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Original Article

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Analysis of *Helicobacter pylori* Genotypes Amongst Jordanians' Dental Plaque Samples

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection has been associated with gastritis, gastric ulcer, mucosa-associated lymphoid tissue lymphoma and gastric cancer. The prevalence of *H. pylori* virulence genes have been studied in different populations and from different sources of samples but their prevalence has not been studied in dental plaque in Jordanian people; therefore, the aim of this study was to determine the genotypes of *H. pylori* isolated from dental plaque samples.

Methods: Dental plaque samples were collected from 60 Jordanian volunteers. The genotypes of *H. pylori* virulence genes including the cytotoxin-associated gene A (*cagA*) and the vacuolating toxin (*vacA*) were determined using polymerase chain reaction (PCR).

Results: The *cagA* gene was detected in 14 (23.3%) samples, while *vacA* was detected in all volunteers enrolled in this study (100%). The most prevalent *vacA* alleles were m2 and s1 in 54 (90%) and 55 (91.7%) of volunteers, respectively. Compared to the other combinations including the most virulent *vacA* genotype s1/m1 which was detected in 11 (18.2%) of volunteers, the most prevalent *vacA* allelic combinations were s1/m2 and s2/m2 in 56 (93.3%) and 27 (45%) of volunteers, respectively.

Conclusions: These results indicate a significant carriage of virulent *H. pylori* strains among Jordanian people in their dental plaques, which increases the possible transmission of these strains among them. In addition, the studying of the genotypic pattern of *H. pylori* virulence genes in the dental plaque could represent an essential tool for infection prevention and predicting the severity and prognosis of *H. pylori* gastric infection.

Keywords: Jordanian; *Helicobacter pylori*; Dental plaque; Virulence genes; The cytotoxin-associated gene; The vacuolating toxin; Alleles; Polymerase chain reaction

Introduction

Helicobacter pylori (*H. pylori*) infection has been found to induce chronic gastritis, gastric ulcer, mucosa associated lymphoid tissue lymphoma, and gastric carcinoma [1]. Gastric carcinoma is categorized as the third most common cause of death worldwide [2]. Bacterial genetic heterogeneity, environmental factors and age of acquisition determine the clinical outcome associated with *H. pylori* infection [3, 4]. The genetic variation within genes encoding virulence factors plays a major role in the pathogenesis of different *H. pylori* strains [5]. The virulence genes of *H. pylori* are located within a DNA segment of 35 - 40 kbp called the pathogenicity island. The cytotoxin-associated gene A (*cagA*) and the vacuolating toxin (*vacA*) are the most studied genes among others [6, 7]. They are used as prognostic factors and as epidemiological markers [8]. It has been found that more than 50% of *H. pylori* isolates carry the *cagA* gene [9]. Upon infection with *H. pylori*, the CagA protein is translocated into the cells by a type IV secretion system. CagA as a virulence factor is known to deregulate several vital signaling pathways of the host including the disruption of tight junctions that increases probability of gastric ulceration and cancer development [10].

The *vacA* gene encodes the vacuolating cytotoxin A which increases the risk of peptic ulceration and gastric cancer [11] through the induction of vacuolation in gastric cells, interfering with mitochondrial functions, and stimulation of apoptosis [12]. Despite the fact that *vacA* gene has been isolated from all *H. pylori* strains, allelic variation was found due to polymorphisms in two significant *vacA* regions. The first region is a region referring to as signal region (s) and the second region located at the middle of the gene called the middle (m) region [11]. Two alleles of the m region, m1 and m2, and two alleles of the signal peptide, s1 and s2 were identified. Within allele s1, subtypes s1a and s1b have been distinguished [1]. Studies have proved that the different combinations of *vacA* al-

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Table 1. PCR Primers for Amplification of *cagA* and *vacA* Genes

