Filarial Infection is Resisted Differentially by Subjects Having Different Blood Group Phenotypes

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This study was aimed to elucidate the relationship between major blood group antigens (BGAs) and susceptibility or resistance to human lymphatic filariasis. A total of 492 human subjects, living in Raipur city of Chhattisgarh, endemic for bancroftian filariasis in Central India, were screened for the presence of *Wuchereria bancrofti* microfilariae and disease manifestations. The frequency of BGAs was tabulated as a function of the status of filariasis, namely normal (no infection), mf carrier (presence of microfilariae in blood), and elephantiasis (confirmed clinical manifestations). The comparison of ABO phenotype distributions among all the three groups clearly indicated that disease status has a significant relationship with the blood group attributes. The result of χ^2 analysis of the frequencies of ABO

phenotypes observed in microfilaraemic and elephantiasis groups (observed frequency vs. expected frequency computed based on the distribution of the normal population) revealed that there has been a significant alteration in the distribution of ABO phenotypes in microfilaraemic, but not in elephantiasis, group. The susceptibility to filarial infection was computed as a function of blood group phenotypes in the population. The susceptibility and conditional susceptibility for elephantiasis were the least among the subjects with AB phenotypes. The mechanism of association between filariasis and ABO antigens needs to be further explored to understand how the distribution of BGAs affects susceptibility/resistance to infection. J. Clin. Lab. Anal. 23:186–191, 2009. © 2009 Wiley-Liss, Inc.

Key words: blood groups; lymphatic filariasis; microfilaria; *Wuchereria bancrofti*; elephantiasis

INTRODUCTION

Lymphatic filariasis caused by *Wuchereria bancrofti* is a major health problem and the second most important leading causes of permanent disability in India. The disease causes disfigurement of body parts, physical discomfort, and the patients are burdened with significant social stigmatization and psychological trauma. The strong feelings of shame, fear, and embarrassment are common among those with elephantiasis and hydrocele. Psychosexual problems, which are neither openly acknowledged nor discussed, include marriages devoid of physical and sexual intimacy and suicidal thoughts. The infection is rampant throughout coastal India and every third Indian is exposed to the risk of filariasis. About half of the world's filarial patients live in India alone. Among the infected population in India, about 28 million carry microfilariae in their

blood, whereas more than 21 million show clinical manifestations of filariasis (1). The *W. bancrofti* in India is nocturnally periodic and transmitted by *C. quinquefasciatus*. The microfilariae (mf) released by the fertilized female reach peripheral circulation during night time and enter a mosquito's body during its blood meal. Further development of microfilariae in the vector host involves exsheathment of ingested mf, two successive moultings in thoracic musculature, which lead to

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Grant sponsor: Council of Scientific and Industrial Research (CSIR); Grant number: 9/248/(30)/2002-EMR-1.

Received 7 February 2008; Accepted 5 February 2009

DOI 10.1002/jcla.20317

Published online in Wiley InterScience (www.interscience.wiley.com).

the passage of larvae to L3 through L1 and L2. The L3 larvae when deposited on the skin of humans through mosquito bites migrate into the lymphatic system where they develop into adult worms. It usually takes 8–16 months after infection for the symptoms of the disease to appear. Bancroftian filariasis involves a wide spectrum of clinical features that range from asymptomatic amicrofilaraemic prepatent infection to elephantiasis.

Endemic normal group: A majority of population living in a filaria endemic region is amicrofilaraemic without any clinical symptoms of filariasis. It is not known whether these subjects remained unexposed to the infective mosquito bites or they are exposed but developed resistance in due course of time or they may be carrying a prepatent or a subclinical infection. About 10–15% of this group exhibits antibodies reactive to filarial larval antigens (2).

Asymptomatic microfilaraemia: Few individuals in an endemic area develop microfilaraemia but with no recognizable clinical manifestations of filariasis. Some remain microfilaraemic for years together without any symptoms of filariasis.

Acute and chronic manifestations: The acute clinical manifestations of lymphatic filariasis are characterized by episodic attacks of adenolymphangitis associated with fever and malaise. Hydrocele, lymphodema, elephantiasis, and chyluria are the main clinical pathological consequences of bancroftian filariasis.

Occurrence of several communicable diseases has been found to be associated with human blood group antigens (BGAs). Patients with blood group A are more susceptible to a severe hepatosplenic form of Schistosomiasis than those with blood group O who developed only milder form of the disease (3,4). Intensity and annual incidence of *S. haematobium* infection and related organ pathology was significantly higher among children of blood group A and lower among blood group O.

