

Original Research Article

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## A Study on the Rate of vacA Genotypes of *Helicobacter pylori* in Gastric Biopsies obtained from Patients with Symptomatic Upper GIT Disorders

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### ABSTRACT

Vacuolating cytotoxin (vacA) gene is one of the most studied virulent genes of *Helicobacter pylori* which plays a major role in causing inflammation in the human gastric epithelium. We have studied the rate of vacA genotypes of *H.pylori* in gastric biopsy samples from patients with upper GIT disorders. Antral biopsies (60 nos.) were collected from patients undergoing upper GIT endoscopy at our hospital. Rapid urease Test and Dilute Carbol Fuchsin staining were used to detect *H.pylori* from the biopsy tissue. The rate of vacA genotypes were studied by PCR, targeting the intermediate region (vacAi) of the vacA gene. 31 of the 60 biopsies were positive for *H.pylori* by RUT and DCF. vacAi region of the vacA gene was amplified from 9 (29%) of the *H.pylori* positive cases. The rate of vacA genotypes determined in symptomatic upper GI disorders is very low. This study reveals that vacA is not the one and only factor for *H.pylori* induced inflammation and further consequences. Gene diversity and combination of virulent genes and their association with other risk factors should be studied to divulge the role virulent genes in *H.pylori*.

#### Keywords

*Helicobacter pylori*,  
VacA, GIT disorders,  
Rapid urease Test (RUT).

#### Article Info

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### Introduction

*Helicobacter pylori* is the largest human pathogen which is present in more than half of the world's population. It is widely distributed in all regions of the world. *H.pylori* is found in the human gastric mucosa and is linked with gastritis and ulceration (Marshall *et al.*, 1991 Francis *et al.*, 2007). *H.pylori* is also found to be associated with the development of gastric

carcinoma (Ashton-Key *et al.*, 1996). *H.pylori* is classified as a group I carcinogen by the International Agency for Research on Cancer (IARC) (Schistosomes *et al.*, 1994). Even when the studies evidence that *H.pylori* induces gastric cancer, most of the *H.pylori* positive people do not develop it (Martin *et al.*, 1995). This may be due to the absence of virulent factors which determine

the risk of developing *H.pylori* induced adenocarcinoma. The strains of *H.pylori* are more divergent, two distinct strains could co-exist in the gastric mucosa of an individual person. So the risk of developing inflammation and its further complications like gastric cancer is associated with the virulence properties of the *H.pylori* strain type that an individual harbors in his/her gastric mucosa (Helena *et al.*, 2000). Bacterial virulence and their role in the pathogenesis of *H.pylori* has been widely studied (John, 1998). The genome of *H.pylori* is composed of a range of virulence genes, among which, vacuolating cytotoxin (VacA) is a well known gene of *H.pylori* which codes for a 95-kDa (VacA) protein. This protein causes vacuolization of epithelial cells and plays an important role in the development of gastritis and ulcers. The Gene is composed of a Signal sequence region (s1, s2), a mid-region (m1, m2) and an intermediate-region (i1, i2, and i3). The allelic forms of signal sequence (s1, s2) and mid region (m1, m2) exist in combinations (except s2, m1) and bring about allelic diversity among the VacA gene (Anna *et al.*, 2008; Samuel *et al.*, 2012). The aim of the present study is to find the rate of vacA genotypes present in gastric biopsies collected from patients with upper GI disorders by PCR amplification of the VacA intermediate region (VacAi).

## **Materials and Methods**

### **Study subjects**

60 patients suggested for upper GIT endoscopy after clinical examination in Sri Manakula Vinayagar Medical College and hospital puducherry were included in this study. Subjects were selected based upon the inclusion criteria; 1) a well defined clinical symptom like dyspepsia, dysphagia, abdominal pain, etc, 2) clinically diagnosed

with a minimum of one GIT disorder caused by *H.pylori* like, gastritis, duodenitis, peptic ulcer disease, etc. subjects with all other endoscopic findings and endoscopic normal studies were excluded from the study. Samples were collected only from those who have agreed to co operate this study. Detailed consent was obtained from each patient. Among the 60 selected subjects, 32 were male and 28 were female with age starts from 17 to 63.

### **Sample collection**

Antral gastric biopsies were collected as +/- 5mm tissues in sterile normal saline. Three bits of tissues were collected from each patient for RUT (Rapid urease Test), DCF (Dilute Carbol Fuchsin) and PCR (Polymerase Chain Reaction) respectively.

