RESEARCH ARTICLE

TLR1 Polymorphism Associations with Gastric Mucosa Morphologic Patterns on Magnifying NBI Endoscopy: a Prospective Cross-Sectional Study

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Abstract

Background: *Helicobacter pylori* is now recognized as a causative factor of chronic gastritis, gastroduodenal ulcers, gastric cancer and mucosa-associated lymphatic tissue lymphoma. Toll-like receptors are important bacterial receptors in gastric epithelial cell signaling transduction and play critical roles in gastric carcinogenesis. Materials and Methods: A total of 400 patients undergoing esophagogastroduodenoscopy for investigation of chronic abdominal pain were genotyped for single-nucleotide polymorphisms (SNPs) in TLR1 (rs4833095) using TagMan SNPs genotyping assay by real-time PCR hybridization. Relationships with susceptibility to *H. pylori* infection and pre-malignant gastric mucosa morphological patterns, classified by magnifying NBI endoscopy, were investigated. Results: The percentages of TLR1 rs4833095, CC homozygous, CT heterozygous and TT homozygous cases were 34, 46.5 and 19%, respectively. CC showed statistical differences between *H. pylori* positive and negative cases (P<0.001). CT and TT correlated with type 1 and type 2 gastric mucosal morphological patterns (P <0.01) whereas CC correlated with types 3 and 4 (P<0.01). Conclusions: This study demonstrated good correlation of TLR1 rs4833095 genotype with severity of inflammation in *H. pylori* infected gastric mucosa according to gastric mucosal morphologic patterns with magnifying NBI endoscopy

Keywords: TLR1 (rs4833095) - gastric mucosal morphologic patterns - *H. pylori* - Magnifying NBI endoscopy

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Introduction

Helicobacter pylori is the major cause of gastritis (80%) and gastroduodenal ulcer disease (15%-20%) (Amieva and El-Omar et al., 2008). Gastric cancer (GC) results from infection of the gastric mucosa by *H. pylori* which initially induces acute inflammation and progresses over time to chronic inflammation, gastric atrophy, intestinal metaplasia, dysplasia, and finally intestinal-type GC in a subset of patients (Castano et al., 2014). There are several factors that contribute to the development of GC such as *H. pylori* infection, host and environmental factors (Pinto and Salama, 2005).

Several host factors such as inflammatory proteins including cytokines, growth factors, and chemokines have been known to control adaptive immune response against *H. pylori* infection (Macarthur et al., 2004). Toll-like receptors (TLRs) belong to innate immune response and provide first line of host defense against several conditions including *H. pylori* infection. TLRs consist of 11 transmembrane proteins. Some studies have reported the role of TLR1 in activation of innate immune responses to *H. pylori* (Yokota et al., 2007). Polymorphisms in TLR1 signaling pathways have been shown to modulate the risk of *H. pylori* infection, gastric precancerous lesions, and/or GC. For example, TLR1 rs4833095 CT genotype or T allele has been shown to be correlated with a decreased risk of *H. pylori* infection and decreased risks of chronic atrophic gastritis and intestinal metaplasia in Chinese patients (Yang et al., 2013). TLR1 rs5743618 appears to impair the surface expression of TLR1 of NK cells and NK cell derived IFN-γ production (Yang et al., 2013). TLR1 I602S variant is the most prevalent functionally relevant SNP in Europeans with a frequency of the variant G allele of 75%, while its frequency in Africans and Asians is 25% and 1%, respectively (Hawn et al., 2007). In Thailand, we first host genetic models for Mdm2 SNP309 with *H. pylori*-related gastritis in Thai patients and play roles in promoting gastric diseases (Tongtawee et al., 2015; Tongtawee et al., 2015; Tongtawee et al., 2016).

