

A Study of Red Cell Genetic Markers in Pulmonary Tuberculosis

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INTRODUCTION

Infectious diseases have played a significant role in altering the course of human populations in addition to bringing about change in their genetic constitutions. In India, tuberculosis remains the single major communicable disease affecting adults with more than 10 m persons suffering, a quarter being openly infectious; some 0.5 m persons die each year with a far fewer number effectively detected and treated. While BCG immunization protects from the most devastating forms of infantile tuberculosis (meningitis and disseminated tuberculosis), it does not appear to offer long term protection against the adult form of disease, which occurs from adult to child in millions of households throughout the country today.

Earlier studies have shown associations between some genetic markers viz., ABO blood groups, HLA antigens, serum proteins and tuberculosis both in India and abroad (Mourant et al., 1978; Bhasin, 1994). However, information on various other red cell markers and the disease is negligible in Indian populations. Therefore, this pilot study was planned to fill this void by providing phenotype and allele frequency distributions of as many as 10 different red cell genetic markers including 2 blood group systems (A_1A_2BO , RhD), 7 red cell enzyme polymorphisms (ADA, AK1, ESD, PGM1, GLO1, ACP1, GPI) and haemoglobin (Hb) variants in pulmonary tuberculosis patients and controls from Patiala district of Punjab, North-West India.

MATERIALS AND METHODS

The data were collected from a total of 100 unrelated patients and controls attending the Rajendra Hospital, T.B. Hospital and private diagnostic laboratories in Patiala city. All the subjects were between 26 and 67 years of age.

Finger prick blood samples were collected at random from 50 patients of pulmonary tuberculosis and an equal number of healthy controls. From each subject about 1 ml blood was drawn using disposable blood lancet and

collected in a microcentrifuge vial containing E.D.T.A.K₂ as an anticoagulant. Blood samples were immediately transported to the laboratory at Department of Human Biology, Punjabi University, Patiala where they were processed for the preparation of haemolysates by freezing and thawing method (Bhasin and Chahal, 1996).

A_1A_2BO and RhD blood groupings were done by the tube method using antisera and lectin procured from J. Mitra, New Delhi. Haemolysates were typed for isozymes of as many as seven red cell enzymes as well as haemoglobin, following standard electrophoretic techniques. Adenosine Deaminase (ADA) and Adenylate Kinase locus 1 (AK1) enzymes were separated together on the same agarose gel and simultaneously stained as described by Murch et al. (1986). Similarly, isozymes of Esterase D (ESD) and Phosphoglucomutase locus 1 (PGM1) were electrophoresed together on one agarose gel and stained in the same order (Wraxall and Stolorow, 1986). For typing Acid Phosphatase locus 1 (ACP1), the method of Wraxall and Emes (1976) was followed with a modification in the technique i.e., electrophoresis was performed in the agarose gel instead of thin starch gel. Mixed agarose/starch gel technique of Scott and Fowler (1982) was used for Glyoxalase locus 1 (GLO1) typing. Glucose Phosphate Isomerase (GPI) variants were characterized by the method of Deter et al. (1968) but using agarose as a medium of separation and staining procedure of Papiha and Chahal (1984). Screening of Haemoglobin (Hb) variants was done in conjunction with GLO1 typing (Divall and Greenhalgh, 1983). In each case the run was horizontal, carried out in a refrigerator at 4-10°C.

Allele frequencies in the A_1A_2BO blood group system were calculated using the maximum likelihood estimates; in the Rh(D) system the square root method was used. In enzyme polymorphisms and Hb system the frequencies were estimated by the gene counting method. Chi-square tests were performed to assess significance of deviations from Hardy-Weinberg equilibrium proportions. A heterogeneity Chi-square analysis (Workman and Niswander, 1970) was done to determine the overall differences in

allele frequencies between the patients and controls.

RESULTS AND DISCUSSION

Phenotypic distributions of blood groups, red cell enzymes and haemoglobins in tuberculosis patients alongwith controls are shown in Table 1. In both the series, all the distributions were found to be in Hardy-Weinberg equilibrium. Allele frequencies of the studied marker systems are listed in Table 2.

In the A_1A_2BO system, blood group B predominated in the patients with highest frequency (46%), followed by A_1 and O both with an identical value (20%), and AB (12%). In controls also a similar pattern was observed, B being the most common blood group (40%), followed by O (30%), A_1 (22%) and AB (2%). Chi-square comparison of the two series for the A_1A_2BO blood groups did not reveal any statistically significant difference ($\chi^2 = 2.019$, d.f. 3, $0.60 > p > 0.50$), suggesting perhaps no association between this serological marker with tuberculosis. Regarding the Rhesus system, the frequency of Rh(D) negatives was recorded 10% in patients and 6% in controls, the difference being statistically non-significant.

