ORIGINAL RESEARCH

PREVALENCE OF DUFFY BLOOD GROUPS AMONG THE POPULATION OF THE DESERT REGION OF INDIA

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ABSTRACT

Objectives: The distribution of vivax malaria is uneven in India and *Plasmodium vivax* is predominant in the North-Western part. Distribution of Duffy blood groups and its prevalence are important for regions where *P. vivax* predominates, because this molecule on the surface of the red blood cell acts as a receptor for *P. vivax*. The tribal (aboriginal) and desert area of the state Rajasthan contributes 70% to the malaria disease burden. The study was undertaken to identify the Duffy phenotypes present in the Rajasthan population and their prevalence. Methods: The study population was divided in to tribal and non-tribal groups and into healthy and *P. vivax* malaria infected groups. Duffy blood groupings of 96 unrelated subjects were carried out by the indirect antiglobulin technique. Simultaneously, malaria status was assessed for all participants using peripheral blood. Results: In the tribal population the prevalence of Duffy phenotypes Fy(a+b-), Fy(a-b+) and Fy(a+b+) were 18.5%, 11.1% and 70.4%, respectively. The prevalence of Duffy phenotype Fy(a+b+) was 15.6% lower in the non-tribal population. The percentage of both tribal and non-tribal population with Duffy phenotype Fy(a+b+) were significantly ($p<0.001$) higher than the population with Duffy phenotype Fy(a+b-) and Fy(a-b+). The Duffy phenotype Fy(a-b-) was absent in the population of the desert region. A higher prevalence of Duffy phenotype Fy(a+b+) was recorded for malaria patients (83.3%) than for healthy participants (59.0%). Conclusions: The population of the North-Western part of India was susceptible for *P. vivax* malaria infection and the tribal population seemed to be more susceptible than the non-tribal population.

Key Words: Desert; Duffy blood group; Malaria; *Plasmodium vivax*; Tribal population; India.

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INTRODUCTION

Plasmodium vivax is globally distributed and the second most prevalent malaria parasite affecting more than 75 million people each year (Imwong et al., 2005). In India, *P. vivax* malaria constitutes about 45% to 50% of all malaria cases (NVBDCP, 2009). The distribution of *P. vivax* malaria is uneven in India. *P. vivax* is endemic in the North-Western part of India and it contributes nearly 92% of all malaria cases within the state of Rajasthan. On the other hand, the tribal and desert area of Rajasthan contributes about 70% of the malaria disease burden (MOHFW, 2007). According to a 1987 estimate, there were about 54 million tribal peoples of various ethnic origins residing in India and accounting for about 8% of the total population but contributed approximately 30% of all malaria cases and 50% of malaria deaths in the country (Sharma, 1996; Singh et al., 2009).

Tribal (aboriginal) communities in India constitute the largest tribal population in the world (Balgir, 2006). A total of 635 tribes are present in the country, which constituted 8.08% (about 84 million) of the total population of India (Census of India, 2001). Among these tribes, 12 are present in the state of Rajasthan and contribute about 12.6% of the total population of the state. The desert region is spread over 62% of the total land of the state and is home to 12.8 million people (Tyagi, 2004). The largest tribal group of the desert is Bhil who form more than 97% of all tribal people living in the desert (Census of India, 2001).

Risk factors for *P. vivax* malaria infection in the non-tribal and tribal population of the desert region require investigation. The erythrocyte surface receptor molecule Duffy antigen plays a crucial role in the *P. vivax* invasion into the erythrocyte (Chitnis, 2001). As a consequence, the Duffy blood grouping, besides its relevance in transfusion medicine, is of major interest for population genetics. The two alleles *FYA* and *FYB* present four Duffy phenotypes; Fy(a+b+), Fy(a+b-), Fy(a-b+) and Fy(a-b-) and have been identified by the corresponding anti-Fya and anti-Fyb antibodies (Parasol et al., 1998). Although a number of genetic studies have been carried out among tribal groups in India, data on Duffy blood group antigen in indigenous tribal people of Rajasthan are limited. The current study was undertaken to investigate the prevalence of Duffy blood groups in the desert population of Rajasthan, India.

METHOD

Study area

The study was carried out in Jodhpur district. The district is in the desert part of Western Rajasthan. The state Rajasthan is situated in the North-Western part of India.

