ORIGINAL ARTICLE

Gut and Liver, Vol. 8, No. 6, November 2014, pp. 632-636

Investigation of -308G>A and -1031T>C Polymorphisms in the *TNFA* Promoter Region in Polish Peptic Ulcer Patients

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See editorial on page 575.

Background/Aims: Tumor necrosis factor α (TNF-α) encoded by *TNFA* is a key mediator in inflammation, a precursor condition for peptic ulceration. Promoter polymorphisms of *TNFA* that influence its transcriptional activity and TNF-α production are known. *TNFA*-308G>A (rs1800629) and *TNFA*-1031T>C (rs1799964), which are responsible for increased *TNFA* transcription, could influence the risk of peptic ulceration. This study aimed to investigate these polymorphisms and to evaluate their association with peptic ulcer disease and *Helicobacter pylori* infection in the Polish population. **Methods:** Gastric mucosa specimens obtained from 177 Polish peptic ulcer patients were used to conduct rapid urease tests and to assess the investigated polymorphisms by polymerase chain reaction-restriction fragment length polymorphism. Genotyping data were compared with the results obtained from healthy individuals of Polish origin. **Results:** There were no significant differences in genotype and allele frequency of the investigated polymorphisms between peptic ulcer patients and healthy individuals. No associations between the frequencies of particular genotypes and alleles for both single-nucleotide polymorphisms (SNPs) and the presence of *H. pylori* infection in peptic ulcer patients and in subgroups of men and women with peptic ulcer disease were found. **Conclusions:** The investigated SNPs are not risk factors for either peptic ulcer or *H. pylori* infection development in the Polish population. The results require verification in a larger cohort. **(Gut Liver 2014;8:632-636)**

Key Words: Tumor necrosis factor-alpha; Genetic polymorphism; Peptic ulcer; Restriction fragment length polymorphism

INTRODUCTION

Helicobacter pylori infection is the main etiological factor of peptic ulcer disease (PUD). Also, as a cause of the gastroduodenal ulceration, an excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs) is of growing importance.[1] Peptic ulcer formation is inevitably connected with mucosal inflammation, a process modulated by several cytokines. One of them is tumor necrosis factor α (TNF-α) encoded by *TNFA* gene. This multifunctional cytokine was originally identified in mouse serum after injection with *Mycobacterium bovis* strain bacillus Calmette-Guerin and endotoxin.[2] TNF-α is secreted mainly by activated macrophages, and its production is stimulated by bacterial lipopolysaccharide. The elevated level of this cytokine was found in the gastric mucosa of patients with *H. pylori* infection.[3,4] Since agents reducing TNF-α concentration in gastric mucosa prevented indomethacin-induced gastric damage, TNF-α could also play a role in NSAID-triggered peptic ulcer formation.[5]

Considering the vital role of TNF-α in the pathogenesis of PUD, it is reasonable to expect that genetic polymorphisms influencing the *TNFA* expression and the cytokine production could affect individual susceptibility to the disease. Single nucleotide polymorphisms at positions -238, -308, -857, -863, -1031 of *TNFA* have been investigated as potential risk factors of PUD and other *H. pylori*-induced disorders like gastritis, intestinal metaplasia, or gastric cancer.[6-15] Unfortunately, these studies have produced conflicting results. For this reason, and since there is no such report from Polish population available, the purpose of this study was to investigate whether polymorphisms -308G>A (rs1800629) and -1031T>C (rs1799964) of *TNFA* promoter region are associated with PUD and *H. pylori*

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Received on June 21, 2013. Revised on September 27, 2013. Accepted on October 21, 2013. Published online on April 23, 2014
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl13224

infection in the Polish population.