Region	Primer	Primer sequence	PCR product size (bp)
cagA	F1	GAGCAATCGCTTACGCTCAG	250
	R1	GTGAATGGAACCCTAGTCGG	
vacA			
m1	VA3-F	GGTCAAAATGCGGTCATGG	290
	VA3-R	CCATTGGTACCTGTAGAAAC	
m2	VA4-F	GGAGCCCCAGGAAACATTG	352
	VA4-R	CATAACTAGCGCCTTGCAC	
s1b	SS3-R	AGCGCCATACCGCAAGAG	187
	VA1-R	CTGCTTGAATGCGCCAAAC	
s1a	SS1-R	GTCAGCATCACACCGCAAC	190
	VA1-R	CTGCTTGAATGCGCCAAAC	
s2	SS2-R	GCTAACACGCCAAATGATGC	199
	VA1-R	CTGCTTGAATGCGCCAAAC	

leles determine the degree of cytotoxicity [13, 14]. The bacterial isolates possessing s1/m1 have higher cytotoxicity, which enhances the possibility of gastric inflammation, atrophy, and gastric carcinoma compared to bacterial isolates with s2/m2 or s2/m1 combinations [2, 13].

The patterns of *H. pylori* genotypes were identified in different populations from gastric tissues, oral cavities, and other sources worldwide. We previously demonstrated the prevalence of *H. pylori* in dental plaque samples from Jordanian people [15], the aim of the present study was to determine the patterns of *vacA* and *cagA* genotypes of *H. pylori* from dental plaques among Jordanians.

Materials and Methods

Sample collections

A total of 60 Jordanian volunteers were invited to participate in this study as published before [15]. Using sterile curette, dental plaque samples were collected from the dentistry clinic at the medical center of Mutah University. Thirty samples were collected from students and the others were collected from the employees working at Mutah University and other visitors. The ages of the participants ranged between 18 and 52 years old with a mean age of 25. Forty one (68.3%) samples were from man and 19 (31.7%) from woman. All participants were non-smokers, not under antibiotic treatment for the previous 4 weeks of the sample collection, and all showed good general health status including healthy oral cavity, which was important to have similar buccal environment for all collected samples. Samples were collected after signing an informed consent from all participants in this study, which was approved from The Scientific Research Ethical Committee in The Faculty of Medicine at Mutah University under the reference number 20170.

DNA extraction

Genomic DNA from the dental plaque samples was extracted using DNA extraction mini kit (Omega, Bio-Tek, USA) according to the manufacturer's instructions. The isolated DNA was eluted in 100 µL nuclease free water, quantified on a UV-nano spectrophotometer (Quawell Technology Inc, USA), and stored at -20 °C until tested [15].

H. pylori genotyping for *cagA* and *vacA*

Polymerase chain reaction (PCR) was carried out as described previously [1]. In brief, a volume of 50 µL containing 1 µM of each primer, 1 µL of genomic DNA (approximately 200 ng), 1 mM of dNTPs mix (1 µL), 0.5 µL Phusion High-Fidelity DNA Polymerase (Thermo Scientific, USA) were used to detect the presence of *H. pylori cagA* and *vacA* s and m regions. PCR amplifications were carried out using Applied Biosystems Thermal PCR Cycler (Thermo Fisher, USA). Table 1 summarized the details of the primers sequences used in this study and Figure 1 showed the PCR results of the amplified genes. After optimization, the following cycle conditions were used for *cagA*: 35 cycles of 7 s at 98 °C, 30 s at 56 °C, and 30 s at 72 °C, while, the following conditions were used for all *vacA* primers: 35 cycles of 7 s at 98 °C, 30 s at 53 °C, and 30 s at 72 °C. Negative controls and positive samples were included in each run. Amplified PCR products were then resolved by electrophoresis on 2% agarose gels run and visualized using Thermo Fisher Scientific gel documentation system.

Results

A total of 60 dental plaque samples were analyzed to study the genetic patterns of *H. pylori cagA* and *vacA* virulence genes.

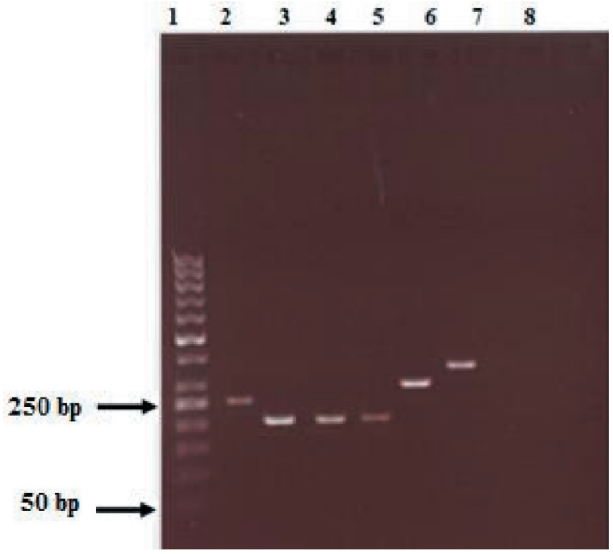

Figure 1. PCR genotyping of *vacA* and *cagA* genes from the volunteers' samples (lanes- 1 = 50 bp molecular weight marker; 2 = *cagA*$^+$; 3 = S1a; 4 = S1b; 5 = S2; 6 = m1; 7 = m2 and 8 = negative control (without DNA)).