Study population

The study population was divided in to tribal (Bhil) and non-tribal. Both male and female participants were included in the study between the ages of 1 to 60 years. Participants formed a convenience sample coming from different unrelated families. Fever cases reported to the Mahatma Gandhi Hospital of S. N. Medical College and

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Fidusar Primary Health Centre during the study period were screened for being malaria positive. Those found to be malaria positive and ready to give their consent were asked to participate in the study. However, patients with complications or other concurrent infections were excluded from the study. Blood samples of healthy individuals were collected by door to door household surveillance. The healthy participants were asked about their malaria history and those who reported having had malaria infection previously were excluded from the study.

Procedure

Blood samples were taken by finger prick or from tubes of the venous blood collected for clinical studies of the hospitalized patients. The whole blood samples of 200µL were kept in EDTA containing tubes for Duffy blood group testing. Duffy antigen identification was carried out within 24 hours of blood collection using the indirect antiglobulin technique. Commercially available antisera anti-Fya, anti-Fyb (M/s DiaMed GmbH, 1785 Cressier s/Morat, Switzerland) and anti-human globulin (M/s DiaMed GmbH, 1785 Cressier s/Morat, Switzerland) were used according to manufacturer's instructions. Blood samples were washed twice with phosphate buffer saline (0.9%) before testing. One ml of phosphate buffer saline (PBS) was dispensed into the 100µL of whole blood for the preparation of 3% to 5% red cell suspension. 50µL the of 3% to 5% red cell suspension was mixed separately with 50µL of anti-Fya and anti-Fyb antisera. The red cell suspension and antisera were mixed properly by shaking and incubated at 37ºC for 30 minutes. Then, the supernatant was removed carefully and the content in the tube was washed thrice with the PBS. Thereafter, 100µL of anti-human globulin was added to the content in the tube and centrifuged at 125 g for 1 minute. The red cells were observed macroscopically over an indirect light source for agglutination. Agglutination of red cells with Duffy antisera anti-Fya and anti-Fyb was interpreted as Duffy positive Fy(a+b-) and Duffy positive Fy(a-b+) respectively. Agglutination of red cells with both of the Duffy antisera was interpreted as Duffy positive Fy(a+b+). When there was no agglutination of red cells with both anti-Fya and anti-Fyb, the result was interpreted as Duffy negative Fy(a-b-). The test result was independently examined by two persons. Samples of Fy(a+b-) and Fy(a-b+) erythrocytes were used as controls while performing the tests.

Malaria parasite diagnosis

For the diagnosis of malaria a thick and thin film was prepared in the same slide from the peripheral blood. Thin film of the slides was fixed with methanol before staining. The blood slides were stained with Jaswant Singh Bhattacharjee (JSB) stain (Singh et al., 1953) and microscopically examined under an oil-immersion lens. A thick smear was regarded as negative on initial review if no parasites were seen in 100 high power fields (Valecha et al., 2009). The diagnosis of malaria was done simultaneously for all the participants including the healthy participants during the Duffy blood grouping. The malaria positive patients were promptly treated with anti-malarial therapy in accordance with National drug policy.

Statistics

The frequencies of both the alleles were calculated from the observed phenotypes following the method described by Mourant et al. (1976). Data were analyzed by one-way analysis of variance (ANOVA) to find significant differences using the software M. S. Excel.

Ethical considerations

The project was approved by the Scientific Advisory Committee and Ethical Committee of the Desert Medicine Research Centre. Consent was obtained from all the patients. Consent from parents/guardians was obtained in case of children. In the case of tribal participants, consent was obtained from their respective group leader.

RESULTS AND DISCUSSION

A total of 96 subjects participated in the study, of which 54 (56.3%) belonged to the tribal group and 42 belonged to the non-tribal group. Blood samples of all participants were screened for the presence of malaria parasites and analysed for the determination of the phenotypic variants of Duffy antigen (Table 1). When the Duffy phenotypes of tribal and non-tribal participants were compared, the Duffy phenotype Fy(a+b+) was 15.6% lower in the non-tribal than the tribal group. Furthermore, the prevalence of the Duffy phenotype Fy(a+b+) was significantly higher than the other Duffy phenotypes Fy(a+b-) and Fy(a-b+)($F=16.31$; $df=2, 3$; $P=0.02$). The Duffy phenotype Fy(a-b-) was not reported in the existing study population.

