

Frequency of *sabA* Gene in *Helicobacter pylori* Strains Isolated From Patients in Tehran, Iran

Zahra Pakbaz¹, Mohammad Hasan Shirazi^{1,*}, Reza Ranjbar², Mohammad Reza pourmand¹, Mohammad Khalifeh Gholi¹, Amir Aliramezani¹, Ziba Vaise Malekshahi³

¹Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

²Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, IR Iran

³Master of Sciences, Microbial Biotechnology

*Corresponding author: Mohammad Hasan Shirazi, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran, Tel: +98-2188953021, E-mail: mhshirazi@tums.ac.ir.

Received: April 08, 2013; Revised: January 16, 2013; Accepted: August 04, 2013

Background: The importance of sialic acid binding adhesin (*sabA*) as a new outer membrane protein in gastroduodenal diseases has been recognized. The prevalence rate of *sabA* gene varies in different geographic areas.

Objectives: The aim of this study was to determine the frequency of *sabA* gene in *Helicobacter pylori* (*H. pylori*) strains isolated from different clinical outcomes in Tehran, Iran.

Patients and Methods: The study included 120 patients with dyspeptic symptoms admitted to the endoscopy suite of gastroenterology section of Firouzgar University Hospital, Tehran, Iran from March to August 2011. Gastric biopsy specimens were evaluated for the presence of *H. pylori* using standard microbiological method and polymerase chain reaction (PCR) assay. The *sabA* genopositive was determined by PCR in *H. pylori* strains.

Results: *H. pylori* isolates were recovered from 82 patients with duodenal ulcer (DU; n=17), gastric ulcer (GU; n=15), gastric cancer (GC; n=13), and gastritis (G; n=37). The frequency of *sabA* gene in *H. pylori* strains was 100% in gastric cancer, 86.7% in gastric ulcer, and 83.3% in both gastritis and duodenal ulcer.

Conclusions: This is a report on the prevalence of *sabA* gene in *H. pylori* isolated from different gastric patients in Iran. The results showed a high prevalence of *sabA* in our clinical *H. pylori* isolates.

Keywords: *SabA* protein, *Helicobacter pylori*; Gene Frequency; Gastric Ulcer

1. Background

Helicobacter pylori as a gram negative, curved, micro-aerophilic and motile organism with multiple polar flagella is the most common cause of gastric diseases including gastritis, duodenal and gastric ulcer, gastric atrophy, adenocarcinoma, and mucosa associated lymphoid tissue (MALT) (1). *H. pylori* infection is a major health problem in many parts of the world, occurring in 40 - 50% and 80 - 90% of the population in developed and developing countries respectively (2).

Several studies have shown that host and bacterial factors can play essential role in the appearance of clinical and pathologic complications. Among the bacterial factors, the ability of bacteria to bind to gastric epithelial cells is a crucial step for a successful infection, because it protects the bacteria from cleaning mechanisms such as fluid flow or pouring the mucous layers (3-7).

H. pylori has a wide range of adhesion components to bind to different carbohydrates. Sialic acid binding adhe-

sin (*sabA*) plays a critical role in the initial colonization of *H. pylori*, and this molecule has an important role in the establishment of persistent infection and chronic inflammation, which causes tissue damage (8, 9).

sabA, a 70-kDa (651-aa protein) outer membrane protein, is essential for attachment and activation of human cells via sialyl-Lewis x/a antigens (sLex and sLea) (10).

2. Objectives

The clinical prevalence of the *H. pylori* *sabA* genotype has not yet been determined in clinical isolates in Iran. In this study, we investigated the presence of *sabA* gene in Iranian clinical *H. pylori* isolates with different clinical outcomes.

3. Patients and Methods

3.1. Study Population

One hundred twenty patients with dyspeptic symptoms

Implication for health policy/practice/research/medical education:

No specific implication has been declared by author.

Copyright © 2013, Iranian Red Crescent Medical Journal; Licensee KowsarKowsar Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

were enrolled in the endoscopy suite of gastroenterology section of Firouzgar University Hospital, Tehran, Iran from March to August 2011. All patients were given informed consent for gastroscopic biopsy samples, which were obtained from the antrum.

3.2. Detection of *H. pylori* and the Presence of *sabA* Gene

Two gastric biopsy specimens were recovered from patients with gastrointestinal disease. One biopsy was

evaluated for rapid urease test (RUT), and the other was sent to laboratory for DNA extraction. DNA was extracted using the DNeasy blood & tissue kit (Qiagen Germany). The sample was considered *H. pylori* positive when a 411bp fragment of urease A gene (*ureA*) was amplified and RUT made positive. For the *H. pylori* -positive samples, the presence of *sabA* in *H. pylori* clinical isolates was analyzed by PCR using two primer pairs. PCR primers are listed in Table 1. Amplification was performed as previously described (11-13). The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide staining.

