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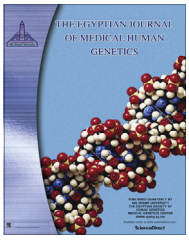

ORIGINAL ARTICLE

Analysis of *TLR* polymorphisms in typhoid patients and asymptomatic typhoid carriers among the schoolchildren

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KEYWORDS

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Abstract *Background:* *Toll like receptor* (*TLR*) plays a critical role in recognition and activation of both innate and adaptive immune responses against microbial pathogens. Several studies have implicated the genetic variations (polymorphisms) in *TLR* genes to influence the host susceptibility to infectious diseases. However, the available literature on *TLR* polymorphism and susceptibility to typhoid fever is unclear.

Aim: This study aimed to investigate the polymorphism of *TLRs* 1, 2, 4 and 5 in typhoid patients and convalescent phase asymptomatic typhoid carriers among the schoolchildren.

Subjects and methods: *TLR* genes were amplified by PCR from peripheral blood leukocytes of schoolchildren with typhoid ($n = 20$) or asymptomatic typhoid carrier ($n = 30$) state, and normal healthy individuals ($n = 50$). The RFLP analyses for *TLR*1, 2, 4 and 5 genes using restriction enzymes such as *AluI*, *AciI*, *NcoI* and *DdeI*, respectively, were performed to determine the single nucleotide polymorphism.

Results: *TLR*1 polymorphism was observed in 5% (1/20) of typhoid patients and 6.6% (2/30) of typhoid carriers. *TLR*2 polymorphism was observed in 10% (2/20) of typhoid patients and 6.6% (2/30) of carriers. *TLR*4 polymorphism was not observed in typhoid patients, but 6.6% (2/30) of typhoid carriers exhibited a polymorphism. As well, *TLR*5 polymorphism was not observed in typhoid patients, while 13.3% (4/30) of typhoid carriers had polymorphism. None of the control healthy individuals had evidence for *TLR* polymorphisms.

Conclusion: The study reports polymorphisms of *TLR* genes in a lower proportion among the schoolchildren with typhoid or convalescent typhoid carrier state.

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1. Introduction

Typhoid fever is a major public health problem worldwide and it is considered as a major enteric illness in the developing countries. It is an acute febrile disease caused by *Salmonella enterica* serotype Typhi [1]. Infection with *S*. Typhi occurs by fecal-oral route through ingestion of food and water contaminated with feces of typhoid patients and asymptomatic typhoid carriers [2–6]. The disease is endemic in the Indian subcontinent, Southeast Asia, Latin America and sub-Saharan Africa.

During its intracellular life in macrophages, *Salmonella* induces multiple regulatory components that are responsible for its endurance inside the host [7]. *Salmonella* has evolved remarkable strategies to avoid the host immune response. One of these strategies is a modification in lipopolysaccharide (LPS) structure, which facilitates *TLR*4-mediated downstream signaling cascade inducing host immune response [8,9]. Further, membrane remodeling blocks detection by host *TLR*4 and also increases the resistance of bacteria against host antimicrobial [10]. A study in Malay population has identified 8.9% *TLR*4 Asp299Gly and 7.2% *TLR*4 The399Ile polymorphisms in typhoid susceptible populations. In addition, *TLR*5 mediates the innate immune responses to *Salmonella* through binding to flagellin [11]. A polymorphism in the *TLR*5 gene introduces a premature stop codon ($TLR5^{392STOP}$), which might play a role in variation for binding to flagellin and mediating immune responses [12]. However, in our previous study, we have identified that *TLR*5 gene polymorphism was not associated with susceptibility to typhoid fever, as there was no significant correlation between *TLR*5 gene polymorphism and clinical parameters in typhoid patients and typhoid carrier [13]. As such, we sought to identify whether nucleotide polymorphisms in other *TLR* genes (e.g., *TLR*1, 2, 4 or 5) are involved in susceptibility *Salmonella* infection among the schoolchildren. We have recently reported the clinical correlates and serotyping of *Salmonella* isolates during typhoid fever and asymptomatic carrier state among the schoolchildren [14]. In this study, we report the association between polymorphism of *TLR* genes and occurrence of typhoid fever among the schoolchildren.

