh & Veerraju 2000) and 0.2700 in Kshathriya (Gracymilan 2000), which is in agreement with the present study. No rare variants were reported from Andhra caste populations.

Considering ceruloplasmin (CP), the electrophoretically detectable polymorphism consists of six different phenotypes, which are controlled by three autosomal co-dominant alleles: CP*A, CP*B and CP*C. In addition to these three alleles two more are known. One of them - CP*TH - seems to be restricted to the Thai population, the other one - CP*NH - to Africans. NH stands for New Haven in the United States, where this allele was observed for the first time (Bhasin and Walter 2007). From the present studies it is evident, that the CP*B allele is the most frequent one in all so far tested populations including India, whereas the CP*C allele is everywhere either completely absent or very infrequent. The ceruloplasmin locus was monomorphic in the Reddy population showing only the BB phenotype. It is interesting to note that all the Andhra caste populations so far screened (Table 8) are monomorphic for this system. Whereas, Walter et al., (1970) and Kellermann and Walter (1972) found a frequency of 0.012 for the CP*A allele in "Brahmins" from India and Indians from West Bengal, and a relatively lower frequency of 0.005 in a sample of Pakistanis (Bajatzadeh & Walter 1969).

The ALB locus was found to be monomorphic with the common allele, ALB*N in the present study as is the case with most of the populations from Andhra Pradesh (Table 9). Sporadic cases of slow moving rare variants were reported from Brahmins (Char & Rao 1983).

To summarize, the genetic variation in the distribution of these polymorphic loci was in accordance with the data available for local populations of Andhra Pradesh. The Reddy, a peasant community falls well in the middle of the other peasant castes. Several studies have been made among tribal and caste populations to understand their affinities and phylogeny. Each of these populations is endogamous from times immemorial and genetic admixture is less possible. Hence, a comprehensive understanding using more polymorphic loci is required to explore the evolution of huge number of endogamous populations from this geographical area.

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