Table 1. The Primers used for the Amplification of *ureA* and *sabA* in This Study

Target gene	Primer Sequence (5-3)	Size of PCR product	Reference
<i>Urea</i>	F-CCCAATGGTAAATTAGTT	411bp	(11)
	R-CTCCTTAATTGTTTTAC		
<i>sabA</i> (Pair 1)	F-CTTAAAGGAACATTTATGAAA	785bp	(12,13)
	R-CACCGCGTATTGCGTTGGGTA		
<i>sabA</i> (Pair 2)	F-CCGCTAGTGCCAGGGTAAC	1330bp	(12)
	R-CGCGCTGTAAGGGTTATTGAAC		

3.3. Data analysis

Fisher's exact test was used for analyzing categorical data. A P-value < 0.05 was considered statistically significant.

4. Results

From 120 patients, *H. pylori* isolates were recovered from 82 patients with duodenal ulcer (DU; n = 17), gastric ulcer (GU; n = 15), gastric cancer (GC; n = 13), and gastritis (G; n=37). The mean age of the patients (50 male and 32 female) was 46 years. The presence of *sabA* gene was examined in all 82 of the *H. pylori* -infected patients with gastrointestinal diseases. The strains were considered *sabA* positive if they had one or two fragments of the gene. Two PCR assays yielded different positive rates for the *sabA* gene in 82 *H. pylori* isolates as shown in Table 2.

Table 2. Detection of *sabA* Gene by PCR in 82 *H. pylori* Clinical Isolates

Primer pair 1	Primer pair 2	Number
+	+	60
-	+	7
+	-	4
-	-	11

Seventy one strains (86.6%) had positive results for *sabA*. Table 3 shows the frequency of *sabA* gene among different clinical outcomes. The *sabA* genotype was detected in all patients with gastric cancer (100%), in 86.7% of patients with gastric ulcer, and in 83.3% of patients with gastritis

and duodenal ulcer.

Table 3. The Prevalence of *sabA* in the Different Disease Groups

Gene	Clinical outcomes			
	DU	GU	GC	G
PositivesabA	15	13	13	30
SabANegative	3	2	0	6
Total	18	15	13	36

Figure 1 and 2 show the results of *sabA* gene amplification in some strains of *H. pylori* using primers pair 1 and 2 respectively.

5. Discussion

The adherence of *H. pylori* to gastric epithelial cells has a crucial role in the specific tropism and pathogenicity of the organism in the human gastric epithelium and is a key step in creating a successful infection in gastric mucosa (3, 14-16). *sabA* is an important adhesion molecule in *H. pylori* which adheres to the host gastric epithelium (17).

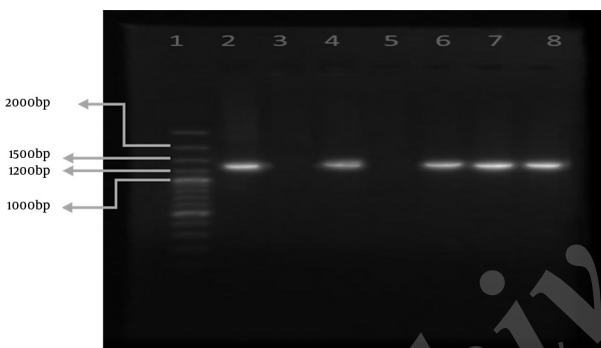
In a previous study performed on 200 *H. pylori*-infected patients, including 120 from Colombia and 80 from the United States, the prevalence rates of *sabA*-positive isolates were 44% in duodenal ulcer, 66% in gastritis, and 70% in gastric cancer (18). In a study in Taiwan, 80% (116 of 145) of *H. pylori* strains had positive results for *sabA* (19). In two other studies the frequency of *sabA*-positive isolates was reported 86% (37 of 43) and 93% (89/96) in French and Dutch respectively (20, 21). Moreover, the prevalence of *sabA* gene was 23.6% in Iran (22).

Figure 1. Amplification of *sabA* Using Primer Pair 1 in Representative Strains of *H. pylori*.



Lane 1 is the 100 bp marker. Lanes 2 and 3 are positive and negative control strains respectively. Lanes 4 to 7 are representative clinical *H. pylori* strains.

Figure 2. Amplification of *sabA* Using Primer Pair 2 in Representative Strains of *H. pylori*.



Lane 1 is the 100 bp marker. Lanes 2 and 3 are positive and negative control strains respectively. Lanes 4 to 8 are representative clinical *H. pylori* strains.

When compared to previous studies, our results showed that the *sabA* gene has high frequency in Iranian clinical isolates (86.6% (71 of 82)). The frequency of *sabA* in different clinical outcomes was 100% in gastric cancer, 86.7% in gastric ulcer, and 83.3% in both gastritis and duodenal ulcer. However no significant association was seen between the prevalence of *sabA* genotype and clinical outcomes ($P > 0.05$).

Two PCR assays produced different positive rates for the *sabA* gene in *H. pylori* isolates, with the 81.7% and 78% using primer pairs 1 and 2 respectively; indicating the possibility of sequence diversity in the *sabA* gene (12).

