



Determination of the Status of *Helicobacter pylori sabA* Gene in Relation to Clinical Findings

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ABSTRACT

Background: Many *Helicobacter pylori* strains express adhesin proteins that bind to specific host-cell macromolecule receptors, like sialic acid binding adhesion (*sabA*). *SabA*-expressing strains have been associated with gastric cancer and negatively associated with duodenal ulcers. The aim of this study was to determine the status of *sabA* gene of *H. pylori* and its association with the clinical diseases in Iranian dyspeptic patients.

Methods: Eighty six biopsy block samples that were positive for *H. pylori* according Geimsa staining were included in this study. Genomic DNA was extracted from paraffin-embedded gastric biopsies obtained from dyspeptic patients. The identity of *Helicobacter* genus was determined through amplification of 16S rRNA which followed by *sabA* PCR using the gene-specific primers. The prevalence of *sabA* gene in three clinical groups including gastritis, gastric ulcer, and gastric atrophy was determined. The association of *sabA* gene and clinical outcomes was assessed statistically using Chi-square test. A *p*-value less than <0.05 was considered statistically significant.

Results: Total of 86 patients was included in this study. Seventeen cases out of 86 (23.6%) were yielded a positive result for *sabA* gene. The prevalence of *sabA* gene was 28.6% in both dyspeptic and Gastric atrophy patients as compared with peptic ulcers (19.2%).

Conclusion: For a first time the frequency of *sabA* gene using PCR methods was reported. The current study demonstrated that the *sabA* gene status was not associated with clinical diseases. In limited number of studied samples, higher frequency of *sabA* gene among dyspeptic and atrophic patients was found.

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Introduction

Helicobacter pylori is a Gram-negative and microaerophilic bacteria that is colonized in gastric tissues and cause chronic gastritis, peptic ulcer disease and gastric adenocarcinoma (1-3). The rate of infection in developing countries is high so that more than 80% of people are infected with *H. pylori* (4-9). The interaction of Host genetics, environment, and the bacterial virulence factors play a critical role in development of *H. pylori* associated diseases. Molecular studies on *H. pylori* pathogenesis have resulted in identifying the major virulence factors. Amongst the virulence factors, the cytotoxin associated geneA (*cagA*) and vacuolating cytotoxin A (*vacA*) are associated with severe clinical outcomes, especially in Western countries. However, such association has not been observed in East Asian countries (10). Many studies in various regions of Iran demonstrated the role of *cagA* and *vacA* (11, 12). However, the status of other virulence markers which play a significant role in early stage of pathogenesis like sialic acid binding A (*sabA*) has not been studied. Most of *H. pylori* strains express adhesion proteins that bind to specific host-cell macromolecule receptors (13, 14). *sabA* is an outer membrane protein which interacts with sialylated structures of human neutrophil, induces phagocytosis and oxidative burst and finally leads to damage of the gastric epithelium (14, 16). With sequencing of whole genome of J99, researchers found out that *sabA* gene is out of frame and have 9 CT repeat sequencing and according the number of CT repeat, *sabA* gene becomes

on or off (10, 14). The aim of current study was to determine the status of *sabA* gene of *H. pylori* in dyspeptic pateints.

Methods

Patients

In this descriptive study, gastric blocks biopsies of 84 patients who underwent gastric endoscopy were obtained during 2008-2010. The samples were taken from patients with different gastric disorders. Among 84 biopsies, 34 samples were form dyspeptic patients, 28 samples were from peptic ulcers patients, 18 from patients with gastric atrophy and 4 samples from NUD (Non Ulcer Disease) patient. Mean age (SD \pm 16.5) of patients was 56.6 years; ranging from 15 to 75 years included 52 females and 32 males.

Patients with history of treatment for *H. pylori* eradication, use of aspirin or other no steroidal anti-inflammatory drugs (NSAIDs), or antibiotics two weeks before the study were excluded from the study. A questionnaire including demographic characteristics such as age, gender, chief complaint, medical history, already used medication and family history of gastric polyp/cancer was completed for each patient using the medical records.

Histopathology

During endoscopy, two biopsy specimens were taken from the antrum for histologic evaluation. These specimens were fixed in 10% buffered formalin, embedded in paraffin and were cut in sequential 4 μ m sections.

One specimen stained with modified Giemsa stain for determination of the

presence of *H. pylori*. Morphological characteristics of *H. pylori* were seen under light microscope: curved and spiral form and intense blue coloring. Other sections were used for DNA extraction.

DNA Isolation

The micro section paraffin-embedded biopsies was deparaffinized with Xylen, and DNA was extracted using the QIAamp Tissue Kit, (QIAGEN, Hilden, Germany) according to the manufacturer's instruction, then DNA stored at -20°C until analysis.

PCR Amplification and Detection of amplified DNA Products

For determination of helicobacter, genus-specific primers (16S rRNA) described by Fox *et al* were used for the PCR amplification (17). The presence of *sabA* and 16S rRNA were determined by polymerase chain reaction (PCR). Primer sequences are listed in Table 1 (17, 18). All PCR mixtures were prepared in a volume of 25 µl, containing 1x PCR buffer, 500 nM of each primer, 1.5 mM MgCl₂; 200µM from each dNTP, 1.5U Taq DNA polymerase, and 300ng DNA sample. The mixtures were placed in a thermocycler (Eppendorf, Hamburg, Germany). PCR amplification was performed under the following conditions: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min. Annealing temperatures were 54°C for 16S rRNA and 56°C for *sabA* and extension at 72°C for 1 min, and final extension at 72°C for 10 min. Negative and positive controls were used in all reactions. PCR products were

visualized by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and examined under UV illumination.

