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Association of G-6-PD Deficiency, Sickle Cell Haemoglobin and Blood Groups with Resistance to Malaria Infection. A Survey on Different Castes in Southern Nepal

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Abstract

A survey was conducted in order to investigate on possible relationship between genetic factors and malaria contraction. Different castes (Brahman, Kshatriya, Vaishya and Sudra) of rural Southern Nepal were the subjects of this survey. The distribution of ABO blood groups and sickle cell haemoglobin was studied using a total of 669 blood samples from both sexes, and G-6-PD deficiency was studied using a total of 311 male blood out of the above samples. The malaria parasitaemia was determined by microscopical examination of blood smear. Out of 669 individuals 29 (4.3%) were positive. High frequency of G-6-PD deficiency was observed in people of Sudra origin (15.4%) and lowest in Brahman (5.3%). The sickle cell haemoglobin was also high in Sudra (2.98%) and lowest in Brahman (2.05%). The distribution of sickle cell between male (3.53%) and female (1.95%) did not show any significance. Whereas, significant association of ABO groups with *P. vivax* and total malaria cases was observed. Blood group O showed the lowest frequency of malaria parasitaemia. The high incidence of malaria parasitaemia was found in male than female. No malaria cases were detected in G-6-PD deficient and sickler people. These results suggest that genetic factors may be associated with resistance to malaria.

Key words: G-6-PD deficiency; sickle cell; blood group; malaria; castes; Nepal.

Introduction

Nepal, a Himalayan country, is a kingdom lying between China on the north and India on the south. Over an area of 147,181 km^2 not less than 26 different ethnic groups speak different languages (Bista, 1972). The Southern part of Nepal has been known to be heavily malarious (Banerjee *et al.*, 1994). The incidence of malaria had once decreased due to a control programme, however it began to increase again (resurgence) in the recent years (Sherchand *et al.*, 1995). Malaria is posing a serious problem in the health and economical activities of Nepalese people, and effective control measures against it are strongly required.

Natural resistance which is associated with genetic factors is suspected to be playing an important role in protecting individuals from malaria. Among genetic factors, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and certain haemoglobin have been found to be associated with protection against *Plasmodium falciparum* malaria (Luzzatto *et al.*, 1983). However, it is known that certain antimalarial drugs may cause haemolytic anaemia in G-6-PD deficient individuals. Therefore, WHO has stressed the necessity of examining the population for these genetic abnormalities before administration of such drugs (WHO, 1989).

Investigation on the relationship between G-6-PD deficiency and malaria susceptibility have been reported elsewhere (Pant *et al.*, 1992; Ishii *et al.*,

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1994). However, in Nepal, only one report of investigations on genetic abnormalities and factors associated with resistance to malaria has been available in Tharu communities (Vaishya origin) of Chitwan district (Terrenato *et al.*, 1988), but no report is available on different castes of various ethnic communities of Nepal. Therefore, the present study was conducted to find out the distribution of G-6-PD deficiency, sickle cell haemoglobin and ABO blood grouping along with malaria incidence and their possible inter-relationship among different castes of Nepalese communities.

Materials and Methods

Subjects and period of study

This study was conducted from January to August, 1995 in Southern part of Nepal selecting three