In conclusion, this is a report on the prevalence of *sabA* gene in *H. pylori* isolated from different gastric patients in Iran. More studies in other parts of country with a higher sample size are necessary to be performed to obtain a complete evaluation on the prevalence of *sabA* gene in Iran. Further tests should be performed to determine the exact role of *sabA* in *H. pylori* infection.

Acknowledgements

None declared.

Authors' Contribution

None declared.

Financial Disclosure

None declared.

Funding/Support

None declared.

References

1. Marshall B, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *The Lancet*. 1984;**323**(8390):1311-5.
2. Bittencourt PFS, Rocha GA, Penna FJ, Queiroz DMM. Úlcera péptica gastroduodenal e infecção pelo *Helicobacter pylori* na criança e adolescente. *Jornal de Pediatria*. 2006;**82**:325-34.
3. Hessej SJ, Spencer J, Wyatt JI, Sobala G, Rathbone BJ, Axon AT, et al. Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. *Gut*. 1990;**31**(2):134-8.
4. Logan RP. Adherence of *Helicobacter pylori*. *Aliment Pharmacol Ther*. 1996;**10 Suppl 1**:3-15.
5. Odenbreit S. Adherence properties of *Helicobacter pylori*: impact on pathogenesis and adaptation to the host. *Int J Med Microbiol*. 2005;**295**(5):317-24.
6. Segal ED, Falkow S, Tompkins LS. *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. *Proc Natl Acad Sci U S A*. 1996;**93**(3):1259-64.
7. Wen S, Moss SF. *Helicobacter pylori* virulence factors in gastric carcinogenesis. *Cancer Lett*. 2009;**282**(1):1-8.
8. Aspholm M, Olfat FO, Norden J, Sonden B, Lundberg C, Sjostrom R, et al. *SabA* is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. *PLoS Pathog*. 2006;**2**(10).
9. Ota H, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T, et al. *Helicobacter pylori* infection produces reversible glycosylation changes to gastric mucins. *Virchows Arch*. 1998;**433**(5):419-26.
10. Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, et al. *Helicobacter pylori* *SabA* adhesin in persistent infection and chronic inflammation. *Science*. 2002;**297**(5581):573-8.
11. Podzorski RP, Podzorski DS, Wuertth A, Tolia V. Analysis of the *vacA*, *cagA*, *cagE*, *iceA*, and *babA2* genes in *Helicobacter pylori* from sixty-one pediatric patients from the Midwestern United States. *Diagnostic microbiology and infectious disease*. 2003;**46**(2):83-8.
12. Shao L, Takeda H, Fukui T, Mabe K, Han J, Kawata S, et al. Genetic diversity of the *Helicobacter pylori* sialic acid-binding adhesin (*sabA*) gene. *Biosci Trends*. 2010;**4**(5):249-53.
13. Yanai A, Maeda S, Hikiba Y, Shibata W, Ohmae T, Hirata Y, et al. Clinical relevance of *Helicobacter pylori* *sabA* genotype in Japanese clinical isolates. *J Gastroenterol Hepatol*. 2007;**22**(12):2228-32.
14. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*. 1995;**55**(10):2111-5.
15. Hamlet* ‡ A, Thoreson‡ AC, Nilsson§ O, Svennerholm‡ AM, Olbe* L. Duodenal *Helicobacter pylori* infection differs in *cagA* genotype between asymptomatic subjects and patients with duodenal ulcers. *Gastroenterology*. 1999;**116**(2):259-268.
16. Magalhães A, Reis CA. *Helicobacter pylori* adhesion to gastric epithelial cells is mediated by glycan receptors. *Braz J Med Biol Res* 2010;**43**:611-8.
17. Yamaoka Y. Increasing evidence of the role of *Helicobacter pylori*

- SabA in the pathogenesis of gastroduodenal disease.* 2008.
18. Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, et al. Helicobacter pylori outer membrane proteins and gastroduodenal disease. *Gut.* 2006;**55**(6):775-81.
 19. Sheu BS, Odenbreit S, Hung KH, Liu CP, Sheu SM, Yang HB, et al. Interaction between host gastric Sialyl-Lewis X and H. pylori SabA enhances H. pylori density in patients lacking gastric Lewis B antigen. *Am J Gastroenterol.* 2006;**101**(1):36-44.
 20. de Jonge R, Pot RG, Loffeld RJ, van Vliet AH, Kuipers EJ, Kusters JG. The functional status of the Helicobacter pylori sabB adhesion gene as a putative marker for disease outcome. *Helicobacter.* 2004;**9**(2):158-64.
 21. Lehours P, Menard A, Dupouy S, Bergey B, Richey F, Zerbib F, et al. Evaluation of the association of nine Helicobacter pylori virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma. *Infect Immun.* 2004;**72**(2):880-8.
 22. Goudarzi H, Rezaee H, Rafizadeh M, Taghavi A. Determination of the Status of Helicobacter pylori sabA Gene in Relation to Clinical Findings. *J Med Bacteriol* 2012;**1**(1).

Archive of SID