



## **Association of dupA, iceA, homB Genes of *Helicobacter pylori* with Gastritis, Peptic Ulcer Disease and Gastric Cancer**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MAB designed the study, performed, collect the data, wrote the protocol and first draft of the manuscript. Author MI give the supervision. Author PKM managed the literature searches and analysis. All authors read and approved the final manuscript.*

### **Article Information**

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### **ABSTRACT**

**Objective:** To determine the association of dupA, iceA, homB genes of *Helicobacter pylori* with gastro-duodenal diseases such as gastritis, peptic ulcer disease PUD and gastric cancer.

**Materials and Methods:** This cross-sectional analytical study was conducted at Gastroenterology Department, Shaikh Zayed Hospital Lahore. Patients with gastro-duodenal diseases and positive *H. pylori* were included. Gastric biopsies were taken from fundus, body and antrum. *H. pylori* DNA were removed utilizing Genra DNA extraction Kit (Life Technologies, USA) as per the technique and Qualitative PCR for the recognition of *H. Pylori* DNA. The PCR primers sets were designed for the specific detection of dupA, iceA and homB genes of *H. pylori*. All the data was recorded in proforma and analyzed by SPSS version 20.

**Results:** Mean age of the cases was 41.22±8.04 years. Males were more affected 118(60.2%). HomB was the most common 76(38.8%) followed by dupA and iceA 28.6% and 24.5%

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respectively. Peptic ulcer disease and gastritis were higher among patients having dup A and iceA positive strains as compared to homB gene patients, while gastric cancer was significantly higher among HomB gene infected patients, p-values were quite significant.

**Conclusion:** It was concluded that homB gene was most frequent in *H. pylori* infected population. Peptic ulcer disease and gastritis are markedly associated with dupA and iceA genes, while homB gene infected patients are at high risk of gastric cancer.

**Keywords:** *H. pylori*; dupA; iceA; homB genes; gastric diseases.

## 1. INTRODUCTION

*H. pylori*, is a micro-aerophilic, helical and a gram negative bacterium, found in gastric mucosal mucus and gastric epithelium or within mucous membrane. It results in chronic inflammatory response of low-level in stomach lining and is closely correlated with duodenal and gastric ulcers and peptic ulcer disease (PUD) [1,2]. *H. pylori* is colonize in the stomach cavity to inhibit the acid-secretion by parietal cells, which are found in the corpus of stomach. The inflammatory reaction to bacteria activates the secretion of gastrin hormone by antral G cells which passes to the corpus via the circulation [3]. Gastrin induces parietal cells within the corpus for the secretion of even additional acid into stomach lumen. Constantly raised gastrin levels increases the quantity of secreted acid. The raised acid load injures the duodenum, and can possibly eventually result into ulceration [3]. On the contrary, gastric ulcers are frequently correlated with reduced or normal production of gastric acid. This signifies that the systems which shield the gastric mucosa are not working [4]. *H. pylori* in such patients are capable of also colonizing the stomach corpus, where the parietal cells which secrete acid are sited. Though chronic inflammatory response stimulated by the bacterium leads to additional decline in acid production, thus ultimately causes stomach lining atrophy that can possibly bring about gastric ulcer as well as promotes the risk for cancer of stomach [5]. Two *H. pylori* correlated mechanisms promoting cancer are under exploration. One of these mechanisms enhances the free radicals production by *H. pylori* as well as amplified mutation rates for host cells. Other projected process is labeled as "perigenetic pathway" [6] that affects the development of the altered phenotype of host cell through modification in proteins of cell, like linkage proteins. It is also projected that *H. pylori* stimulates the inflammatory response as well as in the vicinity high levels of IL-6 and/or TNF- $\alpha$ .<sup>7</sup>In proportion to the projected epigenetic

mechanism, inflammatory response signaling molecules, for instance TNF- $\alpha$ , is capable of altering cell adhesion of gastric epithelium and causes the distribution as well as relocation of mutated cells of epithelium without requiring the further mutations within suppressor genes of tumor, for example cell adhesion proteins codinggenes [7].

Occurrence of various *H. pylori* genes correlates with gastro-duodenal-disease development in *H. pylori* infected population. Significant correlation of dupA gene appeared among north Indian populace. However in Iranian populace it exhibited no correlation with gastro duodenal syndromes [8]. HomB is believed to be co-marker for strains of *H. pylori* correlated peptic ulcer disease in 61.0% cases of western population and 90.0% cases of East Asian population. Occurrence of homB gene was considerably greater in isolated strains of cases with gastric cancer as compare to duodenal ulcer (DU) cases [9,10]. By taking above controversial findings according to different populations and unavailable data in Pakistani population, this study we designed to assess the correlation of dupA, iceA as well as homB genes of *H. pylori* among Pakistani population with gastro-duodenal diseases.

