

Full Length Research Paper

Distribution of red blood cell antigens in drug-resistant and drug-sensitive pulmonary tuberculosis

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Tuberculosis (TB) is still a widespread world infectious disease. *Mycobacterium tuberculosis* kills more people than any other single pathogen, with an estimated one-third of the world's population being infected. According to the World Health Organization (WHO), two billion people are infected with the causative bacillus of *Mycobacterium tuberculosis*. Tuberculosis is rather prevalent in the country of Georgia today. Frequency distribution of ABO, Rh-Hr, MN, Kell blood group system antigens were studied in 277 TB patients (151-drug-sensitive and 126 drug-resistant) of pulmonary tuberculosis to know whether there was any association between them, and also between drug resistance and sensitiveness. They were compared with 485 healthy subjects. Tests were carried out by standard test-tube technique and plate reaction for the following antigens: A, B; C, c, D, E, e; K and k, M and N. The research materials were collected from Tuberculosis and Lung Diseases National Centre of Georgia and Ptisio-pulmonological Hospital of Adjara Region (Georgia). Present serological study has shown high frequency distribution of O and B phenotypes and r allele from ABO system, D antigen from Rh-Hr system and M antigen from MN blood group in TB patients then healthy subjects. This might explain the sensitivity of these antigens with pulmonary tuberculosis in Georgia. No significant difference between drug resistant and drug sensitive TB was found, thus the research in this direction has no importance. No association existed even when divided by age group in incidence of pulmonary tuberculosis.

Key words: Drug-resistant and drug-sensitive pulmonary tuberculosis, red cell markers, alleles, frequency, haplotypes.

INTRODUCTION

Tuberculosis (TB) is still a widespread world infectious disease. *Mycobacterium tuberculosis* kills more people than any other single pathogen (Leandro, 2009). According to WHO, there were an estimated 8.8 million incident cases (range, 8.5 million to 9.2 million), 12.0 million prevalent (range, 11.0 million to 14.0 million) and 1.1 million deaths (range, 0.9 million to 1.2 million) occurred among HIV-negative cases of TB globally in 2010 (WHO, 2010). It is estimated that one-third of the World's population is infected with *M. tuberculosis*, but that only one in 10 (10%) of those infected ever develop clinical disease.

The global burden of tuberculosis remains enormous, mainly because of poor control in Southeast Asia, sub-clinical disease. Saharan Africa, and Eastern Europe, and

and because of high rates of *M. tuberculosis* and HIV coinfection in some African countries (Dye, 1999). Tuberculosis has emerged as an enormous public health problem in former Soviet republics due to economic decline and the general failure of tuberculosis control and other health services following the breakup of the Soviet Union (Weinstock, 2001; Zalesky, 1999). The country of Georgia, an independent nation that was previously part of the former union has one of the highest rates of tuberculosis among former Soviet republics. Drug resistant Tb, especially MDR-TB (multidrug resistant) Tb, is associated with significantly higher morbidity and mortality than drug susceptible disease and emerged serious public health problem in the country of Georgia (Mdivani, 2008; Espinal, 2000). There were estimated 9.5 (8.2 to 11) % new and 31(27 to 35) % retreatment cases of MDR-TB in Georgia in 2010 (WHO, 2010).

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It is known that genetic and non-genetic factors of both - the bacterium and the host have impact on the immune response to *M. tuberculosis* (Saha, 1968). Twins studies and variations in the outcome of tuberculosis infection after exposure to similar environmental risk suggest genetic heterogeneity among individuals in the susceptibility to disease (Leandro, 2009; Harward and Hauge, 1965). Nowadays, the host genetic makeup in relation to susceptibility or resistance to infections has gained importance. There are several demonstrations that susceptibility to tuberculosis is linked to host genetic factors; inherited predisposition plays an important role in development of tuberculosis (Kramnik, 2000; Gra, 2010; Abel, 2000). Among the various factors that contribute to a person's genetic individuality are antigens that are the major alloantigen in humans, attached to surface of red blood cells and naturally occurring antibodies that circulate in the serum. The various combinations of their antigens and antibodies determine various blood groups (Saha, 1968; Naumov, 1993).

Earlier studies have shown association between some genetic markers such as ABO blood group antigens, HLA antigens, serum proteins with tuberculosis (Selvaraj, 2004; Seth, 2003). It was also reported earlier that there was a change in the pattern of the immunological response to *Mycobacterium tuberculosis* in individuals with different blood groups (Lewis, 1961; Naumov, 1993; Garraty, 2000). Studies on association of blood groups with pulmonary tuberculosis date back to 1926 when Halber and Hirzfeld first reported their negative findings. Since then conflicting reports have been published by different authors from different countries (Thamaria, 1972; Rao, 1994). Information about various red cell markers association with the disease, especially separately drug-resistant and other features of pulmonary tuberculosis is unknown in Georgian population.

