The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Role of MyD88-adaptor-like gene polymorphism rs8177374 in modulation of malaria severity in the Pakistani population

Asima Rani[a,*], *Syed Kashif Nawaz*[a], *Shazia Irfan*[a], *Muhammad Arshad*[b], *Razia Bashir*[a], *Najma Shaheen*[c]

[a] *University of Sargodha, Department of Zoology, Sargodha, Pakistan*
[b] *University of Education, Lower Mall Campus, Lahore, Pakistan*
[c] *University of the Punjab, Department of Zoology, Lahore, Pakistan*

ARTICLE INFO

Article history:
Received 23 November 2016
Accepted 7 April 2017
Available online 6 May 2017

Keywords:
Malaria
Plasmodium
rs8177374
MAL
Pakistani population

ABSTRACT

Introduction: The present study was designed to investigate the association between rs8177374 polymorphism and malaria symptoms due to exposure of *Plasmodium vivax* and *Plasmodium falciparum*.

Materials and methods: A total of 454 samples were included in the study (228 malaria patients and 226 healthy individuals). Malaria patients, divided into *P. vivax* and *P. falciparum* groups on the basis of the causative species of *Plasmodium*, were categorized into mild and severe on the basis of clinical outcomes according to WHO criteria. Healthy individuals were used as controls. Allele specific PCR based strategy was used for the identification of rs8177374 SNP.

Results: MyD88-adaptor-like gene polymorphism was associated with susceptibility to malaria ($p < 0.001$). C allele frequency (0.74) was higher in the population compared to T allele frequency (0.26). CT genotype increased the susceptibility of malaria (OR: 2.661; 95% CI: 1.722–4.113) and was positively associated with mild malaria (OR: 5.609; 95% CI: 3.479–9.044, $p = 0.00$). On the other hand, CC genotype was associated with severe malaria (OR: 3.116; 95% CI: 1.560–6.224, $p = 0.00$). *P. vivax* infection rate was higher in CT genotype carriers compared to other genotypes (OR: 3.616; 95% CI: 2.219–5.894, $p < 0.001$).

Conclusion: MyD88-adaptor-like/TIR domain containing adaptor protein polymorphism for single nucleotide polymorphism rs8177374 is related with the susceptibility of malaria.

* *Corresponding author.*
E-mail address: primer.snp@gmail.com (A. Rani).
http://dx.doi.org/10.1016/j.bjid.2017.04.002

Introduction

Malaria is a mosquito borne serious infectious disease of the tropical and subtropical world. Being endemic in tropical and subtropical regions, it marks the major health burden in developed and underdeveloped countries of this region. *Plasmodium vivax* and *Plasmodium falciparum* are known to be the main agents responsible for malaria infection in Pakistan.[1,2] *Plasmodium* parasite elicits dynamic immune response in the host.[3] Toll like receptors (TLRs) regulate the immune system through recognition of pathogen-associated molecular patterns (PAMPs).[4] TLRs identify various ligands from pathogens and initiate downstream signaling through various adaptor proteins. This signaling cascade leads to production of inflammatory cytokines.[5] In humans at least 10 TLRs have been identified with different specificities for PAMPs. Signaling of TLRs occurs through Toll/IL1R (TIR) domain. Signaling of TLR4 and TLR2 requires an additional adaptor protein MyD88-adapter-like (MAL) also known as TIRAP.[6] TLR4 plays important role in *Plasmodium* recognition. Once TLR4 recognize the PAMPs, it triggers the recruitment of adaptor molecules (MAL) and initiates downstream signaling which leads to the activation of nuclear factor kappa B (NF-κB). This results in the production of various inflammatory cytokines like TNF-α, IL-1, IL-6, IL-8, and IL-12.[7] Inflammatory cytokines mediate host immunity to Plasmodium infection and are beneficial for the host as they have anti-parasitic effects. However, enhanced inflammatory response leads to devastating effects causing tissue injury. Uncontrolled inflammatory response has been associated with severe malaria outcomes and deaths particularly with *P. falciparum* infection.[8] *P. falciparum* has long been considered to cause severe and complicated malaria. More recently, it has been reported that *P. vivax* infection can also result in similar severity and complications as *P. falciparum* infection.[9] MAL/TIRAP being important adaptor protein of various TLRs regulates inflammatory response. It is a cytoplasmic protein of 221 amino acids. Gene that encodes MAL is located at chromosome 11q24.2. Single nucleotide polymorphisms (SNPs) affecting the function of MAL regulate the level of inflammation and plays a crucial role in various infectious diseases. rs8177374 cause nucleotide substitution of C to T at position 991 bp. It results in MAL protein with a leucine instead of a serine at position 180 (S180L). This polymorphism has been proposed to have protective effect against various infectious diseases. This variant attenuates the MAL functioning, thus resulting in reduced production of inflammatory cytokines.[10] Host immune response to the invading *Plasmodium* parasite shapes the disease outcome. Genes involved in parasitic recognition and inflammatory signaling modulates the level of inflammation. SNPs affecting the MAL functioning plays important role in immune response against *Plasmodium* infection. Apart from type of *Plasmodium* infection, host inflammatory response determines the disease severity. There is a need to correct and study the exaggerated chronic inflammation and possible mechanisms of this inflammation in patients suffering from malaria. This study aims to investigate the role of rs8177374 polymorphism of MAL/TIRAP gene as controller of severity of malaria in the Pakistani population.

