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Preferential feeding success of laboratory reared *Anopheles stephensi* mosquitoes according to ABO blood group status

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ABSTRACT

Recent epidemiological evidences revealed a higher rate of O blood group in the residents of malaria-endemic areas suggesting that groups A, B, and AB associated with a higher disease severity and fatality. Also recent data showed the low prevalence of AB group within the malaria-endemic residents in south of Iran and India. The aim of this study was to determine the ABO blood groups preference of *Anopheles stephensi* which is the main malaria vector in Iran, southwest Asia, and India. *An. stephensi* mosquitoes were fed either artificially on A/B/O/AB membrane blood feeders or directly on human volunteer hands and forearms of A/B/O/AB groups in a cage under lab conditions. Phenotype and genotype analyzes of 450-blood-fed mosquito specimens using agglutination and multiplex-allele-specific PCR revealed a significant blood preference of *An. stephensi* to AB group (40%) than other groups of A (24%), B (21%), and O (15%) in combination of both experiments. High preference of *An. stephensi* to AB group might increase malaria infection and fatality in this blood group and resulted in low frequency of AB group in the residents of malaria endemic areas. The data suggested that malaria vectors, like parasites may have selection pressure on human genotypes.

1. Introduction

Malaria is widespread globally with a great impact on health and economic particularly in developing countries (Murray et al., 2012; Sachs and Malaney, 2002). According to WHO estimate, only in 2010, 219 million malaria cases were reported with 660,000 deaths mostly in children age less than 5 years (WHO, 2012). Malaria is endemic and a major concern in southern provinces of Iran including Hormozgan, Sistan & Baluchestan and Kerman (Basseri et al., 2010; Edrissian, 2006; Hanafi-Bojd et al., 2012). The annual malaria cases have been declined from 66,075 to 3200 during 1995–2011, which classified the country in an elimination stage (WHO, 2012). There are five malaria vectors in the region including *Anopheles stephensi, Anopheles culicifacies* s.l, *Anopheles superpictus* s.l., *Anopheles fluviatilis* s.l, and *Anopheles dthali* where *An. stephensi* and *An. culicifacies* s.l. regarded as the most important vectors (Hanafi-Bojd et al., 2012; Manouchehri et al., 1976; Mehravaran et al., 2011a, 2012; Oshaghi et al., 2006a,b, 2007, 2008; Vatandoost et al., 2002, 2011). The predominant species is *Plasmodium vivax* (88%) followed by *Plasmodium falciparum* (12%) (Edrissian, 2006). Different studies showed various anthropophilic indices for *An. stephensi* that ranges from 5.4% to 15.8% in Kazeroun and Bander-Abbas (Manouchehri et al., 1976), 11.8% in Jiroft (Mehravaran et al., 2011b), and 0.5% in Kahnouj (Basseri et al., 2005), in south of Iran.

There are reports on possible association between ABO blood groups and malaria severity, as an example individuals with blood group O are relatively resistant to severe *P. falciparum* infection which might be a reason for a high distribution of O blood group in malariuos regions of the world (Adegnika et al., 2011; Deepa et al., 2011; Panda et al., 2011; Wolofsky et al., 2012). It was previously reported by the same authors that O group with 36.7% of the population was the most frequent ABO blood group in south of Iran followed by 27.6, 26.7, and 9.0% of A, B, and AB groups, respectively (Anjomruz et al., 2014). A similar situation was reported from endemic areas of India in which a high prevalence of individuals

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with O blood group (42%), compared with individuals with blood group A (21%), B (29%) and AB (8%) (Balgir, 2006; Panda et al., 2011).

It was suggested that blood type O and the mosquitoes which transmit the parasites are linked in evolutionary history (Cserti and Dzik, 2007). A significant association was demonstrated between severe *P. falciparum* infection and blood group A in Gabon (Lell et al., 1999), Ethiopia (Tekeste and Petros, 2010) and Zimbabwe (Fischer and Boone, 1998), blood group AB in Sri Lanka (Pathirana et al., 2005), Mali (Rowe et al., 2007), and Ethiopia (Tekeste and Petros, 2010), and blood group B in India (Panda et al., 2011).

Three mosquito species of *Anopheles gambiae*, *Aedes aegypti*, and *Culex pipiens* are responsible for annual infection of half a billion people with malaria, dengue fever, and West Nile virus, respectively (Hill et al., 2005). Human blood meal identification methods in mosquitoes are previously described (Oshaghi et al., 2006c,d and references herein), but there is a few reports on preference of *An. gambiae* (Wood et al., 1972) and *Ae. aegypti* (Wood, 1976) to O group. It was also shown that mosquitoes preferentially bite individuals with blood group A (Gupta and Rai Chowdhuri, 1980).