When the Duffy phenotype of the *P. vivax* malaria patients (n=18) and healthy population (n=78) were compared, a higher percentage of people with Duffy phenotype Fy(a+b+) were recorded in the malaria patients (83.3%) than the healthy population (59.0%) and the inverse was noted for Duffy phenotypes Fy(a+b-) and Fy(a-b+) (Table I).

The Duffy blood group of the malaria patients and blood donors of the Brazilian population were compared by Cavasini et al. (2007) and the authors recorded an almost 8% higher prevalence of Duffy phenotype Fy(a+b+) in the *P. vivax* malaria patients than the healthy blood donors. In the present study, we have recorded a 24.3% increased prevalence of Duffy phenotype Fy(a+b+) in malaria patients compared to the healthy participants. However previously, equal numbers of Duffy phenotype Fy(a+b+) were recorded for *P. vivax* malaria patients and healthy Muria Gond tribal people of Central India by Verma and Thakur (1993). The Duffy phenotype Fy(a+b+) of Brazilian people and of Brazilian *P. vivax* malaria patients was more prevalent than Fy(a+b-) and Fy(a-b+) and was found similar to the present investigation (Cavasini 2007; Storti-Melo et al., 2009).

Our data suggests that the population of the North-Western part of India were susceptible for *P. vivax* malaria infection and the tribal population was more susceptible than the non-tribal population. Kar and co-workers (1991) analysed the Duffy blood groups of malaria patients and healthy individuals among the Ao Nagas tribal population of India and recorded a complete absence of Duffy negative Fy(a-b-) individuals. Verma and Thakur (1993) also recorded a complete absence of Duffy negative Fy(a-b-) individuals in the Delhi population. Similarly, no Duffy negative Fy(a-b-) individuals were identified in the present study of North-Western India. This suggest that selection of resistance against *P. vivax* infection is also like to be absent in the population of North-Western India.

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Table 1: Duffy phenotypes and gene frequencies, stratified by tribal as well as malaria status, of 96 participants recruited from the North-Western desert part of India.

Name of the Category	Number tested (n)	Duffy phenotypes (%)			Gene frequencies (%)	
		Fy (a+b-)	Fy (a-b+)	Fy (a+b+)	*FYA*	*FYB*
Tribal Population	54	18.5 (n=10)	11.1 (n=6)	70.4 (n=38)	0.54	0.46
Non-tribal population	42	19.0 (n=8)	26.2 (n=11)	54.8 (n=23)	0.46	0.54
P. vivax malaria patients	18	5.6 (n=1)	11.1 (n=2)	83.3 (n=15)	0.47	0.53
Tribal *P. vivax* malaria patients	12	0 (n=0)	8.3 (n=1)	91.7 (n=11)	0.46	0.54
Non-tribal *P. vivax* malaria patients	6	16.7 (n=1)	16.7 (n=1)	66.7 (n=4)	0.50	0.50
Healthy population	78	21.8 (n=17)	19.2 (n=15)	59.0 (n=46)	0.51	0.49

For the Muria Gond tribal population of Central India the prevalence of Duffy phenotype Fy(a+b+) was recorded to be lower than the Fy(a+b-) and Fy(a-b+) (Verma and Thakur, 1993); a result which is in disagreement with the present study. The Muria Gond tribal population were less infected with *P. vivax* infection than with *P. falciparum.* This difference may be due to the lower prevalence of Duffy phenotype Fy(a+b+). This tribal group had a prevalence of 11.3% Duffy negative Fy(a-b-) individuals which might have protected them against *P. vivax* infection (Verma and Thakur, 1993). Similarly, a majority (82.7%) of individuals from the Jarawas population of the Andaman Nicobar Islands of India were found to be protected from *P. vivax* infection believed to be due to the presence of the Duffy negative Fy(a-b-) phenotype (Das et al., 2005). In addition, the roots of the Jarawas tribe are with the Negritude group which might be the reason behind their Duffy negative selection. On the other hand, the Indian Bhil tribe belongs to the Australoid groups who were found to be more susceptible for *P. vivax*.

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