### **Rapid urease Test (RUT)**

A bit of freshly collected biopsy tissue was transferred to 1.5 ml of Christenson urea broth and incubated at 37<sup>0</sup>C. The samples were observed for every one hour for the development of pink color in Christenson urea broth which is considered as positive for *H.pylori*. Sterile Christenson urea broth without biopsy tissue served as a negative control.

### **Dilute Carbol Fuchsin (DCF) staining**

Samples were collected and transferred to the laboratory within one hour for further processing. A smear was made by rubbing the tissue against slide and fixed with methanol for 3 minutes. The fixed smear was stained with carbol fuchsin (1:10 dilution) for 10 minutes and viewed under 100X oil immersion microscope for the presence of spiral bacilli.

### **DNA isolation**

DNA isolation was done manually by phenol chloroform extraction method. The biopsy tissue was minced with the help of sterile scalpel and forceps. The minced tissue was incubated overnight at 56<sup>0</sup>C in 200µl of tissue lysis buffer (tris-HCL 100mm+ 100mm EDTA+ 100 mm NaCl+ 1% SDS) and 10µl of proteinase K. Equal volume of phenol(24): chloroform(24): isoamylalcohol(01) was added to the digested tissue, mixed well and centrifuged at 10000RPM for 5 minutes. DNA was precipitated from the aqueous phase using absolute ethanol.

### **Polymerase Chain Reaction (PCR)**

All the 60 biopsies were taken for the detection of vacA genotypes. PCR was performed using primers, VacA-1.SE CAATCGTGTGGGTTCTGGAGC, VacA-3. As GCCGATATGCAAATGAGCC GC described by Monstein et.al, 2002, targeting the 678bp intermediate region of vacA gene. 500µm of each forward and reverse primer, 5µl of template DNA were used in a 50 µl of 1X master mix (Takara- EmeraldAmp). Amplification was done in BIO-RAD T100 Thermal Cycler with 35 cycles of initial denaturation at 93<sup>0</sup>C for 5 minutes, cycle denaturation at 93<sup>0</sup>C for 1 minute, annealing at 58<sup>0</sup>C for 1 minute, cycle extension at 72<sup>0</sup>C for 1 minute, final extension at 72<sup>0</sup>C for 5 minutes and hold at 4<sup>0</sup>C for 5 minutes. The resulting product was viewed in 1% agarose gel with 100bp DNA marker.

### **Results and Discussion**

31 (52%) out of 60 samples were positive for *H.pylori* by RUT and DCF staining in which, 14 were male and 16 were female (Figure.1). 9(29%) of the samples got amplified (678) for vacA gene, none of the RUT & DCF negative samples gave positive

result (Figure.2). Two of the 9 vacA samples were sequenced and blasted (Query ID: lcl|Query\_190267 & lcl|Query\_115345) against the sequences available in the NCBI database. The sequences have shown 95% and 98% similarities against the NCBI *Helicobacter pylori* strain PG218 VacA (vacA) gene, complete cds; accession number: GQ331974.1 and *Helicobacter pylori* SNT49, complete genome; accession number: CP002983.1 respectively.

The rate of *H.pylori* in patients with upper GI disorders by RUT and DCF staining is 51.6% which is compatible with the studies reported from many parts of India (12, 13) It is important to know the genotype of *H.pylori* present in the patient to understand the risk of developing *H.pylori* induced gastritis and/or adenocarcinoma. It also reveals information about the importance of rapid eradication of *H.pylori* (Kidd *et al.*, 1999; Mahsa *et al.*, 2010).

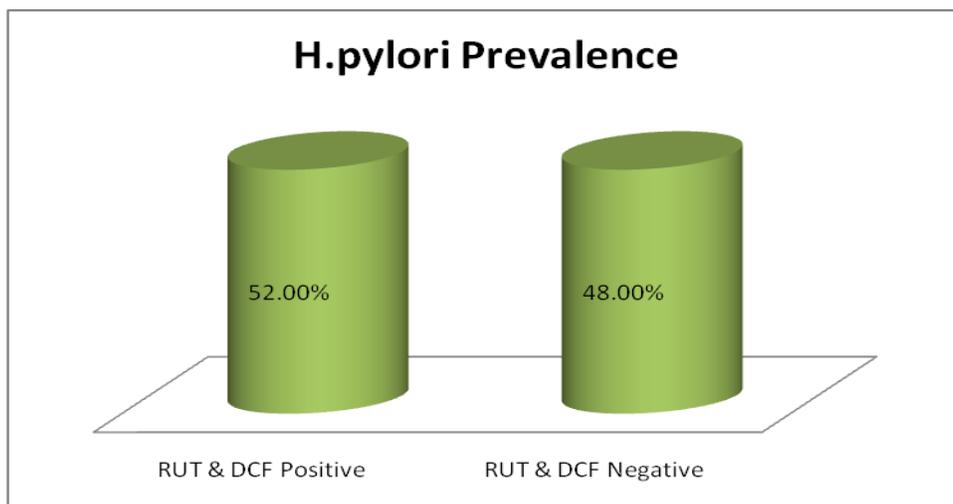
The vacA gene is found to be closely associated with *in vitro* cytotoxicity and also to the clinical consequences of the patient. Studies also show that vacA positive genotypes were isolated from patients with no defined clinicopathological condition. This phenomenon can be explained by the fact that the vacA gene is highly divergent and sequence variations may contribute to the pathogenicity of the particular strain. Amplification of *H.pylori* DNA from direct gastric biopsies is crucial due to the presence of very less amount of bacterial DNA and also the presence of PCR inhibitors in the biopsy tissue.