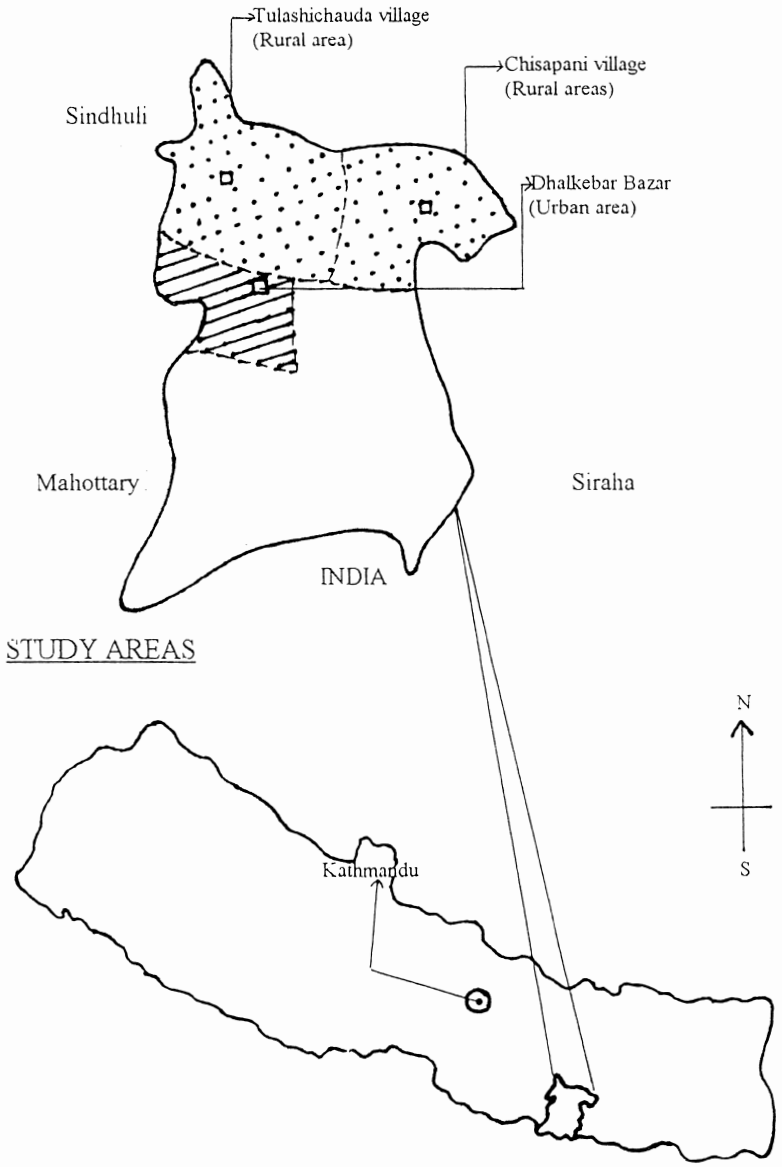

Fig. 1 Map showing two study areas in Dhanusha district of Southern Nepal.

areas of Dhanusha district (Fig. 1). The individual who had been living in the area for at least one year or more were included for this study. 250 persons from Tulashi Chaura village, 250 persons from Chisapani village and 169 from Dhalkebar health post from the same area. In these areas a total of 669 blood samples from different castes (146 Brahman, 205 Kshatriya, 251 Vaishya and Sudra 67) were collected during malaria survey from the randomly selected persons with and without fever.

The caste structure in Nepal is based on the Hindu Varna system and are divided into 4 major castes: Brahman (priest), Kshatria (warrior), Vaishya (trader) and Sudra (untouchable). These castes define social status and are influencing many aspects of life even today. The origin of each caste is, generally speaking, the same ethnic group. Therefore, each caste group is regarded to share similar genetic properties. Under the above castes many sub-ethnic groups exist as shown in the note of Table 1.

Table 1 Distribution of G-6-PD deficiency, sickle cell and malaria parasitemia in four different castes

Ethnic groups	Malaria parasitemia				G-6-PD		Sickle cell	
	Sample size	P. V.	P. F.	Total	Sample size	Deficient	Sample size	Sickler
Brahman-1 Origin	146	7 (4.8)	3 (2.1)	10 (6.84)	75	4 (5.3)	146	3 (2.1)
Kshatriya-2 Origin	205	9 (4.4)	2 (0.97)	11 (5.4)	84	6 7.2	205	6 (2.9)
Vaishya-3 Origin	251	(1.2)	2 (0.79)	5 (2.0)	126	11 (8.7)	251	7 (2.8)
Sudra-4 Origin	67	2 (2.9)	1 (1.5)	3 (4.5)	26	4 (15.4)	67	2 (2.9)
Total	669	21 (3.13)	8 (1.2)	29 (4.33)	311	25 (8.0)	669	18 (2.7)

Figures in parentheses are the percentages.

Note: Different castes representation from study areas of Southern Nepal:

1– Brahman Origin	2– Kshatriya Origin	3– Vaishya Origin	4– Sudra Origin
– Upadhyaya	– Basnet	– Tamang	– Damai
– Jha	– K.C.	– Lama	– Kami
– Paudel	– Thapa	– Rai	– Sharki
– Pokharel	– Thakuri	– Limbu	– Pode
– Dhungana	– Mandal	– Magar	– Biswakarma
– Bhandari	– Mahato	– Tharu	
– Jaishi	– Das	– Thakali	
– Lamsal	– Singh	– Gurung	
– Yadav	– Mukhiya	– Newar	
– Adhikari	– Giri	– Chauhan	
– Sharma	– Karki	– Maghi	

Collection of blood specimens

Venous blood was collected in heparinized tubes and filter papers from each individual. These samples were kept in an ice box and transported to the research laboratory of Tribhuvan University Kathmandu, and stored in refrigerator until tested. Both thick and thin smears were also prepared. These smears were stained with Giemsa solution and observed microscopically for malarial parasites carefully on the same day as blood collection.