Laha and Dutta (1963) found a very high frequency of blood group O in pulmonary tuberculosis patients while Bhosale and Kulkarni (1971) observed that group A showed highest frequency (36%) in case of tuberculosis patients of Bombay. Reddy et al. (1978) considering the proportions of ascariasis, cataract, corneal ulcer and tuberculosis occurring in Badaga patients, found that 47.65% of them belonged to the O group as compared to 31.25% of normals. Marok and Bhanwer (1989) observed higher incidence of pulmonary tuberculosis among blood groups AB and B individuals while Singh et al. (1983) found lower proportion of blood group O in patients as compared to controls. Kshatriya and Kapoor (1991) observed appreciably high frequency of blood group B and Rh(D) negatives in tuberculosis patients, an observation similar to the present findings. Jain (1970) found a significantly higher incidence of group AB among the patients and Nayak (1971) observed a preponderance of blood group O and B in patients as compared to controls. On the other hand, in several other studies reported in literature (Kothare, 1959; Shenoy and Daftary,

Table 1: Phenotype distribution of genetic markers in tuberculosis patients and controls

Genetic marker	Phenotypes	Number observed	
		Tuberculosis patients	Controls
A_1A_2BO	A_1	10(20)	11(22)
	A_2	1(2)	0
	B	23(46)	20(40)
	A_1B	6(12)	3(6)
	A_2B	0	1(2)
	O	10(20)	15(30)
	Total	50	50
Rh(D)	D+	45(90)	47(94)
	D-	5(10)	3(6)
	Total	50	50
ADA	1	37(74)	34(68)
	1, 2	13(26)	15(30)
	2	0	1(2)
	Total	50	50
AK1	1	38(76)	43(86)
	1, 2	11(22)	5(10)
	2	1(2)	2(4)
	Total	50	50
ESD	1	33(66)	34(68)
	1, 2	15(30)	15(30)
	2	2(4)	1(2)
	Total	50	50
PGM1	1	26(52)	26(52)
	1, 2	16(32)	19(38)
	2	8(16)	3(6)
	1, 6	0	1(2)
	1, 7	0	1(2)
	Total	50	50
GLO1	1	3(6)	6(12)
	1, 2	15(30)	19(38)
	2	32(64)	25(50)
	Total	50	50
ACP1	A	5(10)	8(16)
	A, B	18(36)	23(46)
	B	27(54)	17(34)
	B, C	0	2(4)
	Total	50	50
GPI	1	50(100)	49(98)
	1, 5	0	1(2)
	Total	50	50
Hb	A	48(96)	49(98)
	V*	2(4)	1(2)

Figures in parentheses are percentages.

* Variant

1962; Navani and Narang, 1962; Nath et al., 1963; Saha and Banerjee, 1968; Sidhu et al., 1974; Ramachandraiah et al., 1984) no such differences were observed.

As far various red cell enzymes (ADA, AK1,

Table 2: Allele frequencies of genetic markers in tuberculosis patients and controls

Genetic	Allele	Tuberculosis patients	Controls
ABO	A	2.019	3
Rh(D)	D	0.543	1
ADA	A	5.890	1
AK1	AK1*1	0.810	1
ESD	ESD*1	0.170	1
PGM1	PGM1*1	0.220	3
GLO1	GLO1*1	0.220	1
ACPI	ACPI*1	0.220	1
GPI	GPI*1	0.220	1
Hb	Hb*	0.310	1
Rh(D)	D+	0.310	15
ADA	ADA*2	0.130	1
AK1	AK1*2	0.870	1
ESD	ESD*2	0.190	1
PGM1	PGM1*2	0.680	1
ESD1	GLO1*1	0.220	1
ACPI	ACPI*2	0.790	1
PGM1	PGM1*3	0.720	1
GPI	GPI*2	0.000	1
Hb	Hb*V	0.020	1
GLO1	1	3(6)	6(12)
	1, 2	15(30)	19(38)
	2	32(64)	25(50)
	Total	50	50
ACPI	A	5(10)	8(16)
	A, B	18(36)	23(46)
	B	27(54)	17(34)
	B, C	0	2(4)
	Total	50	50

However, some differences were apparent, as for instance, a raised frequency of the *GLO1**2 allele (0.790) in patients compared to controls (0.690). Similarly, in AK1, PGM1 and ACPI enzyme systems also, the frequencies of the *AK1**2, *PGM1**2 and *ACPI**B alleles were consistently higher in the patient series (Table 2). However, with the sole exception of ADA system ($\chi^2 = 5.890$, d.f. 1, $0.025 > p > 0.01$), none of these differences attained statistical significance (Table 3). The overall differences in allele frequencies of the 10 red cell genetic systems considered here between the patient and control series also fell a little short of attaining statistical significance ($\chi^2 = 22.46$, d.f. 15, $0.10 > p > 0.05$).

In conclusion, the present serological and biochemical study apparently failed to find much evidence for association of genetic markers with pulmonary tuberculosis. Although this pilot study reports results based on limited sample sizes of the patients and controls, material is being augmented by collecting further data in this ongoing study. In the meantime, the findings may be treated with caution.

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KEY WORDS Red Cell Genetic Markers. Pulmonary Tuberculosis. Association.

ABSTRACT The distribution of various red cell genetic markers including 2 blood groups (A, A, BO, RhD), 7 red cell enzymes (ADA, AK1, EsD, PGM1, GLO1, ACPI, GPI) and haemoglobin (Hb) in patients suffering from pulmonary tuberculosis and controls is reported. Results showed that barring ADA system, the differences between the patient and control series were statistically non-significant, suggesting little association between any of these genetic markers and the disease.

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Table 3: Contingency Chi-square analysis of differences between tuberculosis patients and controls

Genetic	Allele	Tuberculosis patients	Controls
ESD	ESD*1	0.810	1
PGM1	PGM1*1	0.680	1
GLO1	GLO1*1	0.220	1
ACPI	ACPI*1	0.220	1
GPI	GPI*1	0.220	1
Hb	Hb*	0.310	1
Hb*V	Hb*V	0.020	1

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