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ABO blood groups of residents and the ABO host choice of malaria vectors in southern Iran

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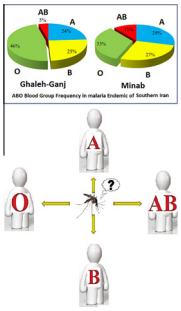
HIGHLIGHTS

- ABO blood group of residents in two malaria endemic areas in southern Iran has been typed.
- Ingested blood meal of Anopheline mosquitoes were typed using allele-specific PCR.
- O blood group was the most frequently observed group in the regions.
- It is the first ABO molecular typing of blood meal in mosquitoes.
- ABO typing of arthropod vectors warrant further researches.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent epidemiological evidences revealed the higher prevalence of 'O' blood group in the residents of malaria-endemic areas. Also some data indicated preference of mosquitoes to 'O' group. The aim of this study was to determine ABO group ratio in the residents as well as ABO group preference of *Anopheles* in two malaria endemic areas in south of Iran. Agglutination method was used for ABO typing of residents. Field blood fed *Anopheles* specimens were tested against vertebrate DNA using mtDNA-cytB PCR–RFLP and then the human fed specimens were tested for ABO groups using multiplex allele-specific PCR. A total of 409 human blood samples were identified, of which 150(36.7%) were 'O' group followed by 113(27.6%), 109(26.7%), and 37(9.0%) of A, B, and AB groups respectively. Analyzing of 95 blood fed mosquitoes revealed that only four *Anopheles stephensi* had fed human blood with A(1), B(1), and AB(2) groups. Result of this study revealed high prevalence of O group in south of Iran. To our knowledge, it is the first ABO molecular typing of blood meal in mosquitoes; however, due to low number of human blood fed specimens, ABO host choice of the mosquitoes remains unknown. This study revealed that ABO blood preference of malaria vectors and other arthropod vectors deserves future research.

1. Introduction

Malaria disease has widespread prevalence in the world which could have great impact on people health and economic cost for communities particularly in developing countries (Murray et al., 2012; Sachs and Malaney, 2002). According to the World Malaria Report of WHO, 219 million cases and 660,000 deaths of malaria were reported in 2010 which was more prevalent among aged group less than 5 years (WHO, 2012). In 2011, totally 3239 malaria cases reported in Iran included 1710 indigenous and 1529 imported cases that had relative decreasing trend than 2010 (WHO, 2012). Totally, about 4 million of Iranian people are endangering to risk of malaria, however, currently, Iran has been classified as country in elimination stage of malaria (WHO,

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2012). Most of malaria cases were residing in southern parts of the country including Hormozgan, Sistan & Baluchestan and Kerman provinces (Basseri et al., 2005, 2010; Edrissian, 2006; Hanafi-Bojd et al., 2010, 2012). In these regions, there are five malaria vectors including *Anopheles stephensi*, *Anopheles culicifacies s.l.*, *Anopheles superpictus s.l.*, *Anopheles fluviatilis s.l.*, and *Anopheles dthali* where *A. stephensi* and *A. culicifacies s.l.* regarded as the most important vectors (Manouchehri et al., 1976; Mehravaran et al., 2011a,b, 2012; Naddaf et al., 2012; Oshaghi et al., 2006a,b, 2007, 2008; Vatandoost et al., 2011, 2002). The most important *Plasmodium* species are *Plasmodium vivax* (88%) and *Plasmodium falciparum* (12%) (Edrissian, 2006). Among the vectors, *A. stephensi* is the main malaria vector in both rural and urban regions, and has shown various anthropophilic indices ranged from 5.4% to 15.8% in Kazeroun and Bander-Abbas (Manouchehri et al., 1992), 11.8% in Jiroft (Mehravaran et al., 2011b), and 0.5% in Kahnouj (Basseri et al., 2005).

Literature review suggested an association between ABO blood groups and malaria disease that individuals of blood group O are relatively resistant to severe disease caused by *P. falciparum* infection. Numerous studies confirmed the low chance of severe malaria for people with O blood group (Adegnika et al., 2011; Deepa et al., 2011; Panda et al., 2011; Wolofsky et al., 2012) which in turn resulted in the high distribution of O blood group in malarious regions of the world. It is suggested that blood type O and the mosquito-transmitted parasite are linked in evolutionary history (Cserti and Dzik, 2007). On the other hand, significant association have been demonstrated between severe *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).