MATERIALS AND METHODS

The investigation was in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee of Medical University of Lodz. One hundred seventy-seven unrelated outpatients (111 females: median age 53 years, range 14 to 85 years; 66 males: median age 55 years, range 20 to 84 years) who visited the Department of Surgery, District Hospital, Łęczyca, Poland for an gastroduodenoscopy because of dyspepsia and diagnosed as peptic ulcer were enrolled in the study. Presence of *H. pylori* was evaluated at the time of gastroduodenoscopy by rapid urease test (Instytut Żywności i Żywienia, Warszawa, Poland). Patients who were treated with NSAIDs were excluded. Control group was 248 healthy individuals, geographically and ethnically matched to the patients with no symptoms of active gastroduodenal diseases. Genotyping data for the control group was published earlier by Bednarczuk *et al*.[16] Data concerning exposure to carcinogens in the patients and controls were not available. All the subjects included in the study gave informed consent.

Genotyping of -308G>A and -1031T>C *TNFA* single-nucleotide polymorphisms (SNPs) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was isolated according to Genomic DNA Prep Plus protocol (A&A Biotechnology, Gdynia, Poland) from endoscopic biopsy specimens of gastric mucosa. PCR mixture consisted of DNA template, 0.2 mM of each primer, 10 μL of Jump Start RedTaq ReadyMix™ (Sigma Aldrich, Munich, Germany) and PCR-grade water to a final volume of 20 μL. Negative controls were included in each experiment (samples without DNA template). The primer sequences and PCR reaction conditions used were published earlier.[17] Amplified DNA fragments for polymorphism -308G>A were digested by restriction enzyme *Nco*I during 16 hours at 37°C, whereas for -1031T>C SNP by *Bpi*I for 16 hours at 65°C. DNA fragments generated by digestion were separated in 12% polyacrylamide gel for -308G>A and in 2% agarose gel for -1031T>C. Electrophoresis pattern showed 126 and 21 bp fragments for wild type -308GG homozygote, 147 bp fragment for mutant -308AA homozygote, and three bands 147, 126, and 21 bp fragments for -308GA heterozygote. 249 and 15 bp bands for wild type -1031TT homozygote, 180, 69, and 15 bp bands for mutant -1031CC homozygote, and 249, 180, 69, and 15 bp bands for -1031CT heterozygote were present.

Statistical analysis were performed using the STATISTICA version 10 (StatSoft Inc., Tulsa, OK, USA) software package. The chi-square test was applied to evaluate conformity between the observed and expected genotype frequencies according to the Hardy-Weinberg rule and to determine the significance of differences in allele and genotype frequencies between the patients and controls. An odds ratio with a 95% confidence interval was estimated by logistic regression. A p-value <0.05 was assumed as significant in all the conducted tests.

RESULTS

All 177 gastric mucosa biopsy specimens were successfully analysed for polymorphisms at positions -308 and -1031 of *TNFA* promoter. All the data obtained and results of its statistical analysis were summarized in Table 1 for -308G>A and in Table 2 for -1031T>C.

All the genotypes for both polymorphisms were distributed in accordance with Hardy-Weinberg equilibrium within both patient and control cohorts which confirmed the cohorts as suitable. Genotype and allele frequencies occurred with similar frequencies in peptic ulcer and control groups for both -308G>A and -1031T>C SNPs. No statistically significant differences between investigated and control group were found (Tables 1 and 2).

According to the results of rapid urease tests, peptic ulcer patients were divided into two groups: *H. pylori*-infected and -uninfected individuals. Because of low occurrence of mutated alleles -308A and -1031C in the investigated cohorts, carriers of genotype -308GA and AA and carriers of -1031CC and CT were combined for analysis. There was no statistical difference between genotype and allele frequencies of the investigated polymorphism and the presence of *H. pylori* infection (Tables 1 and 2). Analogous associations were also examined in subgroups of female and male peptic ulcer cases. Although some dissimilarities in genotype and allele distribution between these subgroups and the whole peptic ulcer cohort for both investigated polymorphisms were demonstrated, no statistically significant association was found (Tables 1 and 2).

DISCUSSION

Host response to *H. pylori*-induced gastric mucosal inflammation is visible as an increased level of cytokines production. This appears to play a significant role in clinical outcome of the infection. The increased level of cytokines can be connected with ethnic diversity and relationship between expression and gene encoding cytokine polymorphisms.