It is known that some humans emit oligosaccharides substances of blood groups on the skin and soluble, A, B, H, and Lewis b blood group antigens were shown in human skin (Kishi et al., 1990). These substances may influence the mosquitoes to select the human host. Blood group O secretors were reported to be preferred more than group O non-secretors by *Ae. aegypti*, and group A non-secretors were preferred over group A secretors (Shirai et al., 2004). Due to a high frequency of O blood group in the residents of malaria endemic regions, we hypothesized that malaria vectors may play an indirect role in the host population genetic structure. In this study, the blood feeding preference of a laboratory strain of *An. stephensi* on different ABO blood groups were checked using direct feeds on human volunteer or artificial human blood feed using glass membrane feeders. In addition to standard agglutination test, multiplex allele-specific PCR (ASPCR) method was used for ABO typing which needs much less amount of blood and is more precise.

2. Materials and methods

2.1. Ethical considerations and bio-safety measurements

The Ethical Committee on Human Research, Tehran University of Medical Sciences, reviewed the protocol of the study. The volunteers were all tested for HIV and hepatitis antigens and were found to be negative. All the volunteers were researchers who fully understand the aims and procedures of the study. Those who were willing to participate signed an informed consent and they were physically examined by a physician.

2.2. Human volunteers and blood group status

Ninety four potential volunteers were interviewed and 32 (F = 16, M = 16) healthy volunteers aged 20-45 years old with no history of illness and proof of negative HIV/hepatitis antigen tests. The volunteers belonged to different ethnic groups including Turk, Kurd, Fars, Turkmen, Arab, Lori, and Shomali who originated from different parts of Iran. There were 8 people (4 males and 4 females) randomly selected from each of the blood group types (O, A, B and AB) to participate in the experiments. In each experiment, four men or women from each blood group were randomly selected. The blood group status of the volunteers was initially determined by interview and then confirmed using standard agglutination test.

2.3. Mosquitoes

A colony of Beech strain of *An. stephensi* originated from India was used in this study. This strain is susceptible to insecticides and is maintained for more than 150 generations in insectarium of School of Public Health, Tehran University of Medical Sciences (SPH-TUMS) at 29 ± 1 oC, 60–70% RH, and a 12:12 (L:D)-h photoperiod. For each experiment, 100–150 starved (male and female) adult mosquitoes of 8–12 day-old (mostly 8 day old) were used and almost half of them were females.

2.4. Mosquito blood feeding tests

A wooden frame (100 × 100 × 100 cm) with netting around the frame was used as the holding cage for the mosquitoes. The sleeves were protruded from the center of the four vertical sides of the cage (total four sleeves). About 100–150 mosquitoes were introduced into the cage for each experiment. The mosquitoes were starved for 24 h and were transferred in to the cage 30 min before the experiments commence. The hands and forearms of four human volunteers each with a specific blood group (A, B, AB and O) were washed with odorless detergent (Golnar, Paxan Iran) and dried thoroughly, then the hands were inserted through the sleeves into the cage with the backside of the hands facing upward, keeping a small space between the palms which is held 40–50 cm above the bottom of the cage. The exposure time was 45 min (for women) to 60 min (for men) minutes and the mosquitoes were allowed to feed from the exposed hands and forearms of the volunteers. The experiments were performed under insectarium condition in darkness to avoid any influence of human color or physical attractants. Each male or female volunteer took part in four experiments. Altogether 32 replicates were conducted for direct feeding on human hands. The sleeves specified for each blood group were changed routinely in each succeeding trial to eliminate the effect of bias toward the cage sides for the mosquitoes. After the exposure time, all fully engorged mosquitoes were aspirated out of the cage, anesthetized and each mosquito was kept in a micro tube separately in dry condition at −18 °C for further serological and/or molecular blood meal analysis.

Similar experiments were conducted using the membrane feeding technique. Batches of 100–150 unfed mosquitoes were fed by membrane feeding method with blood drawn from the four men and women volunteers with A, B, O, and AB blood group randomly selected from the participant list. Blood of each man or woman volunteer was used in four experiments, each time the feeders were positioned at one side of the top of the cage. Again 32 replicates were carried out for the experimental membrane feeds on A, B, AB and O blood groups. Briefly, 3 ml blood was collected from each volunteer and mixed with citrate phosphate dextrose and was placed directly into a water jacketed glass mosquito feeders within 5 min of collection. Circulating water maintained at 37 °C was run through the jacketed membrane feeders to maintain constant temperature. The blood feeders were placed above the cage 50 to 70 cm from each other and 15 cm from the edges of the cage on top of the cage and the mosquitoes were allowed to feed for 60 min.

2.5. Blood meal analysis

2.5.1. ABO phenotyping

In each experiment usually around 30–45 out of 50–75 (60%) female mosquitoes took blood meal. Altogether around 2400 female mosquitoes took blood meal in 64 replicates. Six to nine fed mosquitoes from each replicate were randomly selected for ABO typing. In this study totally the ingested blood meal of 450 (∼20%) fed mosquitoes including 420 (225 from human volunteers and 195 from artificial membrane feeding tests) and 30 (16 from human volunteers and 14 from artificial membrane feeding tests) were typed by serological and molecular methods, respectively (Table 2). The selected mosquito abdomens were removed and dissected individually in a micro tube. Then 30–35 micro liter PBS was added to