2. Subjects and methods

2.1. Demography

Venous blood samples collected from a cohort of schoolchildren previously reported to be positive for typhoid fever ($n = 20$), convalescent phase asymptomatic carriers ($n = 30$) and healthy control individuals ($n = 50$) among the schoolchildren in northern part of Tamil Nadu, India were used for identifying polymorphism in *TLR*1, *TLR*2, *TLR*4 and *TLR*5 genes [14].

2.2. PCR-RFLP analysis of TLR genes polymorphism

Genomic DNA was isolated from 2 ml of EDTA-treated blood using a DNA isolation kit (QlAamp DNA Blood Minikit Cat. No. 51106, Qiagen, Germany) and stored frozen at −20 °C for molecular analysis. The template DNA (200 ng) was amplified in a 50 μl PCR master mix (New England Biolabs) in Thermocycler (Techne, UK) using primers specific for *TLR*1, *TLR*2, *TLR*4 and *TLR*5 genes (Table 1) with slight modification of a procedure described previously. The PCR products were analyzed by electrophoresis on 1.5% agarose gel (Biotech, India). *TLR* genes nucleotide polymorphisms ($TLR1^{602I}$, $TLR2^{753Glu}$, $TLR4^{299D}$ and $TLR5^{392STOP}$) were identified by restriction enzyme (RE) digestion of PCR products with gene-specific RE (Table 1). For RFLP analysis of *TLR* genes, RE digestion of PCR products and analyses for size-specific products by electrophoresis were performed as described previously [13].

3. Results

The normal expression patterns of *TLR*1, *TLR*2, *TLR*4 and *TLR*5 genes were detected in genomic DNA from all the individuals of three groups. The amplified PCR products of *TLR* genes were observed on 1.5% agarose gel electrophoresis (Fig. 1). The PCR products of *TLR*1, *TLR*2, *TLR*4 and *TLR*5 genes from all subjects were subjected to RFLP using restriction enzymes to determine polymorphisms (Fig. 2). Among the typhoid patients, polymorphism of *TLR*1, *TLR*2, *TLR*4 and *TLR*5 genes were found to be 5%, 10%, 0% and 0% respectively. In contrast, the typhoid carriers exhibited a less variations of *TLR* genes polymorphism Viz. 6.6% of *TLR*1, *TLR*2, *TLR*4 and 13.3% of *TLR*5 (Table 2). Consequently, the control healthy individuals had no evidence for *TLR* polymorphisms.

4. Discussion

TLR plays a critical role in recognition and activation of both innate and adaptive immune responses against microbial pathogens [16]. Several studies have implicated the genetic variations (polymorphisms) in *TLR* genes to influence the host susceptibility to infectious diseases [17]. However, the available literature on *TLR* polymorphism and susceptibility to typhoid fever is unclear. *Salmonella* flagellin is one of the major virulence factors and thus genetic polymorphism on it cognate receptor, *TLR5*, might be critical for susceptibility to infection [15]. The study in *TLR*5 gene polymorphism at mature stop codon ($TLR5^{392STOP}$) has implicated for variation in binding ability to flagellin and differing host immune responses during typhoid fever [12]. However, in our recent study with a small cohort of typhoid patient and asymptomatic typhoid carriers, we have identified that *TLR5* gene polymorphism was not associated with susceptibility to typhoid fever, as there was no significant correlation between *TLR5* gene polymorphism and clinical parameters associated with typhoid fever [13]. Interestingly, *Salmonella* LPS is potent stimulator of innate immune cells and thus genetic variation in *TLR*4 gene might also influence the infection state during typhoid infection [8,9]. The prevalence of 12.5% of *TLR*4 polymorphism in typhoid-susceptible Malay populations was reported [11]. This *TLR*4 gene polymorphism in Malay population has been suggested to be a higher risk for typhoid infection. Taken together, it is possible that genetic variations in more than one *TLR* might render the higher susceptibility to typhoid infection. However, a RFLP analysis for polymorphisms in *TLR*1, 2, 4 and 5 genes, in the present study, revealed the