The present study evaluated the effect of *TNFA* gene polymorphisms in patients with peptic ulcer in the Polish population. Frequencies of particular genotypes and alleles observed in the present study were similar to these obtained earlier in other Polish healthy control cohorts[18-22] and in patients suffered from multiple sclerosis[18] or colorectal cancer.[22]

There was no association between occurrence of peptic ulcer and any of investigated polymorphisms. This finding is in agreement with some of previously published results for peptic ulcer patients. No association was stated between PUD incidence and -308G>A *TNFA* polymorphism in Koreans,[13] duodenal ul-

Table 1. Comparison of the *TNFA-308G>A* Allele and Genotype Frequencies between Peptic Ulcer Patients and Healthy Individuals and between *Helicobacter pylori*-Infected and -Uninfected Peptic Ulcer Patients

	Peptic ulcer case (n=177)	Healthy individual (n=248)	p-value	OR	95% CI
GG	121 (68.4)	172 (69.4)	0.8775	1.00	-
GA	54 (30.5)	72 (29.0)		1.02	0.98–1.53
AA	2 (1.1)	4 (1.6)		1.04	0.46–2.35
G	296 (83.6)	416 (83.9)	0.9208	-	-
A	58 (16.4)	80 (16.1)		-	-
HWE p-value	0.3200	0.5878			
	All peptic ulcer cases				
	Infected (n=86)	Uninfected (n=91)			
GG	58 (67.4)	63 (69.2)	0.7981	1.00	-
GA or AA	28 (32.6)	28 (30.8)		1.09	0.57–2.06
G	144 (83.7)	152 (83.5)	0.9586	-	-
A	28 (16.3)	30 (16.7)		-	-
HWE p-value	0.2066	0.9802			
	Female peptic ulcer case				
	Infected (n=54)	Uninfected (n=57)			
GG	33 (61.1)	42 (73.7)	0.1573	1.00	-
GA or AA	21 (38.9)	15 (25.3)		1.78	0.59–5.39
G	87 (80.6)	98 (86.0)	0.2797	-	-
A	21 (19.4)	16 (14.0)		-	-
HWE p-value	0.2171	1.000			
	Male peptic ulcer case				
	Infected (n=32)	Uninfected (n=34)			
GG	25 (78.1)	21 (61.8)	0.1515	1.00	-
GA or AA	7 (21.9)	13 (38.2)		0.45	0.02–10.22
G	57 (89.1)	54 (79.4)	0.1312	-	-
A	7 (10.9)	14 (20.6)		-	-
HWE p-value	0.9200	0.9556			

Values are presented as number (%).
OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

cers and -308G>A or -1031T>C in eastern Indians,[9] or duodenal ulcer disease and -308G>A variation in Chinese Han population.[23] On the other hand, Lu *et al*.[7] showed that -1031T>C and -863C>A are host factors determining the risk of peptic ulceration upon *H. pylori* infection among Taiwanese, which implies the presence of some, not fully known ethnicity-related factors influencing the risk of PUD. In addition, it should be noticed that there are differences among studies in choosing control group (healthy volunteers[9,13,23] or dyspeptic patients[7]) which may produce discrepancies in the results. As the sample size used in the research is relatively small, the results should be considered as preliminary and verified in larger groups.

Also, there was no connection between any of investigated SNPs and *H. pylori* infection in peptic ulcer patients. This is in contradiction with data published elsewhere. Lu *et al*.[7] found significantly elevated risk of peptic ulcer after *H. pylori* infection in carriers of either -1031C or -863A allele from the Taiwanese population. Observed discrepancy could partly be explained by difference in the prevalence of highly virulent strains of *H. pylori*. While almost 100% of the *H. pylori* strains possess virulence factor-encoding cytotoxin-associated gene A (cagA+ strains) in Taiwan,[7] their incidence in Poland is estimated to be approximately 60%.[24] There is some evidence that -308A polymorphism was significantly related to infection with the *H. pylori* cagA+ subtype.[25] On the other hand, *TNFA* gene expression was stated to be independent of *H. pylori* cagA or vacA (vacuolating cytotoxin A) genotype.[24] Taking into account the research findings described above, a lack of data about the