G-6-PD deficiency

A total of 311 blood samples (75 Brahman, 84 Kshatriya, 126 Vaishya and 26 from Sudra peoples) from males were examined for G-6-PD deficiency by fluorescence spot test (Sigma Diagnostics Co. Ltd., G-6-PD Kit®, U.S.A.) following the previous description (Schmidt and Brosious, 1978). Female participants were excluded from this study because heterozygous females were invariably missed by this method, since G-6-PD deficiency is a sex linked genetic disorder, with full expression in males.

Sickle cell haemoglobin

Blood samples from both males and females were assessed for sickle cell haemoglibin following the Dacie and Lewis method (1982).

ABO blood group

ABO blood grouping was done by agglutination slide test using commercially available kit (Orthodiagnostics Co. Ltd., U.S.A.).

Analysis

Epi-info version 6 computer packages and statistical analysis methods such as Chi-square test, Z-test, Fisher' exact probability tests were applied to detect significant association of ABO blood groups with G-6-PD deficient, sickler and malaria contraction in the study areas.

Results

Malaria Parasite Detection

Malaria parasite was detected in 29 subjects out of 669 investigated (*P. vivax* 21 and *P. falciparum* 8). The highest frequency was found in Brahman (6.84%) followed by Kshatriya (5.4%), Sudra (4.5%) and Vaishya (2.0%) (Table 1). However, these difference among caste groups was not statistically significant ($P>0.05$).

G-6-PD Deficiency

Among 311 male individuals from different castes, the highest positivity of G-6-PD deficiency was shown in Sudra origin (15.4%) and lowest in Brahman origin (5.3%) (Table 1). No malaria case was detected in G-6-PD deficient subjects. Blood group-wise distribution of G-6-PD deficiency (Table 2) revealed high positivity of G-6-PD deficiency

Table 2 Blood group-wise distribution of G-6-PD deficiency, sickle cell, and malaria parasitemia

Blood group	G-6-PD deficiency	Sickle cell	Malaria parasitemia		
			PV	PF	Total
A	10 (13.9)	1 (0.67)	9 (6.00)	4 (2.6)	13 (8.6)
B	7 (9.8)	3 (2.02)	7 (4.7)	2 (1.35)	9 (6.08)
AB	2 (4.5)	9 (8.9)	3 (3.0)	1 (1.00)	4 (3.96)
O	6 (4.8)	5 (1.9)	2 (0.74)	1 (0.37)	3 (1.1)

Figures in parentheses are the percentages.

in blood group A 10 (13.9%) followed by B 7 (9.8%), O 6 (4.8%) and AB 2 (4.5%). However, no statistical significance was recognized in the distribution of G-6-PD deficient and non-deficient individuals among ABO blood group (X^2 df3=6.10, P>0.05).

Sickle Cell Haemoglobin

Out of 669 individuals examined, 18 (2.69%) showed sickle cell haemoglobin (Table 1). All 18 sickle cell haemoglobin were sickle cell trait. Among different castes, the positivity of sickle cell trait was highest in Sudra origin (2.98%) followed by Kshatriya (2.92%), Vaishya (2.79%) and Brahman (2.05%) respectively. Blood group-wise distribution of sickle cell individuals is shown in Table 2. Higher incidence was observed in blood group AB (8.91%). There was significant difference in the distribution of ABO among sickler and non-sickler subjects (X^2 df3=18.26 P<0.05).

Sex-wise distribution of sickle cell did not show any significant association with either sex (X^2 df3=1.59, P>0.05). However, higher positivity was found in males (3.53%) compared to females (1.95%) (Table 3). No malaria case was found in the individuals with sickler trait.