Similarly, intensity and incidence of *S. mansoni* infection and related liver lesions were high among children of blood group A (5). Children with O group were found to be more affected by malaria in Nigeria (6). Similar observations were earlier reported by Worlledge et al. (7) in case of adults in Nigeria. Gupta and Raichoudhuri (8) while working on falciparum malaria in India reported that patients having blood group A were more susceptible to infection compared with blood group O. There have been controversial reports regarding the association of blood groups with lymphatic filariasis (9,10). It was reported as early as 1946 that persons with blood group A are more susceptible to filarial infection as compared with persons of other blood groups (11). Nair et al. (12) observed more association between O group and elephantiasis. Gyrokos

et al. (13) reported no association between blood groups and filariasis. Higgins et al. (14) working on Timorian filariasis reported that the prevalence of mf is found to be higher in individuals with blood group A and AB. Pani et al. (15) observed that individuals of blood group B, when microfilaraemic, had a significantly higher probability of developing clinical filariasis. However, persons with AB blood group, when microfilaraemic, had a significantly lower probability of developing clinical manifestations. The same group (16) in another study reported that the ABO blood groups were not risk factors in the occurrence of patent parasitaemia or filarial disease, though age was associated with the occurrence of microfilaraemia and symptomatic disease. Nonetheless, the debate on the association of ABO BGAs with the manifestations of filariasis has been ongoing in the literature for some time. In this study, an attempt was made to find out the relationship, if any, between blood groups and incidence of microfilaraemia and/or elephantiasis in an endemic hitherto unexplored region located on the central India.

MATERIAL AND METHODS

Subjects

The study was conducted at Raipur city of Chhattisgarh, India. Informal oral consent was obtained from 492 subjects who participated in this study.

Study Population and Blood Sampling

Initially, community meetings were held in selected localities of the study region to explain, educate, and create awareness about filariasis, its dynamics, prevention, and therapeutic methods among the masses. The subjects were asked to present at a centrally convenient point on each locality, where the blood sampling was to be carried out. Persons of all age groups, who presented themselves, were registered for the study after taking oral consent. Their biographical characteristics, such as age and gender, were recorded (Table 1).

Finger prick blood samples (20 µl each) were collected between 21:00 and 23:00 hr and thick smears were made for parasitological examination by the first authors throughout the study period. The dried smears were taken to the laboratory and stained with Giemsa following dehaemoglobinization. The slides were rinsed briefly in tap water to remove excess stain and dried. The stained slides were examined under microscope with magnification of 100×. Two smears were collected from each subject and mean mf count was recorded. All subjects were also thoroughly screened for the presence or absence of acute or chronic clinical manifestations of filarial disease by the physician.

TABLE 1. Biographical Characteristics of Normal, Microfilaraemic and Elephantiasis Subjects

Characteristic	Normal	Microfilaraemic	Elephantiasis/ lymphedema	Total
<i>Sample size (number)</i>				
Male	208	22	27	257
Female	142	28	65	235
<i>Age (years)</i>				
Median	13	24.5	45	14
Range	11	53	86	87
Minimum	10	3	24	03
Maximum	21	56	90	90

Determination of BGAs

About 50 microfilaraemic subjects and 92 subjects with clinical manifestations (elephantiasis/lymphedema) and 350 normal subjects from the endemic region were examined for their association with BGAs. BGAs were determined employing latex agglutination test that uses monoclonal antibodies (Stanclone, Monoclonal antibodies, Hyderabad, India) against red cell A and B antigens. Fresh blood was taken on a microscopic slide and a drop of anti-red cell antigens (A or B) was added separately and mixed with the help of a needle. The slide was observed for agglutination under microscope.

Statistical Analysis

The frequency of BGAs was tabulated as a function of the status of filarial infection, namely normal, microfilaria carrier, and elephantiasis (Table 2 and Fig. 1). We performed χ^2 test to find out whether the disease conditions (i.e., microfilaraemic and elephantiasis) are dependent on the distribution pattern of ABO phenotypes that we observed in this study in the normal (disease-free) population (Table 2). The one-sample Kolmogorov–Smirnov test was performed to ascertain normality in the distribution patterns in all three subpopulations. None of the distributions was found to be normal (Normal: disease-free population: $P < 0.001$; microfilaria carrier: $P < 0.001$; elephantiasis: $P = 0.001$). Therefore, we computed expected frequencies of ABO phenotypes in microfilaraemic and elephantiasis groups based on the ratios of blood groups actually observed in normal population. Similarly, we computed expected frequencies of ABO phenotypes in elephantiasis group based on the ratio of blood groups actually observed in the microfilaraemic population. The susceptibility of BGAs to filarial infection, elephantiasis, and the conditional susceptibility for developing elephantiasis once infected were determined and tabulated (Table 3).