Materials and methods

All procedures were in compliance with the declaration of Helsinki. The study protocol was approved from the advanced research and study board University of Sargodha. Permission from ethical committee University of Sargodha was also taken to start the study. All participants were informed about the study and granted approval for using their DNA and related data for research purpose.

Sample collection

Blood samples of 454 individuals were selected for the study. Blood sample (5 cc) was collected from each individual in EDTA coated vacutainer (BD, USA) and stored at −20 °C for further analysis. Blood samples of patients suffering from malaria were collected in different hospitals of Punjab, Pakistan during September 2013 to September 2015. Diagnosis of malaria infection was based on the presence of *Plasmodium* in the blood samples. *Plasmodium* parasite was detected via kit method (ImuMed, China), which involves the addition of 5 μL of blood into the sample well (S) of the test cassette, followed by the addition of three drops of lysis buffer in well B. After 30 min, if a line appeared on control and *Pv* then the parasite in the sample was *P. vivax*. If a line appeared on control and *Pf* then the parasite in the sample was *P. falciparum*. If a line only appeared on control there was no *Plasmodium* species in the sample. Patients were divided in two groups on the basis of parasite species (*P. falciparum, P. vivax*) identified.

Patients were also categorized on the basis of clinical symptoms. Patients were diagnosed with mild or severe malaria based on clinical and physical signs according to the WHO criteria. Severe malaria patients had neurological abnormalities (prostration, lethargy), severe anemia, hyperparasitemia corresponding to >5% parasitemia, gastrointestinal symptoms, hypoglycemia (serum glucose less than 2.2 mmol/L), acidosis with respiratory distress, jaundice, cardiovascular shock, and diffuse hemorrhages. Patients not fulfilling the criteria for severe malaria were categorized as having mild malaria. Blood samples of healthy controls from the local population, matched for gender, with no history of malaria infection were also collected.

Genotyping

Blood samples were used for DNA isolation through standard protocol of Vivantis blood DNA isolation kit (Cat# GF-BD-100). For DNA detection 0.8% agarose gel was used following UV illumination. PCR was performed using allele specific strategy. Primers designed for this study were synthesized from Invitrogen, USA via local representative. Two forward primers, F1 5′TGCACCATCCCCCTGCTGTC3′, F2 5′TGCACCATCCCCCTGCTGTT3′ and one reverse primer R 5′ACCCCGACAGCCTTT3′ were used for amplification of rs8177374 of MAL gene. PCR master mix (Vivantis, product # PL1202) was used for amplification of the targeted sequence. Amplification was performed in a thermocycler (BIOER TECHNOLOGY CO., LTD., TC-XP-G, China). PCR involved an initial step of denaturation at 94 °C. It was followed by 30 cycles of