Helicobacter pylori and Its Virulence Factors' Effect on Serum Oxidative DNA Damages in Adults With Dyspepsia

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Abstract- *Helicobacter Pylori* infection is a common gastrointestinal infection that can cause pathological effects, increase oxidative stress and induce an inflammatory response in gastric mucosa. Inflammatory aspects may prompt the production of radical oxygen substance (ROS) which may damage cells and release 8-hydroxydyoxyguanosine (8-OHdG) to serum. In this study, we evaluate the prevalence of *H. pylori* virulence factors and the association between serum level of 8-OHdG, *H. pylori* infection, and its various virulence factors. The presence of *H. pylori* and prevalence of cagA, babA and oipA genes in samples were determined by rapid urease test (RUT), histopathological exam (HE) and polymerase chain reaction (PCR) and oxidative DNA damage situation were assessed by using serum level of 8-OHdG. There was not any direct relation between *H. pylori* negative and *H. pylori* oipA+specimens by 8-OHdG serum level (P>0.05). In all clinical observations, the presence of cagA and oipA genes was common. There was a statistical relationship between the presence of cagA, babA factors, and high serum level of 8-OHdG (P<0.05). The presence of cagA and babA virulence factors may be associated with increased serum 8-OHdG in dyspeptic patients and may induce the damage to gastric cells.

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Keywords: *Helicobacter pylori*; Oxidative stress; Virulence factors; Dyspepsia

Introduction

Helicobacter pylori (*H. pylori*) is a spiral gram-negative microorganism. *H. pylori* infection is common in humans. The bacteria localize in the gastric mucosa and are known to be the main etiologic reason for gastric disorders such as gastritis, gastric ulcer (1). Many factors have a different role in the development of gastric disorders. Genetic predisposition of the patient and environmental causes and *H. pylori's* virulence factors affect this process (2,3). It was proved that *H. pylori* virulence factors such as cytotoxin-associated gene A (CagA), blood group antigen binding adhesion (babA), and outer inflammatory protein (oipA) increase the risk of gastritis and ulcer more than *H. pylori* without these (4-7). Aerobic organisms produce reactive oxygen species (ROS) in own Cells during their living metabolism. ROS are essential for normal physiologic activity, but its reactive result can be damage to various parts of cells and increase inflammation (8). Oxidative stress is a situation that results from an inequity between the production of ROS and antioxidant defense system. In the previous years, oxidative stress was suggested as one of the basic mechanisms of prolonged illness that causes health and economic problems like diabetes mellitus, cardiovascular disease and cancer pathogenesis (9). *H. pylori* infection attracts immune cells to the gastric mucosa and prompts active inflammation with neutrophilic infiltration and chronic inflammation with infiltration of lymphocytes, monocytes in the gastric mucosa (1,10). These neutrophils and monocytes produce oxygen free radicals that could cause DNA damage to the adjacent cells. ROS can lead to damage in the structure of DNA, but these damages could be repaired. Extreme unrepaired DNA can contribute to the pathogenesis and lead to gene modifications that are potentially mutagenic or carcinogenic (9,11). In nuclear and mitochondrial DNA, 8-hydroxydeoxyguanosine (8-OHdG) is one of the main forms of free radical-induced oxidative lesions and has therefore been generally used

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as a biomarker for oxidative stress. Studies showed that 8-OHdG is a helpful biomarker for risk valuation of various degenerative diseases. This metabolic product can be measured by enzymatic methods in tissues and plasma (12). The immunological procedures which usually progress in the gastric mucosa are probably decisive in the immune response determining the final outcome of the infection. Studies in *H. pylori* infection have discovered that adult *H. pylori* infection is usually in higher levels of gastric inflammation in comparison to children (13). The aim of this study was to investigate the relationship between serum 8-OHdG level, *H. pylori* infection and its virulence factors status in adults with Dyspepsia.