TABLE 2. Distribution of Blood Group Antigens Among Normal, Microfilaraemic, and Elephantiasis Individuals

Blood group	Normal ^a			Microfilaraemic ^a			Elephantiasis ^a			Expected frequency with reference to normal		Expected frequency with reference to mf	
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Mf ^b	Elephantiasis ^c	Elephantiasis ^d	Elephantiasis ^d
A	30 (14.42)	25 (17.6)	55 (15.71)	3 (13.64)	4 (14.29)	7 (14.0)	7 (25.93)	15 (23.08)	22 (23.91)	7.86	14.46	12.88	
B	70 (33.65)	47 (33.09)	117 (33.43)	8 (36.36)	6 (21.43)	14 (28.0)	10 (37.04)	27 (41.54)	37 (40.22)	16.71	30.75	25.76	
AB	20 (9.62)	10 (7.04)	30 (8.57)	9 (40.91)	15 (53.57)	24 (48.0)	1 (3.7)	5 (7.69)	6 (6.52)	4.29	7.89	44.16	
O	88 (42.3)	60 (42.25)	148 (42.3)	2 (90.09)	3 (10.71)	5 (10.0)	9 (33.33)	18 (27.69)	27 (29.35)	21.14	38.9	9.2	

Figure in parenthesis indicates the percentage.

^a $\chi^2 = 77.61$, $df = 6$, $P < 0.001$ (Blood group vs. disease status).

^b $\chi^2 = 24.49$, $df = 3$, $P < 0.001$ (Observed vs. expected frequency with reference to normal in microfilaraemic subject).

^c $\chi^2 = 4.77$, $df = 3$, $P = 0.189$ (Observed vs. expected frequency with reference to normal in elephantiasis subject).

^d $\chi^2 = 42.11$, $df = 3$, $P < 0.0000$ (Observed vs. expected frequency with reference to microfilaraemic in elephantiasis subjects).

Fig. 1. Frequency distribution of ABO blood groups in normal, microfilaraemic, and elephantiasis subjects.

TABLE 3. Ratios of Susceptibility of ABO Blood Groups to Filarial Infection, Elephantiasis, and Elephantiasis Conditional Upon Infection (Based on Table 2)

Blood group	Susceptibility for filarial infection ^a	Susceptibility for elephantiasis ^b	Conditional susceptibility for elephantiasis ^c
A	0.345	0.262	0.759
B	0.304	0.220	0.725
AB	0.500	0.100	0.200
O	0.178	0.150	0.844

^aNumber infected/number sampled.

^bNumber with elephantiasis/number sampled.

^cNumber of elephantiasis/number infected.

This study was approved by Institutional Ethics Committee and prior consent of all human subjects that participated in this study was obtained.

RESULTS

Of the 350 subjects examined from normal population, the majority expressed O blood group (42.30%) followed by B (33.43%) and A (15.71%). Only 8.57% of the normal subjects expressed AB antigens (Table 2). Out of 50 microfilaraemic subjects examined, 48% were found to have AB group antigens, 28% had B, 14% had A, and 10% had O group antigens. A gender variation is associated with B and AB BGAs. While 36.36% males were observed with B group, only 21.43% females were positive for B group. Among AB group, more females (53.57%) were observed as compared with males (40.91%). Out of 92 elephantiasis subjects examined, 40.22% had B BGAs, 29.35% had O BGA, 23.91% had A BGAs, and 6.52% had AB BGA. Gender-associated variation was observed in subjects with AB group. More females (7.69%) than males (3.70%) were noticed with clinical symptoms in AB group (Table 2).

The comparison of ABO phenotype distributions among all the three groups clearly indicated that disease status has a significant relationship with the blood group

attributes (Table 2; Fig. 1). The result of χ^2 analysis of the frequencies of ABO phenotypes observed in microfilaraemic and elephantiasis groups (observed frequency vs. expected frequency computed based on the distribution of the normal population) revealed that there has been a significant alteration in the distribution of ABO phenotypes in microfilaraemic, but not in elephantiasis group (Table 2). Similarly, the expected frequencies of ABO phenotypes in elephantiasis group according to the ratios of blood groups actually observed in the microfilaraemic population showed a statistically significant relationship (Table 2).

