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Original article

Toll-like receptor 4 *Asp299Gly* and *Thr399Ile* polymorphisms and typhoid susceptibility in Asian Malay population in Malaysia

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Abstract

Typhoid fever is a major health problem with frequent outbreaks in Kelantan, Malaysia. Prevalence of TLR4 gene polymorphisms varies with ethnic groups (0–20%) and predisposean individual to gram-negative infections. The prevalence rate of TLR4 *Asp299Gly* and *Thr399Ile* polymorphisms in the Malay population or the influence of these on typhoid fever susceptibility is not yet reported.

250 normal and 304 susceptible Malay individuals were investigated for these polymorphisms using allele-specific PCR and analysed for its association with typhoid fever susceptibility.

The total prevalence of polymorphisms in the normal population was 4.8% in comparison to 12.5% in the susceptible population ($p = 0.002$). An increased frequency of both polymorphisms was observed in the susceptible population ($p < 0.01$) with no homozygous mutants observed. Co-segregation was observed in 2% of controls and 3.6% of the susceptible individuals.

This study, for the first time, reports the prevalence of TLR4 gene polymorphisms in the Malay population and suggests that these polymorphisms confer a higher risk for typhoid, infection. The higher incidence of typhoid fever in Kelantan could be attributed to the higher percentage of Malays (95%) in this state. In order to reduce the incidence of this disease, people with these polymorphisms, can be prioritised for prophylactic strategies.

Keywords: Typhoid fever; TLR4 polymorphisms; Malay ethnicity

1. Introduction

Typhoid fever is caused by the Gram negative bacillus *Salmonella* subspecies *enterica* serovar Typhi (*S.* Typhi) and continues to be a worldwide health problem [1], especially in developing countries, where there exists poor sanitation and poor standards of personal hygiene. According to Crump et al. [1], the worldwide incidence of typhoid fever infection was 21.7 million and was responsible for approximately 217,000 deaths in the year 2000. Asia is reported to have the highest incidence of typhoid fever, with an estimated 90% death rate [2]. The incidence rate recorded in five Asian countries [3] ranged from 15/100,000 in China to 452/100,000 in Karachi, Pakistan. In Malaysia, the incidence rate is recorded to be low in the range of 0.71–4.50 per 100,000 population during the period 1996 to 2006 [Typhoid Fact sheet. pdf - www.dph.gov.my/survelans/PDF.Factsheet/TYPHOID.FactSHEET.pdf.-; Diseases A-Z list http://www.dph.gov.my/cdc/vaccinepreventablediseaseunit/vaccine.preventable.disease/disease.list/typhoid.htm.] However, the State of Kelantan recorded a high incidence rate of 15–33/100,000 during the years 2000–2005 [Source: Jabatan

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Kesihatan, Kelantan, Malaysia. 2005], the reasons for which is not clear.

Typhoid fever results through fecal-oral transmission from an acute or chronic carrier [4] and human beings are the only reservoir for *S.* Typhi [5]. Most acute typhoid patients recover after undergoing a febrile illness which lasts for several weeks [1]. However, a proportion of people who survive the acute phase of the disease harbor *S.* Typhi in the gall bladder and excrete the bacteria intermittently in their stools as chronic carriers [5]. The prevalence of typhoid carriers as evidenced by positive stool cultures is 1–5%. Persistence of bacteria in chronic carriers after the acute response shows that both bacteria and host adopt various mechanisms to reach an 'immune equilibrium'. This persistence could either be due to recurrent infections, especially in those living in the endemic areas [6]. The recurrent infection can also be contributed by the higher genetic susceptibility of the individuals to typhoid infection. Antibodies to *S.* Typhi have been reported to persist for periods ranging from 6 months to 6 years in convalescent individuals [7,8]. The status of persistence of cell mediated immunity, which could be of more importance in typhoid infection, however, is still vague.