## 2. MATERIALS AND METHODS

This cross-sectional analytical study was conducted at Gastroenterology Department, Shaikh Zayed Hospital, Lahore and molecular techniques were performed in Centre of Excellence and Molecular Biology, University of Punjab, Lahore. Study duration was from February 2016 to January 2018. All patients with gastro-duodenal illnesses reporting for endoscopy to the gastroenterology department with age >18 years and both gender were included. Patients with inability to recognize *H. Pylori* by rapid urease test, portal hypertension and history of any treatment for *H. Pylori* destruction in past 4 weeks were excluded. After

taking informed consent, gastric biopsy specimens were acquired from fundus, body and antrum, and inoculated in the CLO or rapid urease test gel and rest was put away in Eppendorf tubes containing typical saline at -20C until DNA extraction.

These specimens were brought through a clean needle into strong urea agar as well as brooded at room temperature. Outcomes were documented until 24 hours after inoculation. On the off chance that urease chemical of *H. Pylori* is available in sample, the subsequent debasement of urea leads the pH to escalate as change the colour of specimens from yellow to pink.

*H. Pylori* DNA was extracted utilizing Genra DNA extraction Kit (Life Technologies, USA) as per the technique given in the pack for convention and Qualitative PCR to detect the *H. Pylori* DNA. Quickly, ordinary saline was expelled from 1.5 ml extraction tubes in the wake of centrifuging the tube at 13000 x g rpm for three minutes. A 300 µl of cell lyses arrangement was added to every tube. Test was emulsified utilizing pipette tips and vortex for 1-2 minutes. One µl of RNAs was included in every tube. Tubes were rearranged a few times to blend the substance and were put at 37°C for 30 minutes. After including hundred micro-litre of protein precipitation tubes were centrifuged for 5 minutes. Supernatant containing DNA was moved into another pre- marked tube containing 500 µl of Isopropanol. Tubes were vortexed for 5 minutes; supernatant was expelled and extracted DNA was rinsed in 70% ethanol/Dried DNA was rehydrated in 20 µl of DNA hydration arrangement gave the unit and was set at 37°C overnight before PCR examination.

Enhancement of all qualities from patient examples was finished by utilizing Taq polymerase (Fermentas). The response blend for a solitary response will contain 10 × PCR Buffer, 25 mM MgCl<sub>2</sub>, 200 µM each of dATP, dCTP and dGTP and 20 pM each of forward and turn around ground works. PCR items were broke down on a 1.5% agarose gel, recolored with ethidium bromide. The coveted groups were extracted from the gel and decontaminated utilizing the Gel Purification Kit. The PCT primers sets were designed for the specific detection of dup A, ice A and homB gene of *H. Pylori* using primer 3 software. All the data was recorded on self-made proforma. Data was analyzed by using SPSS version 20. Chi-square test was applied

and a p-value ≤0.05 was considered as significant.

### 3. RESULTS

Mean age of the cases was 41.22±8.04 years with the range of minimum 25 years and maximum 60 years. Males were more affected 118 (60.2%) as compare to females 78(39.8%). Epigastric discomfort was among all of the cases where as bloating and Belching were noted in 180 (91.8%) patients and Nausea/vomiting was seen in 138 (70.4%) patients Table 1.

HomB gene was the most common in 76 (38.8%) patients followed by dupA and iceA were seen in 56(28.6%) and 48(24.5%) patients respectively Table 2. dupA, Ice-A and homB positive strains were the significant cause of gastritis. All the dupA and iceA positive patients had gastritis, (p= 0.001), while out of 76 homB positive strains 44 had gastritis. Peptic ulcer disease was significantly higher among patients having dup A and iceA positive strains, (p= 0.001). Gastric cancer was significantly higher among homB gene positive patients (p=0.001). No case of gastric cancer was found among homA and iceA positive patients Table 3

**Table 1. Distribution of cases according to age, gender, dyspeptic symptoms and biopsies n=196**

Variables		Frequency /percent
Age	Mean	41.22 years
	Std. Deviation	8.04 years
Gender	Female	78(39.8%)
	Male	118(60.2%)
Dyspeptic symptoms	Epigastric discomfort	196(100%)
	Bloating	180(91.8%)
	Belching	180(91.8%)
	Nausea/vomiting	138(70.4%)

