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Original Research Article

Assessment of *TNFA* polymorphisms at positions -857 and -863 in Polish peptic ulcer patients

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ABSTRACT

Purpose: Peptic ulceration connected with chronic inflammation in gastrointestinal mucosa could be induced by *Helicobacter pylori* infection. Tumor necrosis factor alpha (TNF-α) encoded by *TNFA* gene is a key mediator in the inflammation process. There are several polymorphisms in the promoter of *TNFA* influencing its transcriptional activity. -857C>T (rs1799724) and -863C>A (rs1800630) substitutions may be responsible for increased *TNFA* transcription and TNF-α production. The association of these two polymorphisms with peptic ulceration and the development of *H. pylori* infection in peptic ulcer patients in Poles were evaluated.

Material and methods: Polymorphisms were assessed by PCR-RFLP in 203 peptic ulcer patients. *H. pylori* infection was confirmed by rapid urease test. The results of genotyping were compared with those obtained for 248 healthy Polish individuals.

Results: There were no significant differences in genotype and allele frequencies for both investigated polymorphisms between peptic ulcer patients and healthy individuals. No associations between frequencies of particular genotypes and alleles for both SNPs and the presence of *H. pylori* infection in peptic ulcer patients and in subgroups of peptic ulcer women and men were confirmed.

Conclusions: The investigated SNPs are not risk factors for peptic ulcer development. They are not risk factors for *H. pylori* infection in ulcer patients.

1. Introduction

The pathogenesis of peptic ulcer disease (PUD) is complex and usually referred to as the predominance of aggressive factors over protective ones, resulting in inflammation, and then in the damage of gastric mucosa [1]. The strong association of the disease with *Helicobacter pylori* infection is widely recognized, but non-steroidal anti-inflammatory drugs or low-dose aspirin usage are increasingly important causes of PUD. Due to the fact that some individuals show different susceptibility to developing PUD even with the same etiological factor, more and more attention in this context is paid to the host's genetics. In a twin cohort study, Räihä et al. [2] showed that concordance for the diseases was significantly higher in monozygotic twins than in dizygotic twin pairs. The study demonstrated that the familial aggregation of PUD is attributable almost solely to inherited factors and not to the shared environment.

Many polymorphisms of genes potentially affecting inflammation process and immunological response to *H. pylori* are currently being researched to establish risk factors of development of PUD and other gastrointestinal diseases (gastritis, intestinal metaplasia, or gastric cancer). These include *e.g.* genes encoding interleukins (*IL1B*, *IL2*, *IL4*, *IL6*, *IL8*, *IL10*), toll-like receptor 4 (*TLR4*), or mannose-binding lectin 2 (*MBL2*). It also includes *TNFA* gene encoding tumor necrosis factor-alpha (TNF-α) [3–5].

TNF-α is a multifunctional cytokine involved in lipid metabolism, coagulation, insulin resistance and endothelial function. It is also one of most important cytokines involved in the promotion of the immune response secreted by monocytes and macrophages. It plays a key role in protection from microbial infection, and its

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production is stimulated by bacterial lipopolysacharide (LPS). An elevated level of this cytokine was confirmed in the gastric mucosa of patients with *H. pylori* infection [6,7]. The cytokine also plays a part in the pathogenesis of various inflammatory or autoimmune diseases. Its synthesis is controlled at transcription level and is influenced by polymorphic variations in the promoter region of the gene. These polymorphisms of *TNFA* were confirmed as risk factors for various conditions caused by a combination of immune, genetic and environmental factors, i.e. rheumatic heart disease [8], chronic obstructive pulmonary disease [9], chronic rhinosinusitis [10], multiple sclerosis [11] or systemic lupus erythematosus [12].

In 1998, Higuchi et al. [16] reported for the first time three polymorphisms in the promoter region of *TNFA* gene: T to C change at positions -1031, C to A at -863 and C to T at -857, relative to the transcription start site in Japanese. They found that in individuals possessing at least one new allele, concanavalin A (Con A)-induced TNF-α production by peripheral mononuclear blood cells was higher than in those possessing only wild type alleles. Moreover, the transcriptional promoter activity of the -1031C/-863A or -857 T allele in response to Con A stimulation was higher than that of the dominant allele. The researcher suggested that the newly described polymorphic sites could be connected with interindividual differences in the amount of TNF-α produced in immune responses to various stimuli. In the same year, -857C>A polymorphisms were also confirmed in patients in France and Northern Ireland [13], and -863C>A and -857C>T both in Caucasians and Cambodians [14]. Uglialoro at al. [14] stated that the polymorphisms have no effect on *TNFA* gene expression in activated lymphocytes, but they are non-randomly associated with three extended HLA haplotypes. The researchers suggested these polymorphisms likely serve as markers for neighboring genes encoding HLA or other unknown molecules in the MHC that could influence disease susceptibility. Others [15] showed the mutated allele -863A is connected with lowered transcription activity of *TNFA in vitro*, and Swedish male carriers of this allele had significantly lower TNF-α serum level, which was in contrast to data published earlier [16].