The susceptibility for filarial infection was found to be the highest among the individuals with AB blood group phenotypes and the lowest among the O group phenotypes (Table 3). The susceptibility of microfilaraemic subjects for elephantiasis was the highest among A group phenotypes and was of lower order in both AB and O group phenotypes. However, the conditional susceptibility for elephantiasis, i.e., once infected by mf, was of extremely high order in all except AB group phenotypes.

DISCUSSION

The major factors that govern the course of filarial infection include transmission intensity, which in turn is influenced by density of vector population, human infection rate, and rate of exposure to infective bites of mosquito, etc. in the endemic zone. A majority of the population in filaria endemic region is resistant to filarial infection despite the fact that every person is equally exposed to the bites of infected vectors. This is perhaps due to an innate ability of the subjects to resist the infection, which in turn is attributed to variation in genetic factors among endemic community. Similarly, very few microfilaraemic subjects develop clinical manifestations in due course of time, whereas a majority of them remain microfilaraemic for years together and in some cases throughout the life without developing any visual manifestations. The genetic and immunologic factors that govern the course of infection in exposed subjects remain a top priority of filaria research today. In this study, an attempt has been made to study the frequency of major BGAs in subjects with bancroftian filariasis.

The study was carried out in a filaria low endemicity region. The study population (normal, microfilaraemic, and elephantiasis subjects) belonged to low socio-economic group. The filarial subjects were randomly selected from those diagnosed either as microfilaraemic or having the filarial clinical manifestation, elephantiasis. About 42% of normal population bears O BGAs, whereas about 34% exhibited the blood group

phenotype B. The distribution of BGAs among microfilaraemic subjects indicate that while individuals of AB group possess high susceptibility for microfilaraemic infection, blood group O subjects has the least susceptibility for the same. Further, subjects with O group phenotypes have lower susceptibility for elephantiasis and that of AB group is the least.

These findings strongly corroborate our results on the comparison of the distributions of ABO phenotypes in different populations. However, the conditional susceptibility for elephantiasis, i.e., once infected by microfilaria, was of extremely high order in all except AB group phenotypes. Perhaps this confers protection to the subjects with AB group phenotypes, which are most vulnerable to mf infection. It seems that AB antigens offer tremendous resistance to subsequent development of elephantiasis once subjects belonging to AB group phenotypes are infected. Thus, this study reveals that filarial infection may be resisted differentially by the individuals having different blood group phenotypes. Further, the conditional susceptibility of postinfected subjects to manifest elephantiasis is again BGA-dependent. The results of this study contradict the observations of Kumar and Santhanam (17) who observed no association between filariasis and the ABO blood groups. However, the findings of this study corroborate with the initial observations of Pani et al. (15) in Pondicherry, India, who reported that mf carriers with B and AB group, respectively, had significantly higher and lower probability of developing clinical manifestations. Associations between microfilaraemia and blood groups A and AB were observed in earlier studies at other parts of India (9,10). Srisailapathy et al. (18) reported that the relative risk for developing filariasis is high for the B group as compared with subjects with AB group and they further observed that only males showed clearly significant risks.

The results thus suggest that in areas of low filaria endemicity, like in Raipur, preventive and prophylactic measures could selectively be offered to people with AB blood group because of the vulnerability of acquiring microfilaraemia. The highest prevalence of B blood type among elephantiasis subjects warrants special attention on microfilariae carriers with B blood group, as they are prone to develop clinical manifestations. However, this study was carried out in an urban area, which is endemic for filaria, and therefore, the results of this study may not be applicable to other geographic regions with different levels of filarial transmission intensity. Thus, the differences in study findings may be attributed to different degrees of transmission intensities prevalent in different study zones. A long-term study is required to understand the effect of filarial transmission dynamics and the impact of intervention at the vector level and/or

at the human level. Also, the association between filariasis and ABO antigens as well as how BGAs affect susceptibility or resistance to infection requires further study. The origin and molecular mechanism(s) of this phenomenon still remain obscure.

ACKNOWLEDGMENTS

We acknowledge the help received from Rameshwar Parihar during the conduct of the field study. We are obliged to Arti Parganiha, Arvind Agrawal, and Bobby Varghese, all belonging to the School of Life Sciences, Pt. Ravishankar Shukla University, Raipur-492 010, India, who have helped us in many ways for successful completion of the study. Financial assistance from the Council of Scientific and Industrial Research (CSIR), New Delhi, in the form of Research Associateship (9/248/(30)/2002-EMR-1) to one of us (V. D.) is gratefully acknowledged.

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