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Worldwide, studies have shown inconsistent results regarding the association of TLRs polymorphisms with gastric diseases. There is no study on this issue Thailand. In the present study we therefore aimed to evaluate association of TLR1 SNPs (rs4833095) with gastritis, using a prospective cross-sectional study. We also determined a difference in the presence of genotype between *H. pylori* positive and negative patients, as well as the correlations between TLR1 (rs4833095) SNPs and gastric mucosa morphologic patterns in a population from northeast Thailand.

Materials and Methods

Patients

Four hundred patients undergoing esophagogastroduodenoscopy (EGD) for investigation of chronic abdominal pain participated in this study from December 2014 to March 2016.The following exclusion criteria were applied: previous *H. pylori* eradication treatment prior to the previous 2 months, significant medical illnesses, history of previous gastric surgery, and the use of antimicrobials or gastrointestinal medications like PPIs or bismuth compounds within the previous 2 months. The study was performed in accordance with good clinical practice and the guidelines of the Declaration of Helsinki. All patients provided written informed consent and the study protocol was approved by the Ethics Committee for Research Involving Human Subjects, Suranaree University of Technology (EC-58-58 and EC-58-59).

Biopsy Specimens

The esophagogastroduodenoscopy (EGD) procedures were performed using an upper GI video endoscope (Olympus EVIS EXERA III, CV-190). The whole stomach was examined first with conventional endoscopy and then biopsies were performed by using "Site Specific Biopsy" technique (Tongtawee et al., 2015). Gastric tissue specimens for histological analysis were sent to the pathologist. The hematoxylin and eosin stain and Giemsa stain were used for identification of *H. pylori*.

Image evaluation

All gastroscopic examinations were digitally recorded and still images of the observation sites were captured for use in the reproducibility study. The selected images were transferred to a software program without distorting brightness, contrast or color balance. A total of 400 pictures from 400 patients were selected for the inter and intra observer agreement study. All endoscopists were blinded to the results of the *H. pylori* status and histology before reviewing the gastroscopic picture.

DNA preparation

Genomic DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue of 400 gastric patients using the QIAamp DNA FFPE tissue kit (Qiagen, USA). The DNA extraction was performed according to the manufacturer's instructions. Briefly, the paraffin from the tissue subsequently digested by lysis buffer and proteinase K. DNA was purified from the tissue lysate through QIAamp spin column. Then DNA was eluted.

SNPs selection and Genotyping

Four common single-nucleotide polymorphisms (SNPs) in TLR1 rs4833095 (C>T) selected according to the SNP database of the National Center for Biotechnology Information. The genotype of TLR polymorphism was determined by TagMan allelic discrimination using predesigned Custom TagMan SNP Genotyping Assay by real-time PCR. Forward and reverse primers were used along with wild-type probe VIC and probes FAM used for variant allele. Primers and probes were supplied by Applied Biosystems. The real-time PCR system, according to the manufacturer's instructions (LightCycler® 480 II instrument (Roche diagnostics, Neuilly sur Seine, France). Briefly, the PCR conditions were as follows: 95^{O}C for 10 min, 55 cycles of 95^{O}C for 15 s and 60^{O}C for 1 min. The success rate of genotyping for each SNP was more than 94%. Negative controls and duplicate samples were used to check the accuracy of genotyping and initially analyzed with LightCycler® 480 Software 1.5.

Statistical analysis

SPSS for Windows (version 20.0; SPSS, Chicago, IL, USA) was used for the statistical analysis. The differences in detection for the presence of genotypes between the *H. pylori* positive and negative groups were tested using chi-square. The correlation between genotype of TLR1 polymorphism and the Gastric Mucosal Morphologic Patterns of the patients were analyzed, significance was set at $p<0.05$. The patterns of genetic polymorphism were analyzed by using LightCycler® 480 Software 1.5 (Roche diagnostics, Neuilly sur Seine, France).