ABO Blood Groups

The distribution of blood group in different castes showed blood group O is dominant in Brahman (30.1%), Kshatriya (35.1%), and Vaishya origin (55.8%), whereas in Sudra people, blood group A (38.8%) was dominant (Fig. 2). Sex-wise distribution of blood group showed that blood group O is dominant in both male (39.9%) and female (40.5%) (Table 3). No significant difference in ABO blood group between two different sexes was recognized (P>0.05). The incidence of total malaria parasite detected cases was comparatively higher in blood group A (8.6%) than B (6.08%), AB (3.96%) and O (1.1%) as seen in Table 2. Similar trend was also observed in *P. falciparum* and *P. vivax* malaria.

Table 4 shows a significant difference in the distribution of ABO system among *P. vivax* cases versus normal and total malaria cases versus normal individuals (X^2 df3=10.27 P<0.05, X^2 df3=14.50 P<0.05). However, no significant difference was found in the distribution of ABO groups among *P. falciparum* versus normal subjects and *P. vivax* versus *P. falciparum* (X^2 df3=4.31 P>0.05, X^2 df3=0.26 P>0.5).

Discussion

In Nepal, only one study on genetic factors in

Table 3 Sex-wise distribution of G-6-PD deficiency, sickle cell, blood groups and malaria parasitemia

Sex	Sample size	G-6-PD deficiency	Sickle cell	Blood group				Malaria Parasitemia	
				A	B	AB	O	PV	PF
Male	311	25 (8.03)	11 (3.53)	72 (23.1)	71 (22.8)	44 (14.1)	124 (39.9)	12 (3.8)	4 (1.3)
Female	358	TND	7 (1.95)	79 (22.06)	77 (21.5)	57 (15.9)	145 (40.5)	9 (2.5)	4 (1.11)
Total	669	25 (3.7)	18 (2.7)	151 (22.6)	148 (22.1)	101 (15.09)	269 (40.2)	21 (3.1)	8 (1.2)
X^2 (p-value)		TND	NS (0.307)		NS (0.995)			NS (0.815)	NS (0.841)

Figures in parentheses are the percentages
TND = Test not done
NS = Not significant P>0.05
S = Significant P<0.05

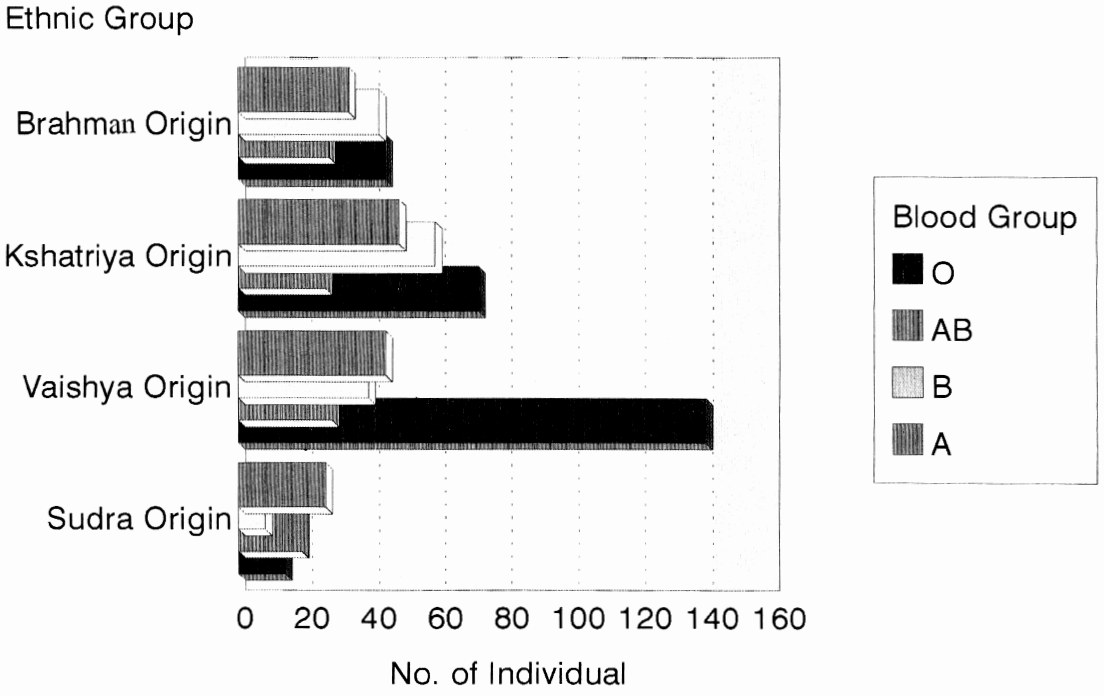

Fig. 2 Distribution of individual by blood group in different castes dhanusha district, Janakpur, Nepal.

