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DETECTION OF HELICOBACTER PYLORI CagA AND VacA GENOTYPES FROM STOOL SPECIMEN AMONG INFECTED AND ASYMPTOMATIC HEALTHY INDIVIDUALS
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ABSTRACT

Background: **Helicobacter pylori(*H. Pylori*) is one of the most common pathogens affecting human kind, infecting more than 50% of the world's population. Invasive and non- invasive methods have been used to diagnose *H. pylori* infection. The polymerase chain reaction (PCR) has been broadly and successfully used to detect** Helicobacter pylori **virulence genes in gastric biopsies and stool specimen due to its high sensitivity and specificity. Genotyping using *H. pylori* genes, the cytotoxin associated gene A (*cagA*) and voculating cytotoxin gene A (*vacA*), offers one of the best options.**
***Objective*: To determine if genotyping of *H. pylori* cytotoxin associated gene A (*cagA*) and voculating cytotoxin gene A (vacA) virulence genes in infected patients as well as healthy individuals can be done from stool specimen.**
Study selection: **A Pubmed/Medline search was carried out using specific key words to retrieve all publications on genotyping of *H. pylori cagA* and *vacA* from stool specimen between year 2003 to 2012.**
Data synthesis:**Ten different studies from different locations fulfilled the selection criteria and were included in the study.**
Results:**This review summarises different protocol used to successfully detect *H.pylori* virulence genes cytotoxin associated gene A (*cagA*) and voculating cytotoxin gene A (vacA) from faecal specimen by PCR from asymptomatic healthy individuals and infected people with *Helicobacter pylori* infection.**
Conclusion: **this review highlights the use of stool specimen as an alternative test for genotyping of *H.pylori cagA* and *vacA* virulence genes as it is non-invasive and easy to collect.**

INTRODUCTION

H. pylori is a Gram-negative microaerophilic bacterium and one of the most common bacterial pathogens of humans. The bacteria has worldwide distribution and the prevalence ranges from 25% in developed countries to more than 90 % in developing areas, but not all infected individuals eventually developed the disease (1-3). *H. pylori* infection is associated with chronic gastritis, peptic ulcer disease, gastric cancer and MALT-lymphoma (4-6). *H. pylori* infection is acquired in childhood and persists as an asymptomatic infection for decades in most individuals , although only a minority develops a clinically significant outcome, such as peptic ulcer or gastric cancer (7).

Culture and histology are the most widely used for diagnosis of *H. pylori* infection due to its high sensitivity and specificity, but they required invasive procedures. Among the non-invasive methods serology has high specificity and sensitivity but does not show current infection with *H.pylori*. The 13C-Urea Breath Test (UBT), is also considered very sensitive and specific but it is not always available in normal clinical settings, moreover it requires expensive equipment (8-10). *H. pylori* stool antigen test provides a simple alternative to the urea breath test due to its simplicity, but does not allow genotyping of the *H. pylori* strains (10).

MOLECULAR METHODS

Molecular methods, such as PCR have been developed for detection of various microorganisms, including *H. pylori,* because they can detect small numbers of organisms and nucleic acids in clinical samples (11).

H. PyloricagA and *vacA* virulence genes have been identified in gastric biopsies by the use of PCR (12-13), other studies have been also able to find these genes by PCR in gastric juice, saliva, dental plaque, and feces (9,14-16).

H. pylori DNA has been detected by means of PCR in feces of infected humans by amplifying genes such as the ureA and ureCgenes and the 16S rRNA (17-19) as well as cagAandvagA virulence genes (12,20).

Genotyping is important as it will help in identifying H. *pylori* virulence genes. Stool samples are easy to obtain and therefore of high potential interest for the development of a direct method of H. *pylori* detection (21).

The current biopsy based method of genotyping is invasive and not suitable especially for children and healthy individuals. Stool specimen can be used as an alternative for genotyping cagA and vacA virulence genes as it is non-invasive and easy to collect and appropriate in genotyping cagA and vacA in infected patients and healthy individuals. This review summarises protocol used in detectingcagA and vacAin stool specimen among asymptomatic healthy individuals as well people infected with *H. pylori.*

H.pylori cagA and *vacA virulence* genes : Two major H .pylori virulence genes cytotoxin associated gene A (*cagA*) and voculating cytotoxin gene A (*vacA*), may play a major role in determining the clinical outcome of Helicobacter infections. Studies conducted in several countries have shown that patients possessing *vacA*-type s1 and *cagA*-positive *H. pylori* strains have higher risk for peptic ulcer disease (PUD) (22).