Table 2. Comparison of the *TNFA-1031T>C* Allele and Genotype Frequencies between Peptic Ulcer Patients and Healthy Individuals and between *Helicobacter pylori*-Infected and -Uninfected Peptic Ulcer Patients

	Peptic ulcer case (n=177)	Healthy individual (n=248)	p-value	OR	95% CI
TT	113 (63.8)	167 (67.3)	0. 9663	1.00	-
CT	61 (34.5)	76 (30.6)		1.02	0.98–1.53
CC	3 (1.7)	5 (2.0)		1.04	0.46–2.35
T	287 (81.1)	410 (82.7)	0.5525	-	-
C	67 (18.9)	86 (17.3)		-	-
HWE p-value	0.2622	0.6228			
	All peptic ulcer case				
	Infected (n=86)	Uninfected (n=91)			
TT	54 (62.8)	59 (64.8)	0.7772	1.00	-
CT or CC	32 (37.2)	32 (35.2)		1.09	0.59–2.03
T	139 (80.8)	148 (81.3)	0.9036	-	-
C	33 (19.2)	34 (18.7)		-	-
HWE p-value	0.3684	0.7816			
	Female peptic ulcer case				
	Infected (n=54)	Uninfected (n=57)			
TT	33 (61.1)	35 (61.4)	0.9748	1.00	-
CT or CC	21 (38.9)	22 (38.6)		1.09	0.59–2.03
T	86 (79.6)	96 (79.8)	0.2797	-	-
C	22 (20.4)	23 (20.2)		-	-
HWE p-value	0.6867	0.5982			
	Male peptic ulcer case				
	Infected (n=32)	Uninfected (n=34)			
TT	21 (65.6)	24 (70.6)	0.6653	1.00	-
CT or CC	11 (34.4)	10 (29.4)		1.26	0.42–3.77
T	53 (82.8)	57 (83.8)	0.8762	-	-
C	11 (17.2)	11 (16.2)		-	-
HWE p-value	0.4748	1.000			

Values are presented as number (%).
OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

cagA status in the investigated *H. pylori*-infected cases could be considered as a limitation of our research. It should be specified in the future.

Recently, we showed that 3435C>T polymorphism of the *ABCB1* gene is a risk factor for *H. pylori* infection development in male peptic ulcer patients but not in female peptic ulcer patients.[26] Due to a significant overrepresentation of female in the investigated cohort of patients (women to men ratio, 1.68), analysis was performed in subgroups of women and men. Obtained results indicated that there was no association between investigated SNPs and risk of *H. pylori* infection in both female and male peptic ulcer cases.

Some researchers postulate the *TNFA* promoter polymorphisms act synergistically, so it is only possible to observe some their effects when many of these polymorphisms are analysed together. For example, Chakravorty *et al*.[9] did not found any association between single polymorphism locus of *TNFA* (-308, -857, -863, -1031) and *H. pylori*-mediated duodenal ulcer, but observed significantly higher percentage of *TNFA* haplotype -308G_-857C_-863A_-1031T in *H. pylori*-infected duodenal ulcer patients than in individuals with infection but without ulceration. Presence of such an effect could not be excluded in the present study. Further study is needes in peptic ulcer patients including other *TNFA* loci and haplotype analysis.

In conclusion, neither -308G>T nor -1031T>C SNP is a factor for genetic susceptibility to peptic ulcer in the population. Moreover, none of the investigated SNPs are the risk factors for *H. pylori* infection development in this group.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

Supported by grants from Ministry of Science and Higher Education/National Science Centre, Warszawa/Kraków, Poland (N-N405-161339) and Statutory Funds of Department of Pharmaceutical Biochemistry, Medical University of Lodz, Poland (503/3-015-02/503-01); and from Faculty of Pharmacy, Medical University of Lodz, Poland (2012 502-03/3-015-02/502-34-021).

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