Materials and Methods

121 adult patients were included in this study, who were 20 to 70-year-old and have dyspeptic complication refer to Hajar hospital internal ward, determined with rapid urease test (RUT), Histopathological exam (HE) and polymerase chain reaction (PCR), that 67 of them been *H. pylori* positive. 4 patients were excluded for lacking demographic data. Patients with all positive tests (RUT, PCR, HE) were considered as positive for *H. pylori* infection. The procedure was approved by Ethics Committee of Shahrekord University of Medical Sciences. Gastritis was investigated by endoscopy. None of the patients had received nonsteroidal anti-inflammatory drugs (NSAIDs) and anticoagulants for 1 month before specimen collection, and none of them had received treatment for *H. pylori* infection, and no autoimmune disease was reported. Endoscopic biopsy samples, which were taken from antrum, were placed separately into a kit of Urease, sterilized water containing tubes and 10% buffered formalin containing tubes immediately transferred to the laboratory for HE and PCR (1,14-17).

Rapid urease test

One specimen of each patient was tested by RUT for detection of *H. pylori* that was performed with a Gastro urease kit (Bahar-Afshan Co, Tehran, Iran) according to manufacturer's instruction.

Genomic DNA extraction and polymerase chain reaction

DNA was extracted from biopsy specimens using a Genomic DNA Extraction kit (Bioflux, Japan) according to manufacturer's guidelines. The 16S rRNA and glmM genes were amplified to confirm the presence of the isolated *H. pylori* strains. For analyses of the presence of target genes (OipA, CagA, and BabA), *H. pylori* extracted DNA was amplified using specific oligonucleotide primers (Table 1). DNA samples from *H. pylori* (D0008; Genekam, Germany) were used as a positive control, and sterile distilled water was used as a negative control.

Table 1. Primers used for PCR analysis of 16SrRNA, glmM, cagA, oipA and babA genes

Reference	Gene	Size (bp) of PCR product	Primer sequence (5'-3')
(1)	*16S rRNA*	109	HP-1: CTGGAGAGACTAAGCCCTCC HP-2: ATTACTGACGCTGATTGTGC
(1)	glmM	161	F: AAGCTTTTAGGGGTGTTAGGGGTTT R: GCA TTC ACA AAC TTA TCC CCA ATC
(1)	cagA	244	F: ATGACTAACGAAACTATTGATC R: CAGGATTTTTGATCGCTTTATT
(1)	babA	272	F: CCAAACGAAACAAAAAGCGT R: GCTTGTGTAAA AGCCGTCGT
(2)	*oipA*	401	F: GTTTTTGATGCATGGGATTT R: GTGCATCTCTTATGGCTTT

Histopathological exam

One of Gastric biopsy specimens were merged in 10% buffered formalin, cut into sequential 4 μm sections and stained with silver and giemsa for *H. pylori* detection (18).

ELISA for recognition of serum 8-OHdG

Venous blood samples were taken from all patients at the admission time. Serums of *H. pylori*-positive and negative patients were obtained by centrifugation of blood samples and were stored at -85° C until assay. Serum 8-OHdG levels were measured with a commercial kit (Japan Institute for the Control of Aging (JAICA), Shizuoka, Japan). The measurement was performed according to the manufacturer's instructions. Serum 8-OHdG levels were compared between *H. pylori*

positive and negative patients.

Data analysis

The data were examined by SPSS software (Version 18.SPSS Inc, USA). Continuous variables were stated as mean or median matching to their homogeneity; categorical variables were described as a ratio. Comparison of variables was made with Student's *t*-test, and *P*.values was analyzed using Chi-square tests to describe any significant relationship between 8-OHdG serum level and virulence factors. *P*.values less than 0.05 were considered statistically significant.

Results

Table 2 gives an overview of the frequent of the cagA, oipA, and babA2 status. The oipA gene was detected in 62% of the *H. pylori* positive biopsies. The babA gene was found in 45% of *H. pylori* positive specimens. The cagA gene was found in 58% of *H. pylori* positive specimens. Six groups were formed according to *H. pylori* and presence of its virulence factors and 8-OHdG level were compared between these groups that shown in table 3.

