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Expression Levels of Vascular Endothelial Growth Factors A and C in Patients with Peptic Ulcers and Gastric Cancer

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Purpose: Vascular endothelial growth factor (VEGF) is one of the most important growth factors for metastatic tumors. To clarify the role of VEGF-A and C in patients with peptic ulcer disease (PUD) or gastric cancer (GC), we evaluated the expression levels of these two molecules. We also analyzed the effect of *Helicobacter pylori* infection on *VEGF-A* and *C* expression levels.

Materials and Methods: Patients with dyspepsia who needed diagnostic endoscopy were selected and divided into three groups: non-ulcer dyspepsia (NUD), PUD, and GC, according to their endoscopic and histopathological results. Fifty-two patients with NUD, 50 with PUD, and 38 with GC were enrolled in this study. *H. pylori* infection was diagnosed by the rapid urease test. After RNA extraction and synthesis of cDNA, the expression levels of *VEGF-A* and *C* were determined by quantitative reverse transcriptase polymerase chain reaction.

Results: The *VEGF-C* expression level in the PUD and GC groups was significantly higher than that in the NUD group. Moreover, the *VEGF-A* expression level in the PUD and GC groups was higher than in the NUD group, although the differences were not statistically significant. Significant positive correlations were also observed between the expression levels of these two molecules in the PUD and GC groups. In addition, the expression levels of these two molecules were higher in *H. pylori* positive patients with PUD or GC than in *H. pylori* negative patients of the same groups; however, these differences did not reach statistical significance.

Conclusions: Up-regulation of *VEGF-C* expression during gastric mucosal inflammation may play a role in the development of peptic ulcers or GC.

Key Words: Vascular endothelial growth factor-A; Vascular endothelial growth factor-C; Stomach neoplasms; Peptic ulcer; *Helicobacter pylori*

Introduction

Dyspeptic disorders such as gastroesophageal reflux, gastritis, peptic ulcer disease (PUD), and gastric cancer (GC) are major medical conditions.[1] PUD is usually associated with a reduced health-related quality of life, whereas GC is the fourth most common cancer and the second leading cause of cancer-related deaths worldwide.[2,3] Host factors such as genetics and nutrition, and environmental factors such as *Helicobacter pylori* infection may be involved in the development of these conditions.[1,4]

H. pylori infection has been shown to be a major risk factor for the development of PUD and GC.[5,6] However, despite several investigations, it is still not completely understood why the major-

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ity of infected people (80%~90%) carry and spread the bacterium while they are asymptomatic, or why only a small percentage of infected people develop peptic ulcers, whereas others develop GC.[6]

Host immune responses against *H. pylori* can result in chronic inflammation in the gastric mucosa, which in turn leads to the development of pathological conditions including PUD and GC.[5,6]

Vascular endothelial growth factors (VEGFs) are glycoproteins secreted by tumor cells that are the most important factors in angiogenesis and tumor metastasis.[7] The VEGF family includes VEGF-A to F and placental growth factor.[7,8] Studies have shown that VEGF-A and B play a key role in blood vessel growth, whereas VEGF-C and D are important for the growth of lymphatic vessels.[9,10] The role of VEGFs, particularly VEGF-A, C, and D in promoting angiogenesis and metastasis of many cancers including GC, has been previously discussed.[11,12] Moreover, inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α) are generally responsible for the epigenetic alteration of gastric epithelial cells.[13] These cytokines induce the mediators of angiogenesis, including VEGF and IL-8, which promote angiogenesis in cancer. These mediators also promote angiogenesis during chronic inflammation such as cardiovascular disease, rheumatoid arthritis, diabetic retinopathy, delayed-type hypersensitivity, and asthma.[14] It has been shown that VEGF-A expression is up-regulated in response to *H. pylori* infection.[15] Indeed, *H. pylori* activates the c-Jun N-terminal Kinases (JNK) signaling pathway, which leads to transactivation of the *VEGF-A* promoter. VEGFs promote angiogenesis, which is a pathophysiological mechanism that can result in inflammatory and ulcerative epithelial lesions and malignant tumor growth and metastasis.[15]

To understand the role of VEGFs in the pathogenesis of *H. pylori*-related gastric abnormalities, the mRNA expression levels of *VEGF-A* and *C* were determined in patients with peptic ulcers or GC, and compared with those with non-ulcer dyspepsia (NUD).

Materials and Methods

1. Patients and sampling

Patients with dyspepsia who underwent esophagogastroduodenoscopy at Imam Hospital or Tooba Outpatient Clinic (Mazandaran University of Medical Sciences, Sari, Iran) were enrolled in the study. All samples were collected between January 2012 and December 2013. The study was approved by the ethics committee of Mazandaran University of Medical Sciences. Clinical history, demographic data, and written informed consent forms were obtained from all study subjects. None of the subjects had a history of chronic inflammatory or autoimmune disorders or treatment with *H. pylori* eradication therapy, nor did they receive any non-steroidal anti-inflammatory drugs for 2 weeks prior to enrollment. Among patients with GC, none had undergone surgery, radiotherapy, or chemotherapy, or received any other medical intervention before sample donation.

Based on the endoscopic and histopathological assessments, the patient samples were divided into three groups: NUD, PUD, and GC. The histological grade of the gastric tumors was determined based on the state of differentiation. PUD was defined as a circumscribed mucosal break (>5 mm in diameter, with apparent depth) in the stomach or duodenum, covered with exudates. *H. pylori* infection was diagnosed by histopathological examination (including Giemsa staining) and a positive result on the rapid urease test performed on at least one additional biopsy sample. Patients were considered *H. pylori* positive if the results of one or both diagnostic methods were positive, and *H. pylori* negative if the results of both methods were negative. Patients in NUD group were then divided into two groups: *H. pylori* positive and *H. pylori* negative. Tissue samples were obtained from all patients during endoscopy and preserved in RNALater (Qiagen, Phoenix, AZ, USA).

2. RNA isolation and cDNA synthesis

Each tissue specimen was homogenized using mortar and pestle at room temperature. Total RNA was extracted from the dissected tissues using commercial RNA extraction kits (RNeasy Minikit; Qiagen), according to the manufacturer's instructions. The quantity and quality of the extracted RNA were assessed using a nanodrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and agarose gel electrophoresis, respectively. RNA (1 μg) was reverse-transcribed into complementary DNA (cDNA) using the RevertAid™ First-Strand cDNA Synthesis Kit (Fermentas, Pittsburg, PA, USA) primed with random hexamers as per the manufacturer's instructions.

3. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

VEGF-A, *VEGF-C* and hypoxanthine-guanine phosphoribosyl transferase (*HGPRT*, for normalization), sequences were obtained from the GenBank (Table 1). Primers for amplification of *VEGF-A*, *VEGF-C*, and *HGPRT* were designed using the Beacon designer 7 software and synthesized by TIBmol (Germany) (Table 1).

qRT-PCR was performed using 96 well plates (Bio-Rad Laboratories Inc., Hercules, CA, USA) in a volume of 20 μl containing