Previously we had confirmed the presence of *H. pylori* in all analyzed samples obtained from dental plaques among Jordanian people [15]. In the current study, *cagA* and *vacA* genes were analyzed and detected from the same dental plaque samples that were used previously [15]. *H. pylori cagA* gene was found in 14 samples, which represented 23.3% of the total number of samples. Our data confirmed the presence of the s and m alleles of *vacA* in all samples (Table 2). The *vacA* m2 allele which was detected in 54 samples (90%) was more prevalent than *vacA* m1 allele which was found in nine samples (15%). Only four samples (6.6%) were found to contain m1 and m2 alleles as shown in Table 2, which indicates, probably, the presence of different *H. pylori* strains. Concerning *vacA* s region, the s1 allele was found in 91.7% of samples in which 33 samples (55%) were subtype s1a and 32 samples (53.3%) were subtype 1b. Moreover, s2 was found in 30 samples (50%) as shown in Table 2. In this study, the combinations of *vacA* regions genotypes were identified as follow: s1/m1 (18.2%), s1/m2 (93.3%), s2/m2 (45%), and s2/m1 genotype was found in 10% of our samples (Table 2). These findings clearly suggest the presence of different genotypes of *H. pylori* in the dental plaques samples among Jordanian individuals.

Discussion

H. pylori is considered the most common causative agent of gastric infections reported worldwide, with a prevalence ranging from 25% to 80% in the developed and the developing countries, respectively [16, 17]. *H. pylori* infection is associated with gastritis, peptic ulcers, and gastric cancer; therefore, *H. pylori* is classified as a carcinogenic bacterium [1]. It is believed that *H. pylori* is transmitted among people through the oral cavity. The hypothesis that the mouth is a reservoir for *H.*

Table 2. Prevalence of *cagA* Gene, *vacA* Alleles, and *vacA* Allele Combinations

Gene status	Number of samples (%)
cagA	
cagA$^+$	14 (23.3)
cagA$^-$	46 (76.7)
vacA	
m1	9 (15)
m2	54 (90)
s1a	33 (55)
s1b	32 (53.3)
s2	30 (50)
m1/m2	4 (6.6)
s1a/m1	4 (6.6)
s1b/m1	7 (11.6)
s1a/m2	29 (48.3)
s1b/m2	27 (45)
s2/m1	6 (10)
s2/m2	27 (45)

pylori and a potential source of gastric infection is supported by several studies proved the presence of *H. pylori* DNA in the saliva and dental plaque [15, 18-21]. Abu Lubad et al proved that the dental plaque is considered as a potential reservoir for *H. pylori* and is might be an important source for the gastric infection and reinfection among Jordanian people [15]. To our knowledge, there are no studies conducted on the prevalence of *H. pylori vacA* and *cagA* genotypes among the Jordanian population from dental plaque samples. The clinical relevance and geographical distribution of the virulent genotypes of *H. pylori* is still a matter of debate. Our current study reported the prevalence and genotypes of *H. pylori* virulence genes *cagA* and *vacA* in addition to the allelic variations of the *vacA* gene from dental plaques from Jordan, in which *cagA* gene was found in 23.3% of dental plaque samples in this study, while *vacA* genotype was found in 100% of samples tested.

H. pylori CagA and VacA are the most extensively studied virulence cytotoxins. CagA and VacA toxins are responsible for the progression to more severe disease conditions and the deregulation of many host cell pathways. The genetic distribution of the *H. pylori cagA* and *vacA* in different populations has been extensively studied using different sources including gastric biopsies, stool, and saliva samples [22-26]. In this study, we aimed to investigate the genotypic distribution of *H. pylori* virulence cytotoxins in dental plaque samples among Jordanian people. Surprisingly, the prevalence of *cagA* gene in dental plaque samples in this study was similar to its distribution in gastric biopsies obtained from Jordanian patients having gastrointestinal diseases [27]. *CagA* gene was found in 23.3% of the Jordanians' dental plaque samples which is similar to that in the dental plaque samples among Brazilian and Iranian people (24.1% and 27.3%, respectively) [26, 28].