Table 4 Comparative Chi-square test values

	G-6-PD defic. vs Normal	Sickler vs Normal	*P. vivax* vs *P. falciparum*	*P. vivax* vs Normal	*P. falciparum* vs Normal	Tot. MP+ve vs Negative
ABO System	6.10	18.26	0.26	10.27	4.31	14.50
X^2 df3	NS	S	NS	S	NS	S
P-value	0.107	0.0003	0.967	0.016	0.229	0.0022

NS = Not significant P>0.05
S = Significant P<0.05.

relation to malaria has been reported in a subethnic group (Tharu community) that belongs to Vaishya origin. It was found that the Tharu groups are more resistant to malaria than the other Nepalese population (Terrenato *et al.*, 1988). In India, the prevalence of G-6-PD deficiency shows much racial variation (Pant *et al.*, 1992; Panich, 1981) with pockets of high prevalence in some areas close to Nepal. They have reported 5.9% and 4.2% of G-6-PD deficiency in scheduled castes and scheduled tribes in India; such scheduled castes and tribes are usually backward, deprived and untouchable. Although discrimination on the basis of castes has been formally outlawed by National code of India and Nepal, it is recognised that it still influences many aspects of life. Inter-caste marriages are not unknown, but are

still unusual, and overt or covert discrimination still play a part in some sections of society.

In Nepal, such scheduled tribes are equal to Vaishya and Sudra origin. The above findings are parallel to the results of the present study; the positivity rate of G-6-PD deficiency was high in the order of Sudra, Vaishya, Kshatriya and Brahman origin with the range of 15.4% and 5.3%. In the present study no malaria case was found in G-6-PD deficient individuals, which corresponds to the previous findings that G-6-PD deficiency may have a protective role against malaria (Usanga and Luzzatto, 1985).

Previous report showed that the frequency of sickle cell anaemia and sickle cell trait in Nepal and Indian subcontinent population varied from 0–30% (Panich, 1981; Sharma, 1983). In the present study, the positivity rate of sickle cell trait was highest in Sudra origin (2.98%), which is considered as lower caste in Nepal, and lowest positivity rate was in Brahman (2.05%) which is superior caste of Nepal. In this study no malaria case was found in sickler samples, which coincides with the evidence that HbS mutation confers a protection against malaria infection. This is probably because of deoxy-HbS aggregates within RBC interfering with the intra-erythrocytic schizogony or initiating sickling of RBCs with developing schizont and subsequently removed by macrophages (Ghosh, 1983). Luzzatto *et al.* (1970) also reported the increased sickling of parasitized erythrocytes as the mechanism of resistance against malaria in sickle cell trait.

It is known that lower caste people acquired high protective immunity and acquired genetic factors that can resist to malaria, after many centuries more efficiently than higher caste people. The living condition of lower caste people are worse than higher caste people and they work mostly in the peripheral areas, to cultivate crops, field and forest. Most of them are alcoholics and usually sleep late, so, lower caste people have opportunity to have frequent exposure to mosquitoes. It is considered that lower caste people acquired high protective immunity and acquired genetic factors that can resist to malaria, after many centuries more efficiently than higher caste people.

A possible relationship between ABO blood groups and malaria has been extensively investigated so far with either negative or contradictory results. Arthey and Coriell (1967) have published that blood group B may have an advantage in malarious regions, suggesting a possible relationship between susceptibility of malaria and the blood group. In the present study, the lowest frequency of malaria parasitemia was recognized in blood group O. This finding also suggests possible association of blood group with resistance to malaria infection.

The results of present investigation contribute to the potential value of the G-6-PD estimation, sickle cell trait, and a possible relationship between ABO blood groups and malaria in Nepalese people. Moreover, persistence of malaria in such multiethnic population could be attributed to their genetic polymorphism, socio-economic and cultural characteristics, inaccessibility in most of the areas and poor health services coverage and utilisation.

However, more detailed epidemiological studies are required to reveal the frequency of the above parameters and their relationship with the incidence of malaria in other communities of the country, so that the outcome could be utilized for better understanding of malaria dynamics in different population groups in Nepal.

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