Table 2. Frequency of the cagA, babA and the oipA in studied patients

Genotype	cagA		babA		oipA	
	Positive	Negative	Positive	Negative	Positive	Negative
Frequency (%)	39(58)	28(42)	30(45)	37(55)	42(62)	25(38)

Table 3. Concentration of serum 8-OHdG levels (ng/ml) in positive and negative specimens

Group	CagA+	babA+	oipA+	CagA+babA+	cagA+oipA+	oipA+babA+	CagA+babA+oipA+	Negative	Total
H. pylori	+	+	+	+	+	+	+	-	67
Male/famale	5/6	5/3	10/12	15/15	14/20	16/17	13/13	28/22	-
8 OHdG concentration (ng/ml)	2.5±0.4	2.3±0.2	1.4±0.4	2.9±0.3	2.8±0.2	2.2±0.3	3.4±0.4	0.8±0.2	-
***P*.value**	<0.03	<0.03	>0.05	<0.02	<0.05	=0.03	<0.01	>0.05	-
Total	11	8	22	30	34	33	26	50	-

Presence of cagA/babA, cagA/oipA, oipA/babA and cagA/oipA/babA virulence factors in specimens was 45%, 51%, 49% and 39% respectly. There was no significant difference between *H. pylori* negative, *H. pylori* cagA positive, *H. pylori* babA positive, *H. pylori* oipA positive, *H. pylori* CagA+babA+, *H. pylori* cagA+oipA+, *H. pylori* oipA+babA+group with respect to gender and age (not mentioned).

The ELISA results for detection of serum levels of 8-OHdG showed relationship differences in levels of 8-OHdG in *H. pylori*+comparing with *H. pylori*-samples. Serum 8-OHdG levels of *H. pylori* oipA+ and *H. pylori* (-) group were closer (1.4±0.4 ng/ml and 0.8±0.2 respectively *P*>0.05). The serum levels of 8-OHdG in other groups were high, and there were statistical differences between *H. pylori* negative, *H. pylori* OipA+ by *H. pylori* cagA+ and *H. pylori* babA+groups (*P*>0.05). Patients infected with *H. pylori* bearing CagA and babA has significantly higher 8-OHdG levels than other groups (*P*<0.05). Figure 1 shows an association between the presence of virulence factors and serum concentration of 8-OHdG in infected and non-infected patients.

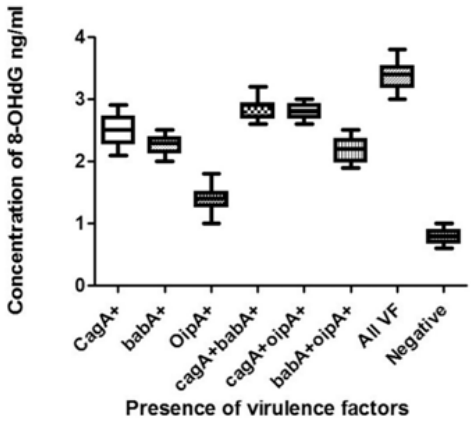

Figure 1. Comparison of *H. pylori* virulence factors genes status and serum level of 8-OHdG in patients

Discussion

H. pylori are known as a main pathogenic factor in humans, which causes active chronic inflammation and induce gastric mucosal disorders. More than 50% of the

world's human population are frequently infected with this bacteria with or without any symptoms (19). Also, there are variations in *H. pylori* prevalence in different countries. *H. pylori* virulence factors and infection are a source for creating free radicals. Previous reports have shown that there was a relation between *H. pylori* infection and generation of ROS in infected areas. Cellular glutathione concentrations in cells may be reduced by several causes such as *H. pylori* infection, and the cells can release the reactive oxygen species (ROS). Oxidative stress can induce the serum 8-OHdG level, and oxidative stress can increase by Elevated ROS production in the gastric mucosa. 8-OHdG is a proved indicator for oxidative DNA damage which makes one of the common changes in DNA induced by ROS (20). This is reported that *H. pylori* infection can induce gastritis and these inflammatory reactions prompt pathogenesis of cancer and this is observed in many studies that ROS may have roles in carcinogens. Chronic infection by *H. pylori* can induce chronic inflammation by attracting immune inflammatory cells such as macrophage and lymphocyte to the gastric mucosa, and these cells can produce ROS for killing the bacteria, but same ROS may damage the host cells structures (21). There are variations in the distribution of *H. pylori* virulence factor genes among strains in different zones of the world (22).