The rate of vacA genotypes found in this study is 29% which is lesser than that of the other studies reported from different parts of Asia viz. palastine, bharian, india. (Tamer *et al.*, 2013). The low level of vacA genotypes in upper GI disorders show that vacA alone is not a risk factor for developing *H.pylori*

associated complications. A study on the combination of other virulent factors along with *vacA* is essential to know the pathogenicity of *H.pylori*. For example, the

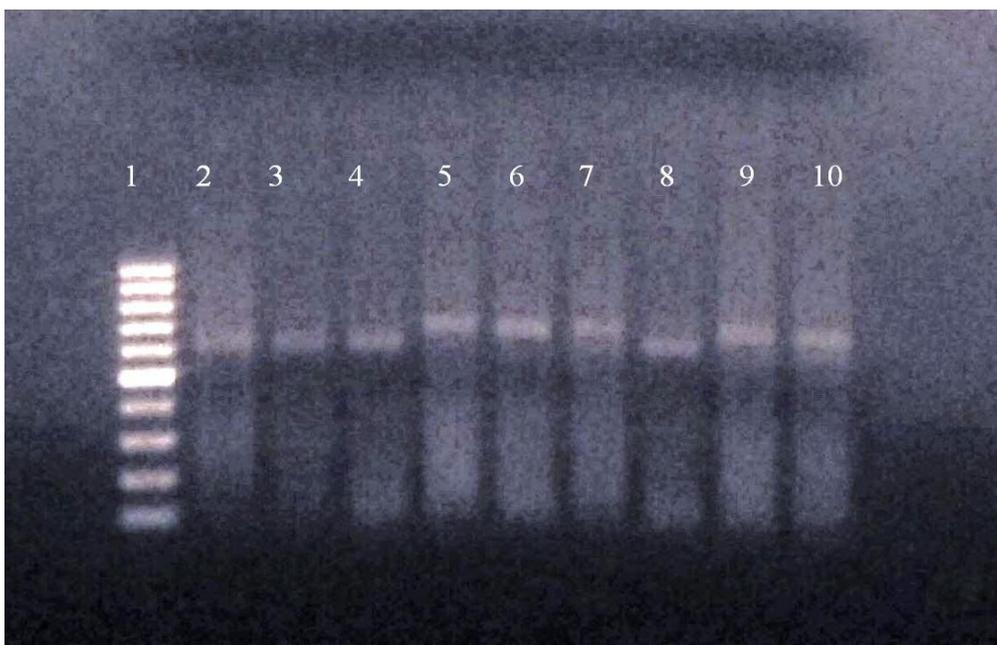
presence of *vacAi* sequence is found to be associated with *cagA* positivity and overall virulence of the strain (Vandenplas, 1999).

**Fig.1** Prevalence of *H.pylori* in GIT disorders detected by Rapid Urease Test (RUT) Dilute Carbol Fuchsin (DCF) staining



The prevalence of *H.pylori* among patients attending a tertiary care hospital in puducherry is found to be 52%

**Fig.2** Agarose gel electrophoresis showing 678bp *vacAi* region.



Lane 1: 100bp DNA marker, Lane 2 to 10 : *H.pylori* samples showing positive result for *vacAi* at 678 (650-700) bp.

Presence or absence of the functional *vacA* gene and its association to the virulence of the strain is controversial. It is also important to analyze the complete sequence (combination of (s1, s2), (i1, i2, and i3) & (m1, m2)) of *vacA* gene for the presence of a functional *vacA* protein.

In conclusion, this study is a reference for the rate of *H.pylori vacA* genotypes of this area. The present study states that *vacA*i positivity in symptomatic upper GI disorders is low. Further studies on the full length sequence analysis of *vacA* gene and its co-existence with other risk factors would give useful information about its diversity and role in *H.pylori* pathogenesis.

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