Data Analysis

Chi-Square test were used in data analysis for categorical data; and *p*-values equal or less than 0.05 were considered statistically significant.

Result

Patients

A total of 84 patients including 32 (48.2%) female and 52 (51.8%) male with the mean age of 56.6 (SD ± 16.5) years old were studied. There was a significant correlation between age of patient and clinical outcomes (*p* < 0.001). Diagnosis of disease was based on pathological data of each patient which gathered from patient's data sheet in pathology laboratory.

H. pylori sabA Gene

The identity of *H. pylori* was confirmed using 16S rRNA gene. Seventy two (85.7%) of patients who yielded a 422 bp band were considered positive for *H. pylori*. Among 72 positive cases for *H. pylori* 16S rRNA, 17 (23.6%) were *sabA* positive.

Association of *sabA* Gene and Gastric Diseases

Among 72 *H. pylori* infected patients, 28.6% were diagnosed as functional dyspepsia, 19.2% as peptic ulcer (PU), and 28.6% as gastric atrophy cases. As showed in Table 2, the *sabA* gene was detected at a higher frequency in patients with gastric and atrophic symptoms, but the presence of this gene was not associated

with any type of disease ($p > 0.05$) (Table 2).

Discussion

H. pylori is one of the most genetically diverse bacterial species; and there are geographic genetic variations among *H. pylori* strains. Recently, the importance of sialic acid binding adhesin (*sabA*), has been increasingly clarified by researchers (10).

When host inflammation increases, expression of sialyl-Lewis x increases and *H. pylori* adhere to the gastric mucosa with *sabA*. Many of the genes encoding outer membrane proteins undergo phase variation, so that not all strains will produce functional proteins, and *sabA* expression is frequently switched on or off to respond acidic changes in the stomach (14). *sabA* production is indeed reported to be associated with severe intestinal metaplasia, gastric atrophy, and the development of gastric cancer in both developed and developing countries, emphasizing the importance of investigating *sabA* in developing countries (19).

In this study we examined 86 paraffin-embedded blocks of gastric biopsies. Patients were mostly more than 50 years old. Between age of patients and clinical outcomes there was a statistical correlation ($p < 0.05$), and gastric disease are mostly seen in elder patients.

Reports from different regions of Iran showed

variation about prevalence of *H. pylori*. In 2000 prevalence of *H. pylori* was reported 37.5% in city of Yazd and 47.5% in the city of Ardebil (20, 21). In 2004 prevalence of bacteria in Tehran was about 70% which showed significant increases (22). Molaei *et al* in 2010 reported the prevalence of *H. pylori* about 86% based on ureC gene (11). In this study for a first time, by using 16S rRNA that was specific for *Helicobacter* species we reported the prevalence of *Helicobacter* strain about 85.7%.

In Europe and America the prevalence of *sabA*-positive *H. pylori* was about 90% whereas in Japan was 80% (23). All reports were based on gene amplification and sequencing. But there wasn't any report from Iran and Middle East countries.

For a first time the prevalence of *sabA* gene was reported from Iran based on PCR technique, that was about 23.6%.

Also reported from Europe and America didn't find any correlation between *sabA* genes with clinical outcomes (24).

But Yamaoka *et al* reported that *sabA* "on" status related with cancer and atrophy and negatively related with neutrophil infiltration and duodenal ulcers (10).

In this study presence of *sabA* in gastric and atrophic patients was more prevalent but there wasn't any statistical correlation between clinical outcomes and *sabA* status ($p > 0.05$).

Table 1. Oligonucleotide primers using for 16S rRNA and *sabA* gene

gene	Primer designation	Sequences	Product size	Ref.
16s rRNA	16s-F	5'-GCT ATG ACG GGT ATC C-3'	422bp	17
	16s-R	5'-GAT TTT ACC CCT ACA CCA-3'		
<i>sabA</i>	<i>sabA</i> -F	5'- TTTTGTGTCAGCTACGCGTTC -3'	622 bp	18
	<i>sabA</i> -R	5'- ACCGAAGTGATAACGCGCTTG -3'		

Table 2. Association of *sabA* gene with diseases

Disease	<i>sabA</i> gene				Total	
	Negative		Positive		Num	Percent
	Num	Percent	Num	Percent		
Gastritis	20	71.4%	8	28.6%	28	100%
Ulcer	21	80.8%	5	19.2%	26	100%
Gastric Atrophy	10	71.4%	4	28.6%	14	100%
NUD	4	100%	0	0	4	100%
Total	55	76.4%	17	23.6%	72	100%

Results from this study showed that this gene can't be a reliable factor for determining the diseases. But the presence of this gene may contribute in development of acute gastritis and accumulations of bacteria in injured tissues and leading to accumulation of inflammatory cells and metaplastic changes of infected tissues.

Our study has several limitations. Only biopsy blocks from the antrum of the stomach were used for identifying the *sabA* gene statues in each patient, and the influence of sampling errors cannot be excluded, because patients may carry different strains in the antrum and corpus. However, the antrum is the predominant site of *H. pylori* colonization and the chance of false-negative results is lower than in corpus biopsies (25). Also *sabA* gene goes under phase variations and may be due to bad situation and changes in acidic condition in sampling and processing of tissues it might switch off or on. Thus for determining the role of this factor, we need to sequence the gene to find out the statue of gene and see if this gene is functional or not to decide about the role of *sabA* in developing of disease.

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Conclusion

For a first time the frequency of *sabA* gene using PCR methods was reported. The current study demonstrated that the *sabA* gene status was not associated with clinical diseases. In limited number of studied samples, higher frequency of *sabA* gene among dyspeptic and atrophic patients was found.

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