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 of different blood group systems in patients with drug resistant and drug sensitive forms of pulmonary tuberculosis in Georgia.

MATERIALS AND METHODS

The research materials (blood) were collected from Tuberculosis and Lung Diseases National Centre of Georgia (The Caucasus) and Ptisio-pulmonological Hospital of Adjara Region (Georgia). Investigations were done on total 277 patients with a clinical and radiological evidence of pulmonary tuberculosis during 1998 to 2011. Blood samples were collected from the patients with drug sensitive (151 subjects) and drug-resistant (126 subjects) TB. All the subjects were between 17 and 75 years of age. As a control series, healthy individuals of Adjara Region were investigated. From each subject about 1 ml blood was taken and collected in a centrifuge vial containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood samples were immediately transported to the laboratory at the Department of Biology, Shota Rustaveli State University where they were processed.

The study was approved by the institutional Ethical Committee of Tuberculosis and Lung Diseases National Centre of Georgia.

Blood groups were tested by standard Immuno-serological methods with monoclonal antibodies and for calculating allele frequencies the data were processed by Statistical methods.

Using immune-serologic methods, we investigated 10 erythrocyte group antigens (two of the ABO system: A, B; five of the RH system: C, c, D, E, e, Kell system: K and two of the MN system: M and N antigens).

In the course of the work, the following specific test systems were used: anti -B, -A, -D, -C, -c, -E, -e, -K, -M, -N (Gemostandart Limited., Moscow), standard 0(I), A(II), B(III) group erythrocytes and standard 0(I), A(II), B(III), AB (IV) serums (the titer of the used reagents was not lower than 1:32). The obtained results were statistically processed. The ABO system genes alleles' frequency was computed by the formula proposed by F. Bernstein and used in investigation of three-allele genetic systems. The frequency of the 0, A and B genes in the given case will be indicated by the letters *r*, *p* and *q*:

$$\begin{aligned} r &= 0; \\ p &= 1 - \sqrt{A+O}; \\ q &= 1 - \sqrt{B+O}. \end{aligned}$$

Where 0, A and B – 0(I), A(II) and B(III) are the ratio of the group carrier people in relation to the total number of the subjects of the study. The frequency of the *Rh*-system genes and haploid types was computed by using the following formulas:

1. $D = 1 - \sqrt{dd}$;
2. $C = 1 - \sqrt{cc}$;
3. $E = 1 - \sqrt{ee}$;
4. $c = 1 - \sqrt{CC}$;
5. $e = 1 - \sqrt{EE}$.

Where, *D*, *C*, *E*, *c*, *e* are the number of the gene-carrying persons in correlation with the number of the study subjects, *dd*, *cc*, *ee*, *CC* and *EE* are the corresponding phenotype frequency. The *Rh*-haplotypes frequency is computed by the formula proposed by A. E. Mourant:

1. $cde = \sqrt{ccddee}$;

2. $Cde = \frac{Ccddee}{cde \cdot 2}$;

3. $cdE = \frac{ccddEe}{cde \cdot 2}$;

4. $cDe = \frac{ccDDee}{cde \cdot 2}$;

5. $cDE = \sqrt{ccDEE + cdE^2} - cdE$;

6. $CDe = \sqrt{CCDee + Cde^2} - Cde$;

7. $cCDE = \frac{CCDEe}{(CDe + cde) \cdot 2}$.

Where, *ccddee*, *Ccddee*, *ccddEe*, *ccDee*, *CCDee* and *ccDEE* are the corresponding phenotypes' frequency. The *RhD* and Kell-system alleles' concentration was computed under the following formula:

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

Genetic marker	phenotype	Number of observed individuals		
		Drug-sensitive	Drug- resistant	Donor
ABO	O	53.6 ± 4.0	48.4 ± 4.4	47.6 ± 5.3
	A	27.8 ± 3.6	35.7 ± 4.2	36.4 ± 5.1
	B	14.5 ± 2.8	9.5 ± 2.6	11.4 ± 3.3
	AB	3.9 ± 1.5	5.5 ± 2.0	4.2 ± 2.0
Rh-Hr (D)	Rh+	85 ± 2.8	81 ± 3.5	78 ± 4.1
	RH-	14 ± 2.8	19 ± 3.4	22 ± 4.1
MN	M	71.5 ± 3.6	59.5 ± 4.3	50.2±
	MN	15.2 ± 2.9	30.9 ± 4.1	29.4±
	N	13.2 ± 2.7	9.5 ± 2.6	20.2±
Kell	K+	5.9 ± 1.9	7.1 ± 2.2	4.2 ± 2.3
	K-	93.3 ± 2.0	92.8 ± 2.3	95.8 ± 3.2

*Figures are in percentages. N = 277.