The first line defense of the host against any pathogen is the innate immune system. Recognition of the pathogen induces an immune response including innate and specific cell mediated and humoral responses to eliminate pathogens [9]. The innate immune system recognizes invading microorganisms based on Pathogen Associated Molecular Patterns (PAMPs) through germ line-coded receptors called Pattern Recognizing Receptors (PRRs) [10]. Toll-like receptors (TLRs) are one of these PRRs, and to date 13 TLRs have been identified in humans and mice [11–13]. The TLRs are present either on the cell membrane or in the cytosol and contain signalling motifs that recognize pathogens leading to activation of the host cells. TLR4 is reported to be a lipopolysaccharide (LPS) recognition receptor, contributing to the early detection and mounting of immune response to gram-negative pathogens [11,14,15]. Mutations in the TLR4 gene or absence of the gene are reported to be associated with a higher susceptibility to infections by Gram negative organisms [14,16–19]. Twenty-eight non-synonymous and 7 synonymous polymorphisms have been described so far in the TLR4 gene among which, two co-segregating mutations of TLR4 *Asp299Gly* and TLR4 *Thr399Ile* polymorphism have been studied most widely. TLR4 *Asp299Gly* is an A/G transition that causes Asp/Gly polymorphism at amino acid 299, and TLR4 *Thr399Ile* is a C/T transition that causes Thr/Ile polymorphism at amino acid 399 [20]. Polymorphisms at these amino acids have been shown to alter the ligand–binding site of the TLR4 receptor [15]. Since TLR4 alterations are thought to be related to susceptibility to Gram negative infections, healthy and patient populations have been screened for the frequency of polymorphisms in this gene. Available reports in the literature show the prevalence rate to be 5–10% even though reports in some Asian populations show no TLR4 polymorphism in the population [16,21]. The prevalence of TLR4 polymorphism in Malaysian population or its status in typhoid infection has not previously been reported.

Malaysia is divided into thirteen states and 3 federal territories. Malaysia's population is made up of many ethnic groups, the major ones being the Malays (50.4%), Chinese (23.7%) and the Indians (7.1%) [Source: National Census 2000. Department of Statistics Malaysia]. Four of the thirteen states have a Malay population of over 70% and the incidence of typhoid is reported to be higher in these states, the reasons for which are not clear. We hypothesise that a genetic susceptibility factor could be involved in this phenomenon. There is wide variation in outcomes following exposure to potentially life-threatening pathogens in different populations pointing to functional genetic diversity of the immune response among these populations [22]. Since TLR4 polymorphisms have been related to enhanced susceptibility to gram negative organisms, the aim of this study was to investigate the prevalence of TLR4 *Asp299Gly* and TLR4 *Thr399Ile* polymorphisms in individuals of Malay ethnicity with regard to history of typhoid infection in this region.

2. Materials and methods

2.1. Study population

The study population included 304 convalescent typhoid patients (individuals belonging to the same endemic environment as the uninfected controls and hence considered 'susceptible' to typhoid) and 250 normal controls with no history of typhoid infection. Samples collected were approved and were in strict adherence to the ethical guidelines of the University Ethical Committee, Universiti Sains Malaysia. Malay ethnicity was decided based on the data from the individuals with no interracial marriages in the last three generations prior to the proband. The typhoid susceptible individuals were those who have had typhoid fever randomly selected from the Kelantan Typhoid Carrier registry consisting of subjects who have recovered from typhoid fever and are routinely tested for carrier status. Typhoid susceptible individuals included 140 males and 164 females. The mean age (±1SD) was 36.31 ± 15.19. The control subjects were unrelated healthy individuals who have never had typhoid infection or typhoid vaccination in their life time, and were negative for stool culture. They were selected from the same geographical environment and ethnic background as the susceptible individuals. The control group included 129 males and 121 females, the mean age being 31.93 ± 15.59.

2.2. DNA extraction

Genomic DNA from patients and control subjects were extracted from blood clots using DNAeasy Tissue Kit (Qiagen, Germany). The DNA concentration was determined by UV absorbance at OD 260 nm and the purity was determined by 260/280 ratio using a Nanodrop spectrophotometer.