In British children, *cagA* gene was detected in six (17%) out of 36 dental plaque PCR positive samples [29]. It is known that *cagA* is present in 40% to 90% of *H. pylori* strains in Western countries and in East Asian countries, respectively [24]. In Taiwanese patients, the *cagA* was positive in 79% and 9% of stomach antrum and body samples, respectively [9]. *CagA* was also detected in 60%, 61.8%, 87.5%, 61.6%, and 46.7% among Chilean, Saudi, Chinese, Tunisian, and Brazilian patients, respectively [1, 2, 23, 26, 30].

VacA is the other important virulence factor of *H. pylori* that has a cytotoxic effect and has an ability interfere with important cellular functions [12]. Our study revealed that all dental samples were positive for *vacA* with variability in the expression of different alleles among different samples. Concerning the s region of *vacA*, the prevalence of s1a (55%) region was almost similar to that of s1b (53%) and s2 (50%) alleles among Jordanian people. Regarding the m region of *vacA*, the m2 allele was found in 90% of samples compared to only 15% for m1 allele. One important determinant of the pathogenicity of *H. pylori* is the presence of certain *vacA* genotypes. The most pathogenic genotype is s1/m1 which is associated with high levels of vacuolation capacity compared to the moderate effect of s1/m2, while s2/m2 and s2/m1 *vacA* alleles lack detectable cytotoxin activity [11, 31, 32]. In our study, 93.3% of samples were positive for the s1/m2 genotype, followed by 45%, 10%, and 18.2% for s2/m2, s2/m1, and s1/m1, respectively. These percentages of allelic combinations indicate the presence of mixed serotypes of *H pylori* in the dental plaque samples among Jordanian people.

On the other hand, a previous report showed that gastritis samples from Jordanian patients showed an equal prevalence of s1/m1 and s2/m2 (46.2%), while 7.7% for s1/m2 and the genotype s2/m1 was not detectable [27]. It has been also demonstrated that the expression of both *cagA* and *vacA* s1/m1 genotypes in gastric samples are involved in more severe disease outcome and are associated with an increased risk of developing distal gastric adenocarcinoma [11, 19, 33]. Other reports linked the *cagA* and *vacA* s1/m1 genotypes in dental plaque samples with more severe gastric disease [34, 35]. However, in our study we found around 5% of individuals tested in this study are positive for both *cagA* and *vacA* s1/m1 genotype and despite this small percentage, those people who are considered a high-risk group for the transmission of a highly virulent strain of *H. pylori* to other people [11, 19, 33, 35]. Therefore, studying the genotype pattern of *vacA* gene can be a tool to help in applying vigorous infection prevention measures in endoscopy units and in the management plan by predicting the severity and prognosis of *H. pylori* infection.

The present study showed that the majority of *H. pylori* detected in the dental plaque samples carry virulence-associated genes, which might make the oral cavity a potential route for the transmission of the highly virulent *H. pylori* strains. It has been proved that the *H. pylori* strains that are associated with stomach reinfection were similar to that present in the oral cavity [36-39]. Because the oral cavity in Jordanian people harbor virulent strains of *H. pylori*, it seems essential that the diagnosis and therapy described for *H. pylori* gastric infection individuals accompanied with additional molecular testing for the dental plaque if their gastric *H. pylori* infection recurs.

In conclusion, our study is the first report with an extensive coverage of the prevalence of the *H. pylori* virulence-associated genotypes from dental plaque samples among Jordanian people. Our results showed that the majority of *H. pylori* from dental samples carry multiple virulence genes that might be easily transmitted among Jordanian people causing severe gastric diseases. The presence of highly pathogenic *H. pylori* in dental plaque samples might be exploited to clarify the reason behind the high prevalence of *H. pylori* associated gastritis in Jordanian people [27]. Finally, studying the prevalence of virulence-associated *H. pylori* genotypes would have profound clinical and epidemiological implications in our understanding of the pathogenesis of *H. pylori* in Jordan.

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Conflict of Interest

The authors declare that they have no competing interest.

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