The cytotoxin-associated gene (*cagA*) is associated with cytotoxin production and the induction of interleukin 8 (IL-8) by gastric epithelial cells. Several studies have suggested that cagA is a useful marker for the most virulent strains that are associated with peptic ulcer, atrophic gastritis and adenocarcinoma (3, 23). The cag pathogenicity island (PaI) encodes a type IV secretory system that enables translocation of cagA protein. After the delivery, CagA protein are phosphorylated on tyrosine residue . Phosphorylated CagA interacts with the phosphatase SHP-2 causing dephosphorylation of cortactin and cytoskeletal rearrangements forming the "hummingbird " phenotype . Deregulation of SHP-2 by CagA may be involved in the induction of abnormal proliferation and movement of gastric epithelial cells, leading to gastritis and gastric carcinoma (24-25).

The VacA gene is present in almost all strains of *H. pylori.* It encodes vacuolating cytotoxin and induce apoptosis in epithelial cells (26). The vacAgene comprises two main regions: the signal zone (s1 or s2) and the middle region (m1 or m2). Type s1/m1 mosaic combination strains have more cytotoxin activity than s1/m2 strains(27-28).

Infected patients: In a study by Sicinschi *et al.*(29) (table 1) among Colombia *H.pylori* infected and uninfected children, DNA was extracted by using QIAamp DNA stool mini kit (Valencia, CA) with modification in the protocol. They detected cagA and vacA genes in stool samples by one step stool PCR with 70-80 cycles and 32P radioactively labelled primers. cagA and vacA was detected in 21 (53.8%) and 36 (92.3%) respectively, the specificity of the test was 100%. There was no DNA amplification from *H.pylori* uninfected children for cagA and vacA. This result indicates that themodified protocol is useful only in infected individuals. This is similar to another study that was conducted among Thailand children where H. pyloricagA gene was detected in 49.2% (table 1). In this study DNA was extracted from stool by using a bead crushing method and amplified by nested PCR (30).

In another study, Sicinschi *et al.* by using previous method for stool samples(20) compared the genotyping of cagA and vacA from paraffin biopsies, frozen biopsies and stool specimen in adult patients and the detection rate was 83.5%, 74.7% and 75.9% respectively (table 1). This study indicates both biopsies and stool specimen are suitable for cagA and vacA identification and none of them provide complete genotype (20).

In another study in Iran among 300 patients undergoing upper gastrointestinal tract endoscopy, DNA was extracted from stool specimens by DNA purification kit (Germany). 77.66 % were positive for H. pylori by PCR assay in gastric samples while cagA and vacA detection rate was 71.67% of stool samples (table 1). In stool specimens, 97 % of *H. pylori* positive specimens were cagAwhile71.85% were positive for s1a/m2 regions of vacA (12).

Bindayna *et al.* compared detection of H.pyloricagA gene from biopsy specimen, clinical isolates and stool specimen among 44 Bahraini adult patients. They used QIAampDNA stool mini-kit and stool PCR for stool specimen. CagA detection rate in biopsy specimen, clinical isolates and stool specimen was 59 (59%), 10 (62%) and 10 (22.7%) respectively (31) (Table 1). This result is lower compared with study done by Zambon *et al.* in adult patient. They alsoused QIAmp Stool Mini Kit (Qiagen , Milan , Italy) for extraction of DNA and managed to detect cagA in 16/48 (33.3%) (32) (Table 1).These low results may be due to some factors that may have affected the stool protocol like presence of faecal inhibitors.

Asymptomatic healthy individuals

H. pylori infection is acquired in childhood and persists as an asymptomatic infection for years in the majority of colonised individuals. Only a small fraction of infected persons develop clinically significant outcomes such as peptic ulcer disease , gastric cancer , and gastric mucosa - associated lymphoid tissue lymphoma . The factors that lead to few

individuals to develop the associated diseases, while the majority of infected people remain asymptomatic, are unknown (33-34).

Invasive and non- invasive method have been used for genotyping of *H. pylori* from asymptomatic healthy subject.

In a study from Colombia conducted among 86 asymptomatic children residing in a high-risk area for gastric cancer, DNA was extracted from stool by using QIAamp DNA stool mini kit (USA) while a single -step PCR with higher number of amplification cycles was used. H. pylori cagA and vacA gene was detected in 66.1% and 84.6% respectively from 65 samples that were positive for 16S rRNA (Table 2). The sensitivity and specificity of vagA gene was 70.6 and 80.0% respectively , while sensitivity and specificity for cagA gene was not determined because the author thinks that cagA gene is not present in all *H. pylori* strains . This study clearly indicates that these genes were common among asymptomatic population and early screening of those in high risk area is crucial (33). This percentage are more higher compared with studies done among symptomatic Portuguese children at risk of gastric cancer, where cagA and vacA s1 detection rate was 36.1% and 32.7% respectively in gastric biopsies sample (35).