Apart from that, the exact influence of *TNFA* promoter polymorphisms on its transcription activity is still a matter of debate, with some studies showing the connection between the various promoter polymorphisms of *TNFA* and susceptibility to PUD. The majority of the studies examined the widely known -308 G>A substitution, which was investigated as a gastric and/or duodenal ulcer risk factor in Spanish Caucasians [17,18], Chinese Han [19], Koreans [20,21], Mexicans [22], Italians [23], Japanese [24] and Taiwanese [25], all giving conflicting results. In contrast, there are few reports revealing the possible connection between -857C>T and -863C>A and susceptibility to peptic ulcer diseases, most of them based on research undertaken on an Asian population. -857C>T and -863C>A were associated with gastric but not duodenal ulcers in Japanese patients [24]. On the other hand, none of the mentioned polymorphisms was found to be connected with duodenal ulcer in Koreans [20] or eastern Indians [26]. The *TNFA* -857TT genotype was found to be linked with gastric and duodenal ulcers in Italians considering both the all-patients group and only *H. pylori*-infected subjects [23], and -863CA or AA genotypes in *H. pylori*-infected hosts in Taiwanese subjects [25].

Since these existing research publications delineate conflicting results, and there is no such report from the Polish population available, the purpose of this study was to investigate if polymorphisms -857C>T (rs1799724) and -863C>A (rs1800630) of the *TNFA* promoter region are associated with peptic ulcer disease and *H. pylori* infection in Poles.

2. Materials and methods

2.1. Patients

Two hundreds and three unrelated outpatients (130 females, 73 males, median age 54, min. age 14, max. age 85) were enrolled in the study, having attended the Department of Surgery, District Hospital, Łęczyca, Poland, for gastroduodenoscopy because of dyspepsia symptoms and diagnosed at this time as peptic ulcer patients. In all investigated cases, gastric mucosa specimens were taken from the antrum of the stomach. Biopsy specimens were collected between 2008 and 2011.

The control group was 248 healthy individuals, geographically and ethnically matched to the patients. Genotyping data for the control group were published earlier [27]. Data concerning exposure to carcinogens in patients and controls were not available. Patients who were treated with non-steroidal anti-inflammatory drugs were excluded. The investigation was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Medical University of Lodz (RNN/49/10/KE). All subjects included in the study gave informed consent.

2.2. Rapid urease test

For the diagnosis of *H. pylori* infection, a rapid urease test was used (Instytut Żywności i Żywienia, Warsaw, Poland).

2.3. DNA isolation

DNA was isolated according to the "Genomic DNA Prep Plus" protocol (A&A Biotechnology, Gdynia, Poland) from material biopsy specimens of gastric membrane mucosa. The purity and concentration of the DNA samples were estimated spectrophotometrically. The samples were stored at −20 °C until analysis.

2.4. PCR-RFLP

Genotyping of -857C>T and -863C>A *TNFA* SNPs was performed by the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. The PCR mixture consisted of the DNA template, 0.2 mM of each primer, 10 μl of Jump Start RedTaq ReadyMix™ (Sigma–Aldrich, Germany) and PCR-grade water to a final volume of 20 μl. Negative controls were included in each experiment (samples without a DNA template). The primer sequences and PCR reaction conditions used were published earlier [15]. Amplified DNA fragments were digested by restriction enzyme *Tai*I for 16 h at 65 °C for -857C>T and at 37 °C for -863C>A. DNA fragments generated by digestion were separated in 12% polyacrylamide gel. The electrophoresis pattern consisted of 106 and 22 bp fragments for wild type -857CC homozygote, 128 bp fragments for mutant -857TT homozygote, and three bands, 128, 106, and 22 bp fragments, for -857CT heterozygote. A 125 bp band for wild type -863CC homozygote, 101 and 24 bp bands for mutant -863AA homozygote, and 125, 101 and 24 bp bands for -863CA heterozygote were formed. Electrophoresis gel pictures for both investigated SNPs are provided in Fig. 1.

2.5. Statistical analysis

Statistical analysis was performed using the STATISTICA version 10 (StatSoft, Inc., Tulsa, OK, USA) software package. The χ^2 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