

Serotyping of Georgian Strains of *H.pylori* Based on Cytotoxin Producing Features (CagA and VacA) and the Role of Different Serotypes in the Development of Surgical Complications and Various Clinical Outcomes of PUD

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Abstract

Several genotypes of VacA are associated with both different levels of cytotoxin activity and different clinical outcomes of complicated PUD. Recent studies from developed countries have suggested that the CagA and VacA genes are more prevalent among the strains of *H.pylori* isolates from the patients with PUD. On the other hand, reports from some authors from the regions like developing countries, have found no association between the presence of cagA and vacA and the clinical outcome of *H.pylori* infection. Have been studied 21 strains of *H.pylori* taken from 16 Georgian patients using PCR. The results of our study suggest similarity to those findings in the prevalence of cytotoxin productive (CagA and VacA) genes and clinical outcomes.

Keywords: *cagA, vacA, peptic ulcer disease, H.pylori*

Introduction

In 1998, first time has been reported that about 50% of *H.pylori* isolates produce a vacuolating cytotoxin (VacA) [1]. VacA causes vacuolation of cells. Has been detected that human gastric epithelial cells have special sensitivity to VacA. Intragastric administration of

purified VacA produces gastric epithelial cell vacuolation and focal necrosis [2].

Several genotypes of VacA are recognized and are associated with both different levels of cytotoxin activity and different clinical outcomes. There are three families of VacA signal sequences (designated s1a, s1b and s2) and two families of middle region alleles (termed m1 and m2). Isolates, containing an s2 signal sequence do

not induce cell vacuolation and produce no detectable cytotoxin activity. Moreover, isolates containing type m1 middle regions have greater cytotoxic activity than those containing type m2 middle regions; consequently, the s1/m1 strains of *H.pylori* have greater cytotoxin activity than do s1/m2 strains [3]. The fact that there are multiple combinations of VacA signal-sequence and middle-region types suggest that recombination of *H.pylori* genomes occurs in vivo and has the wide variety.

The type s1 vacA alleles are significantly associated with the presence of a gene as cytotoxin-associated gene A (cagA) [3]; cagA encodes a 128-kDa protein (CagA) that is present in about 60% of *H.pylori* strains. Most strains that produce detectable vacuolation of cells in vitro are cagA positive (+). However, the correlation between cytotoxicity and presence of CagA is not absolute. cagA is contained within a 40-kDa region of the *H.pylori* chromosome known as the cag pathogenicity island. Other genes within this region encode products required for *H.pylori* to induce cytokine expression in epithelial cells.

Toxin positive strains of *H.pylori* are associated with an increased induction, relative to cytotoxin-negative strains, of the oxidative burst in polymorphonuclear neutrophils [4]. Because neutrophil activation is an important source of oxygen-free radicals, which have been implicated in the pathogenesis of peptic ulceration, the association of cytotoxin production and neutrophil activation by some *H.pylori* strains may be important in the development of peptic ulcers [5]. Moreover, both the strains that produce active cytotoxin and those that produce an oxidative burst in neutrophils are associated clinically with severe inflammation in the gastric mucosa [6].

Certain strains of *H.pylori* are more ulcerogenic than others. Infection with toxin-producing *H.pylori* strains is more common among the patients with peptic ulcer disease than among *H.pylori*-infected patients without peptic ulcer disease [7, 3].

In addition, toxin-producing *H.pylori* strains are strongly associated with both atrophic gastritis and gastric carcinoma [12]. Toxin-producing *H.pylori* isolates are more prevalent in patients with severe atrophic gastritis; the activity of cytotoxin is also much higher in *H.pylori* isolates from the patients with severe atrophic gastritis or duodenal ulcer than in those from patients with mild atrophic gastritis [13]. There is evidence of strong correlation between cytotoxin-producing strains of *H.pylori* and severity of mucosal damage in the gastrointestinal tract [10].

Materials and Methods

According to the above mentioned the aims of our study were to study cytotoxin producing features of *H.pylori* strains isolated from Georgian patients and to evaluate link between the clinical outcomes and presence of VacA and CagA genes. Strains, isolated from the patients suffered with Peptic Ulcer Disease (PUD) were studied using PCR (Polymerase Chain Reaction). All the patients had the surgical complications of PUD. Among them: 5 patients with perforated ulcers (PU); 8-pylorostenoses of different degree (PS); and 3 patients were admitted to the hospital because of the profuse upper GI bleeding (PB). The age of the patients varied from 25 to 58 year (Male-11, Female 6). All the patients applied to the Section of Abdominal Surgery at the Department of Surgical Diseases of Central Clinical Hospital of Tbilisi State Medical University. 5 patients with PU & adjuvant ferment peritonitis were underwent ulceroraphy and adequate course of treatment; 3 patients were operated due to the pylorostenoses (In 1 case Billroth-I technique and in the rest of cases Roux-n-Y technique has been applied); Patients with the PB were treated without surgical intervention (Thermo coagulation and adequate conservative treatment has been applied).