H. pylori produce cytotoxin-associated protein CagA, outer inflammatory protein oipA, and the blood group antigen binding adhesion babA, which are the important virulence factors that cause in the pathogenesis of *H. pylori* (23). Previous studies have shown that cagA, babA and oipA gene is present in 50-70%, 44-91%, 33-71% of *H. pylori* strains in Iranian patients (1,2). In this study, we found 58%, 45% and 62% infected patients with cagA-positive, babA-positive and oipA-positive strains which are similar to some reports from Iran (24). In this study, it was not observed any direct relation between *H. pylori* negative patients and serum level of 8-OHdG as like as previous reports. Also, at the H. *pylori* cagA-negative and babA-negative samples, there were not statistical relations between the presence of oipA virulence factor and serum level of 8-OHdG. However, another study observed that the serum levels of 8-OHdG in patients that infected by *H. pylori* was higher than negative patients (25).

By other observation in this study, the predominance of cagA and babA were observed in all clinical high concentration of 8-OHdG serum level in patients which is in agreement with another study (8,20,21,26). Contrary to our results, Tabatabaei *et al.*, showed that there was not an association between cagA status and 8 -OHdG serum level in patients (27).

To the best of this study, this observation is the first report considering associations between a high concentration of 8-OHdG serum level and babA2 virulence factor. Also, in this study, it is observed that in the combined form of virulence factors, the serum level of 8-OHdG is higher than other groups that these results are reported for the first time in the like studies. In conclusion, this study proposes that babA-positive, cagA-positive strains may damage to the cell and have a direct relation between the presence of these virulence factors and serum level of 8 -OHdG, especially in combined forms. CagA and OipA were common strains in Iranian patients, and there were not observed any statistical association between the presence of oipA virulence factors and clinical outcomes. Finally, it is suggested that involving more patients and virulence factors may be helpful in assessing the samples.

References

1. Shahi H, Reiisi S, Bahreini R, Bagheri N, Salimzadeh L, Shirzad H. Association between Helicobacter pylori cagA, babA2 Virulence Factors and Gastric Mucosal Interleukin-33 mRNA Expression and Clinical Outcomes in Dyspeptic Patients. IJMCM 2015;4:227-34.
2. Salimzadeh L, Bagheri N, Zamanzad B, Azadegan-Dehkordi F, Rahimian G, Hashemzadeh-Chaleshtori M, et al. Frequency of virulence factors in Helicobacter pylori-infected patients with gastritis. Microb Pathog 2015;80:67-72.
3. Lankarani K, Ravanbod M, Aflaki E, Nazarinia M, Rajaee A. High prevalence of Helicobacter pylori infection in Behcet's disease. BMC Gastroenterology 2014;14:58.
4. Matsunari O, Shiota S, Suzuki R, Watada M, Kingo N, Murakami K, et al. Association between Helicobacter pylori Virulence Factors and Gastroduodenal Diseases in Okinawa, Japan. J Clin Microbiol 2011;50:876-83.
5. Karabiber H, Selimoglu MA, Otlu B, Yildirim O, Ozer A. Virulence factors and antibiotic resistance in children with Helicobacter pylori gastritis. J Pediatr Gastr Nutr 2014;58:608-12.
6. Hagymási K, Tulassay Z. Helicobacter pylori infection: new pathogenetic and clinical aspects. WJG 2014;20:6386-99.
7. Matteo MJ, Granados G, Perez CV, Olmos M, Sanchez C, Catalano M. Helicobacter pylori cag pathogenicity island genotype diversity within the gastric niche of a single host. J Med Microbiol 2007;56:664-9.