Gastric cancer incidence among Japanese middle-aged group is high (36) in a study done by Hirai *et al.* among healthy asymptomatic Japanese, they detected cagA gene in 41 of 65 samples (63.1%) (table 2) while in also different study done by Hirai *et al.* with 80 samples of Japanese asymptomatic patients cagA was detected in 18.8% (Table 2).this was low compared to previous study. In both studies they used QIAamp DNA stool mini kit (Qiagen , Valencia, CA) for DNA extraction and nested PCR. Stool is suitable for such populations because of difficulty in collecting gastric specimen from healthy individuals ; this study indicates that gastric cancer poses great threat among healthy Japanese patients (37-38).

Sasaki *et al.* in their study among asymptomatic healthy adults fromEcuador and Panama extracted DNA by QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) and used nested PCR for genotyping CagA. The cagA gene was detected in 28/61 (45.9%) and 7/35 (20.0%) of the H. pyloriDNA-positive specimens from Ecuador and Panama, respectively (39) (table 2).

Table 1
Summary of *cagA* and *VacA* virulence gene detection in *H. pylori* DNA in clinical stool specimens (infected patients)

Target Population	Country	Target gene	Number of samples	Positive rate %	Reference
Adult	Iran	cagA/vacA	233	71.67	[12]
Children	Thailand	cagA	120	49.2	[30]
Adult	Bahrain	cagA	44	22.7	[31]
Adult	Italy	cagA	48	33.3	[32]
Children	columbia	cagA	39	53.8	[29]
		vacA	39	92.3	
Adult	United states	cagA/vacA	20	75.9	[20]

Table 2
Summary of *cagA* and *VacA* virulence gene detection in *H. pylori* DNA in asymptomatic healthy individuals

Target population	Country	Target gene	Number of samples	Positive rate %	Reference
children	Columbia	cagA	65	66.1	[33]
		vacA	65	84.6	
Adult	Japan	cagA	65	63.1	[37]
Adult	Japan	cagA	80	18.8	[38]
Adult	Equador& Panama	cagA	61	45.9	[39]
		cagA	35	20.0	

DISCUSSION

Stool-PCR may also be a very useful method in detection of *H. pylori* infection , but reported success rates of detection of *H. pylori* DNA in feces vary from 25% to 100% (9). This variability is due to a variety of faecal inhibitors such as acidic polysaccharides , metabolic products and large amounts of irrelevant , DNA Detection of *H. pylori* in stool using PCR and standard extraction methods has proved difficult, because of this reason , DNA extraction procedures that remove inhibitors of PCR such as filtration of stool specimens by using polypropylene filters , use of extraction kits QIAamp for tissue and stool for separation of inhibitors were developed . PCR has been successfully used in detection of *H. pylori cagA* and vacA virulence genes from stool specimen especially by using QIAamp DNA stool mini-kit (12, 20).

The detection *H. pylori vacA* and vacA gene among infected patients from stool specimen has been done . The detection rate was found to vary between 22.7% to 92.3% (29, 31). This data shows that biopsy based test that expose lots of risk to already ailing patients can be avoided by using stool specimen.

H. pylori cagA and vacA gene have been detected using stool specimen among healthy subjects. The rate of detection varies between 18.8% to 84.6% (33, 38-39). There are few studies on the different cagA genotype samples healthy from individuals . This is due to difficulty in collecting biopsy specimen mainly from healthy individuals (40-41) as compared to other studies that used stool specimen . Therefore stool PCR being non-invasive is important in screening health subjects.

The stool PCR assay can also be used in the diagnosis of *H. pylori* infection in those patients for whom the invasive methods are not indicated as well as children especially in areas where there is high risk of gastric cancer (33). In study done by Sicinschi *et al.* They found that the distribution of *H . pylori cagA* and vacA among healthy children being 66.1% and 84.6% respectively . This data suggest that there is need for early screening of such population and stool PCR can easily be used as it's easy and practical in such population.

CONCLUSIONS

In conclusion, this review highlights the use of stool specimen as an alternative test for genotyping of *H. pyloricagA* and *vacA* virulence genes especially in children and asymptomatic healthy subjects.

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