Despite numerous studies in this context, a few studies were conducted to test association of ABO blood groups and malaria in Iran. The main purpose of the present study was to find frequency of ABO blood groups of malaria endemic residents as well as to test whether Anopheline mosquitoes has got any preference to each of ABO groups.

2. Materials and methods

2.1. Study area

The study was conducted in the two malaria endemic areas of Minab and Ghale Ganj with a previous history of malaria transmission. Minab located in the Hormozgan province in the southeast of Bandar Abas (capital of Hormozgan province). Its population is 300,000 people including 34% rural and 66% urban residents. Ghale-Ganj district is located in the Kerman province in the south of Iran with a population of approximately 70,000 people. The hot and humid weather of Minab and Ghale-Ganj is an important factor for growth of *Anopheles* spp. as well as malaria transmission. Malaria seasonal and transmission pattern have two peaks in year from June to July and in October. *P. vivax* and *A. stephensi* are the main malaria parasite and vector in this areas, respectively. Implementation of the malaria elimination program leads to the decreasing trend of malaria cases in recent years (Zoghi et al., 2012).

2.2. ABO Blood group determination

A total of 409 human blood specimens including 296 peoples from Minab and 113 peoples from Ghaleh-Ganj were randomly collected from the residents to detect their ABO blood groups. ABO blood groups of the people were typed by standard agglutination method using commercial antisera (CinaGen Co., Tehran, Iran). Three drops of the blood specimens were put in three places of glass slide on which a few drops of antisera A, B and D were employed to detect of blood group A and B. Applicator stick was used to mix of blood cell and antigen in each place. Titled slide was applied for agglutination and their results interpreted according to the guideline.

2.3. Anopheline collection

Anopheles collections were conducted based on the guideline suggested by WHO (1992). Mosquitoes were collected by hand catch, total catch and shelter pits from outdoor and indoor settings during five months from August to November in 2010 and also March 2011. Female specimens were separated and identified based on the morphological key of Shahgudian (1960). The specimens were conserved into 1.5 ml Eppendorf micro tubes and transferred to the laboratory of School of Public Health, Tehran University of Medical Sciences (SPH-TUMS) and stored at −20 °C until molecular examination.

2.4. Anopheles blood fed identification

2.4.1. DNA extraction

Genomic DNA of blood fed mosquitoes was extracted to analyze the blood meal sources, ABO blood groups, as well as the malaria parasite infection in female mosquitoes. DNA extraction was performed using DNeasy® Blood & Tissue Kit (Qiagen), according to the manufacturer's instructions. All of the experiments were carried out with parallel laboratory unfed *A. stephensi* specimen as negative and the known human ABO blood groups as positive controls.

2.4.2. mtDNA cyt B PCR–RFLP

A region of the mtDNA cyt B gene were amplified and digested by *Hae*III for host blood meal identification of the blood-fed specimens following the protocol already introduced by Maleki-Ravasan et al. (2009). The primers of UNFOR403 and UNREV1025 (Table 1) were used to amply a 623-basepair region of the cytB gene of vertebrate mtDNA. Following amplification, the PCR products were digested by *Hae*III enzyme. Incubation of the mixture was performed based on recommendation of enzyme suppliers. Then, 8 μl of the PCR–RFLP product was loaded onto a 2% agarose gel and subjected to electrophoresis. Electrophoresis was performed using a GeneRuler 100-basepair molecular mass marker (Sinaclone, Iran). Gels were stained with ethidium bromide (2 mg/ml) and the RFLP profiles were observed under ultraviolet light.

2.4.3. Multiplex allele-specific PCR (ASPCR)

Following the PCR–RFLP and identification of the mosquitoes fed on human blood, their genomic DNA were also tested for ABO blood groups using the multiplex allele-specific PCR (ASPCR) protocol described by Lee et al. (2009). In this experiments, four-reaction multiplex ASPCR genotyping assays using 10 different primers (Table 1) used to detect specific nucleotide sequence differences between the six ABO alleles A101, A102, B101, O01, O02, and cisAB01.

3. Results

A total of 409 residents were included for ABO blood group identification, of which 296 individuals belonged to Minab and 113 individuals belonged to Ghale-Ganj. The results of the blood groups in Minab showed 100 (33.8%) – 'O' group, 83 (28.0%) – 'A' group, 80 (27.0%) – 'B' group and 33 (11.2%) – 'AB' group (Fig. 1). The results of the blood group typing in Ghale-Ganj showed 52