Results

TLR1 polymorphism and gastritis

A total of 400 patients were enrolled. The comprised 136 males and 264 females 17 to 80 years (mean ± SD, 44.6±15.9). The TLR1 rs4833095 polymorphism was observed in different genotypes. The distribution of the allelic and genotypic frequencies summarized in Table 1. CT was common in this ethnic Thai population showing frequency of 46.5% whereas CC and TT was 34% and 19.5%, respectively. The scatter plot analysis of TLR1 (rs4833095) polymorphism using Tag Man SNPs Genotyping assay shown in Figure 1. Among the 400 cases 204 *H. pylori* positive and 196 negative. Genotype distribution *H. pylori* infection summarized in Table 2 determine difference in the presence of genotype between *H. pylori* positive and negative patients. The percentage of genotype detections of CC, CT and TT was 30.5, 1 and 19.5% in *H. pylori* positive patients and 3.5, 45.5 and 0% in *H. pylori* negative patients (Table 2). The CC genotype majority of *H. pylori* positive patients whereas the CT majority of *H. pylori* negative patients. Results indicate that CC, wild-type genotype a signal *H. pylori* infection. Strong signal might contribute to gastric inflammation.

TLR1 polymorphism and gastric mucosa morphologic patterns

Table 1. Demographics Data of Gastritis Patients and TLR1 Genotypes Frequencies

Characteristics	Total = 400
Male/female (N)	136/264
Median age ±SD (years)	44.6 ±15.9
TLR1 CC genotype	136(34%)
CT genotype	186(46.5%)
TT genotype	78(19.5%)
Gastric Mucosal Morphologic Patterns	
Type 1	108(27%)
Type 2	136(34%)
Type 3	100(25%)
Type 4	56(14%)

Table 2. TLR1 rs4833095 Genotype between *H. pylori* Positive and Negative Cases

Genotype	*H. pylori infection status*		p value
	%Positive	%Negative	
CC	122(30.5)	14(3.5)	<0.001
CT	4(1)	182(45.5)	<0.001
TT	78(19.5)	0	<0.001

Table 3. TLR1 rs4833095 Polymorphism and Gastric Mucosal Morphologic Patterns using Magnifying NBI Endoscopy

Patterns of TLR1	Gastric Mucosal Morphologic Patterns			
	Type1	Type2	Type3	Type4
CC homozygous	5	13	73	45
CT heterozygous	66	94	19	7
TT homozygous	37	29	8	4

The gastric mucosal morphologic patterns were identified using magnifying NBI endoscopy. The type of gastric mucosal morphology was characterized as follows, type 1: the normal pattern is characterized by a regular arrangement of small, round pits (white spots) surrounded by a sub-epithelial capillary network (brown rings), type 2 is characterized by slightly enlarged, round pits with an unclear or irregular sub-epithelial capillary network in mild chronic gastritis mucosa. Type 3 is characterized by obviously enlarged, oval or prolonged pits with increased density of irregular vessels in moderate or severe chronic gastritis mucosa and type 4 is characterized by well-demarcated, oval or tubulovillous pits with clearly visible coiled or wavy vessels in moderate or severe chronic gastritis mucosa with atrophy and intestinal metaplasia. Type 2 was markedly high (34%) whereas type 1, type 3 and type 4 was 27, 25 and 14%, respectively in Thai patients (Table 1).

The observed frequencies of the genotyped SNPs in gastric mucosal morphologic patterns showed CC homozygous accounted for 5 and 13 patients in type 1 and type 2, accounted for 73 and 45 patients in type 3 and type 4, respectively. CT heterozygous showed similar results with TT heterozygous, a higher was accounted in type 1 and type2 than type 3 and type 4 (Table 3). When, we examined the correlation between genotype and type of gastric mucosa morphology, CC homozygous was significantly correlated with type 3 and type 4 ($p<0.01$) whereas CT and TT heterozygous was significantly correlated with type 1 and type 2 ($p<0.01$). Our classification have clearly shown a significant correlation between genotype and gastric mucosal morphologic patterns (Figure 2).