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

Genetic marker	Allele	number of observed individuals		
		Drug-sensitive	Drug- resistant	Donor
ABO	r	0.72	0.69	0.68
	p	0.11	0.09	0.09
	q	0.23	0.25	0.23
MN	p (N)	0.2	0.25	0.35
	q (M)	0.79	0.75	0.65
Kell	p(K)	0.07	0.06	0.02
	q(k)	0.93	0.94	0.97

N = 277.

$$q = \frac{naa}{N};$$

$$P = 1 - q.$$

Where, naa is the recessive homozygotes (dd and kk) by the indicated loci, N is the total number of the studied subjects. In order to establish the concentration of the MN system alleles, the following formulas were used:

$$P = \frac{nA + \frac{1}{2}nAB}{N};$$

$$q = \frac{nB + \frac{1}{2}nAB}{N}.$$

Where, nA is the number of the M -phenotype carriers, nAB - of the MN phenotype, and nB is the number of the N - phenotype carriers. The errors in the frequency of genes were computed by the formula: $M = \frac{P(100 - P)}{n}$ (28), here P is the frequency of antigens in %, n is the number of the study subject.

RESULTS AND DISCUSSION

Investigations were done on different blood group antigens, alleles and haplotypes to ascertain their distribution and correlation with drug-sensitive and drug resistant pulmonary tuberculosis (PTB) patients. Phenotypic distributions of blood groups in tuberculosis patients along with healthy subjects are shown in Table 1. Allele and haplotype frequencies of the studied marker systems are listed in Tables 2 and 3.

Table 3. Distribution of Rh-Hr group system antigens and Rh haplotypes in tuberculosis patients and donors.

Genetic marker	Antigens and haplotypes	Number of observed individuals		
		Drug-sensitive	Drug-resistant	Donor
	D	0.63	0.57	0.54
	d	0.6	0.58	0.43
	C	0.44	0.36	0.28
	c	0.78	0.7	0.74
	E	0.26	0.11	0.11
	e	0.83	0.86	0.87
Rh-Hr	cde	0.37	0.41	0.436
	Cde	0.014	0.004	0.006
	cdE	0.008	-	-
	cDe	0.102	0.15	0.148
	cDE	0.15	0.08	0.126
	CDe	0.033	0.28	0.192
	CDE	0.016	0.003	0.004

N = 277.

Investigations on ABO system phenotype groups have revealed high spread frequency of O(I) ($53.6 \pm 4.0\%$) and B(III) ($14.5 \pm 2.8\%$) phenotype in patients with drug sensitive TB, but not in drug resistant patients. Here, it was observed that they had lower concentration than control subjects, explaining the unequal number of Tb research groups. A(II) phenotype is significantly low in drug-sensitive tuberculosis ($27.8 \pm 3.6\%$) compared with control series ($36.4 \pm 5.1\%$). Differentiated search according to drug sensitiveness has not shown any significant findings. Our finding suggests that high frequency of O(I) and B(III) phenotype points to the association with tuberculosis and may play a role in susceptibility development of active tuberculosis after mycobacterial infection. Due to the limited number of individuals with AB(IV) phenotype, it is difficult to discuss any association with them.

There was analysis of the frequency distribution of r(O), p(A), q(B) alleles of ABO system. Similar patterns were observed between total TB patients and control subjects. No significant association was revealed.

The study of Rh-Hr phenotype system revealed that pulmonary tuberculosis correlates with Rh+ phenotype; higher concentration revealed D antigen ($85.4 \pm 2.8\%$) in both drug-sensitive and drug-resistant ($80.9 \pm 35\%$) patients, compared with control subjects ($78 \pm 4.1\%$).

Red cell single antigen of Rh-Hr blood group is unequally distributed in all of the three research groups. It is noticeable that concentration of e antigen is higher in control subjects.

Among the Rh haplotype, cde is a generally wide spread haplotype but the concentration is lower in diseased people than the control series. It should be mentioned that CDe haplotype is significantly high (0.28) in drug resistant patients. The fixed least amount of cde,

CDe and CDE haplotypes is among Rh blood group system haplotypes.

Regarding the MN system, significant association was revealed between MN system groups and both pattern of TB patients. Research has shown correlation of MM ($71.5 \pm 3.6\%$) phenotype with drug sensitive tuberculosis and the majority of MN phenotype in drug resistant patients ($30.9 \pm 4.1\%$). This suggests that there is significant association between M antigen and pulmonary tuberculosis.

Spreading of Kell blood group phenotypes and alleles was almost equally distributed in research subjects and healthy individuals. It may point out little association between K+ phenotype with drug resistant TB; and that P allele is highly presented in drug sensitive form of tuberculosis.

Conclusions

Present serological study has shown high frequency distribution of O and B phenotypes and r allele of ABO system, D antigen of Resus system and M antigens of MN blood group in TB patients. This be the reason for the sensitivity of mentioned phenotype groups and antigens with pulmonary tuberculosis in Georgia. There was no significant difference between drug resistant and drug sensitive TB, thus the research in this direction has no importance.

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