8. Yeniova AO, Uzman M, Kefeli A, Basyigit S, Ata N, Dal K, et al. Serum 8 Hydroxydeoxyguanosine and Cytotoxin Associated Gene Aas Markers for Helicobacter pylori Infection. Asian Pac J Cancer Prev 2015;16:5199-203.
9. Raza Y, Khan A, Farooqui A, Mubarak M, Facista A, Akhtar SS, et al. Oxidative DNA damage as a potential early biomarker of helicobacter pylori associated carcinogenesis. Pathol Oncol Res 2014;20:839-46.
10. Lee K KA, Jackson C, Oconnor L. Helicobacter pylori CagA Triggers Expression of the Bactericidal Lectin REG3c via Gastric STAT3 Activation. Plos One 2012;7:12-20.
11. Ohnishi S, Ma N, Thanan R, Pinlaor S, Hammam O, Murata M, et al. DNA damage in inflammation-related carcinogenesis and cancer stem cells. Oxid Med Cel Longev 2013;2013:387014.
12. Murata M, Thanan R, Ma N, Kawanishi S. Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. Bio Med Res Int 2012;2012:20-8.
13. Yamaoka Y, Reddy R, Graham DY. Helicobacter pylori virulence factor genotypes in children in the United States: clues about genotype and outcome relationships. J Clin Microbiol 2010;48:2550-1.
14. Shahi H, Reiisi S, Sadeghiani M, Mahsa M, Bahreini R, Moghni M. Prevalence of cagA and babA2 genes in Helicobacter Pylori strains Isolated from Iranian gastrointestinal disorder patients and their gastritis classification. J Biol Today's World 2014;3:256-60.
15. Shahi H, Moghni M, Bahreini R, Reiisi S, Sadeghiani M, Rahimi M. Association Between H. pylori babA Virulence Factor with Clinical Outcome and ABO Blood Groups. JPAM 2014;9:285-90.
16. Espinoza MG, Vazquez RG. Detection of the glmM gene in Helicobacter pylori isolates with a novel primer by PCR. J Clin Microbiol 2011;49:1650-2.
17. Kargar M, Baghernejad M. Compression of three methods of polymerase chain reaction, culture and rapid urease test in diagnosis of Helicobacter pylori in gastric biopsy specimen. Koomesh 2010;11:198-203.
18. Manxhuka-Kerliu S, Telaku S, Devolli-Disha E, Ahmetaj H, Sahatciu-Meka V, Kerliu A, et al. Helicobacter pylori gastritis updated Sydney classification applied in our material. Prilozi 2009;30:45-60.
19. Torres LE, Melián K, Moreno A, Alonso J, Sabatier CA, Hernández M, et al. Prevalence of vacA, cagA and babA2 genes in Cuban Helicobacter pylori isolates. WJG 2009;15:204.
20. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res Rev 2004;567:1-61.
21. Arabski M, Klupinska G, Chojnacki J, Kazmierczak P, Wisniewska-Jarosinska M, Drzewoski J, et al. DNA damage and repair in Helicobacter pylori-infected gastric mucosa cells. Mutat Res 2005;570:129-35.
22. Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H. Helicobacter pylori endemic and gastric disease. Digest Dis Sci 2005;50:2075-80.
23. Chomvarin C, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis 2008;12:30-6.
24. Dabiri H, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, et al. Distribution of Helicobacter pylori cagA, cagE, oipA and vacA in different major ethnic groups in Tehran, Iran. J Gastroen Hepatol 2009;24:1380-6.
25. Khodaii Z, Ghaderian S, Najar RA, Nejati H, Panah AT. cagA and vacA status and influence of Helicobacter pylori infection on serum oxidative DNA damage in Iranian patients with peptic ulcer disease. Ir J Med Sci 2011;180:155-61.
26. Calvino Fernández M, Parra Cid T. H. pylori and mitochondrial changes in epithelial cells. The role of oxidative stress. Rev Esp Enferm Dig 2010;102:41-50.
27. Tabatabaei P, Khodaii Z, Ghaderian S, Akbarzadeh N. Influence of caga and Helicobacter pylori infection on serum oxidative DNA damage in patients with peptic ulcer disease. J Mic Biotech 2012;4:29-34.