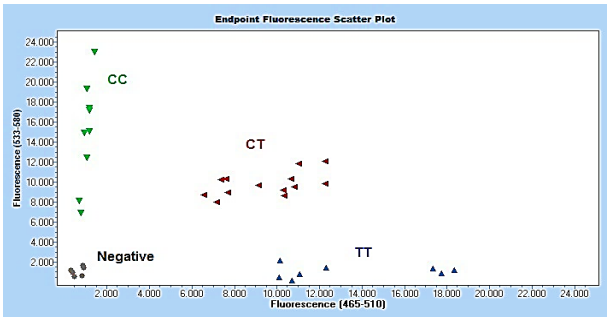

Figure 1. Scatter Plot Analysis of TLR1 (rs4833095) Polymorphism using Tag Man SNPs Genotyping Assay

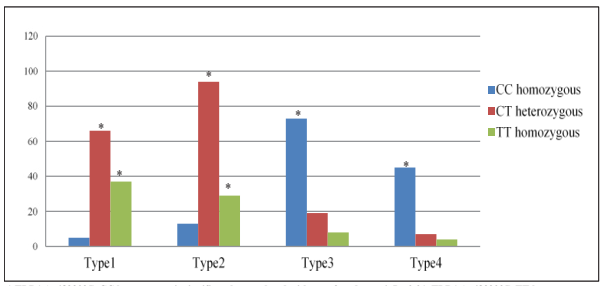

Figure 2. Correlation between TLR1 rs4833095 Polymorphism and Gastric Mucosal Morphologic Patterns

Discussion

In this study, the polymorphism of TLR1 rs4833095 was first identified in Thai population. The percentage of CC homozygous, CT heterozygous and TT homozygous has been reported on relationship between *H. pylori* infection and genotypic frequencies, as well as the correlations between TLR1 rs4833095 SNPs and gastric mucosa morphologic patterns. A previous case-control study of TLR1 rs4833095 in Chinese patients showed that CT genotype or T allele correlated with a decreased risk of *H. pylori* infection and decreased risks of chronic atrophic gastritis and intestinal metaplasia in (Yang CA et al., 2013). Our results support the finding that CT genotype is not related to the presence of *H. pylori* infection, even presence only 1% in *H. pylori* positive patients (Table 2). Most recently, we reported that *H. pylori* is associated with an increased risk of colorectal polyps, especially adenomas with dysplasia in the Thai population (Tongtawee et al., 2016). *H. pylori* infection has been shown to be strongly associated with gastric carcinoma by inducing inflammatory process and progresses over time. CagA is shown to be associated with several biological alterations of gastric epitheliums, including cell proliferation, cell structural and motility changes and alteration in cell adhesion (Basso et al., 2010; Johnson et al., 2012). From the study, the results showed that CC homozygous revealed the presence of the majority of *H. pylori* positive patients. This results indicated that CC, wild-type genotype provides a signal for *H. pylori*

infection and given the strong signal might contribute to gastric cancer. In addition, the CC homozygous was correlated with gastric mucosal morphologic patterns type 3 and type 4 which represent of severe inflammation suggesting that there was a correlation between TLR1 rs4833095 and gastric cancer development. Interestingly, CT heterozygous was correlated with gastric mucosal morphologic patterns type 1 and type 2 which represent of mild to moderate inflammation which was markedly high (34%) (Table 1). As a results, evaluation SNPs among Thai population. The CT heterozygous was common in this ethnic Thai population revealed the presence of the majority of genotypes (46.5%). These results can explain low gastric cancer development in the Thai population or "Thailand enigma" (Singh and Ghoshal, 2006). *H. pylori* infected gastric mucosa can be reliably identified using Magnifying NBI endoscopy and can also predict the histopathological severity of gastritis. This study has demonstrated the good correlation of TLR1 rs4833095 genotype and severity of inflammation in *H. pylori* infected gastric mucosa according to Gastric Mucosal Morphologic Patterns using Magnifying NBI endoscopy.

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