Before the treatment, all the patients were endoscoped. The biopsy samples were obtained in 11 cases during endoscopies and in 5 cases during the surgery. (Resected part of the stomach were opened and pieces of mucosal layer were incised; special intraoperative biopsy forceps were used as well in the cases of perforated ulcers). Biopsies were obtained from the antral part of the stomach and from the middle point of greater curve (see Fig. 1).

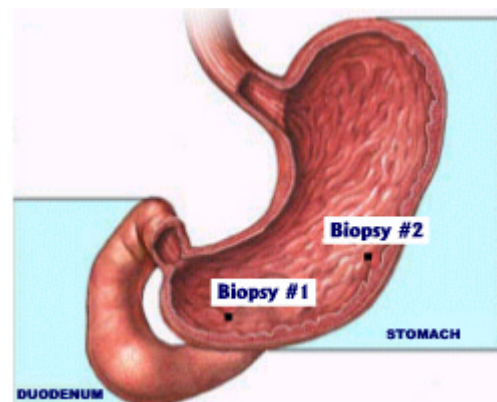


Fig.1. Places of Biopsies Obtained from the Gastric Mucosa

Results

All the isolates were analyzed in this study. *H.pylori* was identified by means of microscopic observation and urease, catalase and oxidase tests. Isolates were finally confirmed by PCR technique. The presence of *cagA* and *vacA* were detected using appropriate primers.

According to the data received, the strains were sorted out by serotypes. Based on containment of *CagA* and *VacA* genes, 4 serotypes were distinguished: Serotype I - *cagA*(+) *vacA*(+); Serotype II - *cagA*(+) *vacA*(-); Serotype III - *cagA*(-) *vacA*(+); Serotype IV- *cagA*(-) *vacA*(-). The prevalence of abovementioned serotypes in the PUD patients is given in the *Tab.1*.

Distribution of abovementioned serotypes were compared to the clinical features of complicated PUD. Mentioned comparison is reflected in the *Tab.2*.

Discussion

The severity of the disease were evaluated according to the criteria as follow: Anamneses, frequency of complications, applied treatment (if any), efficiency of the treatment applied (if any), clinical signs, the features and the trends of disease, appeared complains, results of specific diagnostic and general applicable tests etc.

As from the table #2, the prevalence of both *CagA* and *VacA* genes (Serotype I) is common and typical for the strains we studied; while the Serotype IV is less

prevalent and is typical to the seldom complications of PUD like PS. The strains that cause the upper GI bleeding are characterized with the high level of cytotoxin production. Middle position in the point of view of severity of clinical manifestations is taking by perforated ulcer.

Several studies from developed countries have suggested that the *cagA* gene and the allelic variant *s1* of *vacA* are more prevalent among the strains of *H.pylori* isolates from the patients with PUD (11). On the other hand, reports from some regions like developing countries, have found no association between the presence of *cagA* and *vacA* and the clinical outcome of *H.pylori* infection (12). The results of our study suggests that we have similarity to those findings in that the prevalence of genes and clinical outcomes.

According to our investigation we can conclude that presence of *CagA* and *VacA* genes have a predictive value as a risk markers for the development of PUD and the patients infected with the *vacA* and *cagA* positive strains may increase the risk of development of complicated conditions like: perforation of the ulcer, pylorostenoses and upper GI bleeding.

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The total number of investigated patients – 16			
The total number of isolates investigated – 21 (100 %)			
Serotype I	Serotype II	Serotype III	Serotype IV
<i>cagA</i> (+) <i>vacA</i> (+)	<i>cagA</i> (+) <i>vacA</i> (-)	<i>cagA</i> (-) <i>vacA</i> (+)	<i>cagA</i> (-) <i>vacA</i> (-)
9	7	2	3
42,8 %	33,3 %	9,52 %	14,28 %

Tab.1. *Prevalence of serotypes in the patients with pud.*

Clinical Outcome	Serotype I	Serotype II	Serotype III	Serotype IV
	<i>cagA(+)</i> <i>vacA(+)</i>	<i>cagA(+)</i> <i>vacA(-)</i>	<i>cagA(-)</i> <i>vacA(+)</i>	<i>cagA(-)</i> <i>vacA(-)</i>
PU	3	3	1	-
PB	3	4	1	-
PS	3	-	-	3

Tab.2. Comparison of serotype distribution with complicated clinical outcomes.

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Серотипирование штаммов *H.pylori* на основании продукции цитотоксинов (CagA и VacA) в грузинской популяции и роль различных серотипов в развитии хирургических осложнений пептической язвенной болезни желудка

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Р Е З Ю М Е

Существуют различные генотипы *vacA* и *cagA*, которые по данным литературы чаще встречаются в штаммах, выделенных у пациентов с осложненными формами пептической язвенной болезни желудка. Однако исследователи развивающихся стран, а также некоторые авторы из развитых стран сообщают, что четкой связи между наличием *vacA* и *cagA* с клинически осложненным течением пептической язвенной болезни не существует. Был изучен 21 штамм *H.pylori*, выделенный у 16 больных с помощью PCR. Результаты исследований выявили прямую связь между наличием *vacA* и *cagA* с осложненным течением пептической язвенной болезни.

Ключевые слова: *пептическая язвенная болезнь, H.pylori, cagA, vacA*