**Table 2. Cases distribution according to detection of *H. pylori* genes N=196**

<i>H. pylori</i> genes	Frequency /percent
dupA	56(28.6%)
iceA	48(24.5%)
homB	76(38.8%)
Negative	16(8.16%)
Total	196(100%)

**Table 3. Association of dupA, iceA, homB strains with gastritis, peptic ulcer disease and gastric cancer (n=196)**

	<i>H. pylori</i> gene						P-value
	dupA Positive	Negative	iceA Positive	Negative	homB Positive	Negative	
<b>Gastritis</b>							
Yes	56	96	48	104	44	108	0.001
No	0	44	0	44	32	12	
Total	56	140	48	148	76	120	
<b>Peptic ulcer disease</b>							
Yes	40	52	44	48	22	70	0.001
No	16	88	4	100	54	50	
Total	56	140	48	148	76	120	
<b>Gastric cancer</b>							
Yes	0	44	0	44	32	12	0.001
No	56	96	48	104	44	108	
Total	56	140	48	148	76	120	

#### 4. DISCUSSION

*Helicobacter pylori* (*H. pylori*) is a prevalent gastric pathogen, which causes peptic ulcer disease and gastritis besides gastric cancer (GC) [11]. GC is among the world's most prevalent cancers, and infection with *H. pylori* is regarded to be a causative agent [12]. While it has been revealed that several unique *H. pylori* virulence factors are correlated with various clinical outcomes, much remains to be learned regarding the contribution of various bacterial causes in gastrointestinal carcinogenesis [13]. In current study the severity of the disease may differ due to various genotypes, as it was observed that homB strain appeared to be strongly associated with gastric cancer. These findings are comparable to the finding of Jung SW et al. [14], who stated that existence of a homB gene is an independent factor for GC discrimination and is correlated with inflammatory response and atrophy of the corpus. In contrast, Abadi AT et al. [13] observed analogous findings as the existence of homB was correlated with development of gastric malignancy. However Kang J et al. [15] observed that no correlation was established between the homB gene distribution and the status of disease within strains of East Asian population. However they validated a statistical correlation between the gastric cancer and the presence of homB gene in Western people, who carry a single homB gene at locus A, and not among East Asian people, who carry and only homB gene at locus B, this statistics perhaps highlights significance of homB gene location within genome. Genes passed to a specific locus may be expressed at higher levels; distributors

of homB and homA may vary reasonably to affect gene expression levels, or the specific loci inside the genome might allow to bind and affect overall homotranscript levels to distinct inhibitors /enhancers of every hom gene. Though, a causal correlation between the GC and homB gene and inflammation of the corpus is not yet clear. This local gene point must be centered and more studies must be carried out.

In this study homB, iceA and dupA were also leading triggers of gastritis, and Peptic ulcer disease. These finding are close to the study of Tsang et al. [16] who stated that dupA is correlated with a raised risk for duodenal ulcer. Lu et al. [17]. Found dup A among 42% cases of duodenal ulcer and 21% cases of gastritis. In Colombia, Korea, and Japan, dupA was recounted to be correlated with a raised risk for duodenal ulcer as compare to gastric cancer and atrophy. However in Western nations this correlation remained undetected, [18] which supports our findings, as current study found no correlation between dupA and gastric cancer and PUD was found significantly greater among ice A and dup A positive strain cases.

Babaei et al. [19] revealed a strong correlation of iceA with PUD ( $P < 0.05$ ). This correlation also supported our findings since we also established greater rates of PUD among patients with iceA strain. A few further studies also reported the correlation between peptic ulcer and iceA of *H. pylori* [20,21]. Abu-Taleb et al. [22] found iceA1 gene among 30% cases with mixed lesions, 6.25% cases with peptic ulcer, and among 8.3% cases with gastritis, while no case of gastric cancer was found. We also found no case of

gastric cancer in cases with iceA gene. As reported by Ashour et al. [23] and Benetal, [24] iceA expression had higher correlation with gastric inflammation, that raises the risk of gastric cancer and UD development. Earlier studies from Netherland and the US exhibited a close association between peptic ulcer disease and iceA [25,26].

We found 41.22 years of mean age of patients and infection rate was higher among males 118(60.2%). Similarly Lu et al. [27] found infection rate of *H. pylori* was 1.1 times greater among males than the females. Zhu et al. [28] reported a mean age of 50.15 years for *h-pylori* infected cases with a greater rate of infection among females as compared to males. The effect may be linked to how food is cooked, and dietary salt administration can cause mucosal degradation, including diffused erosion and degradation, and damage the gastrointestinal mucosal barrier. These modifications in gastric mucosa could be correlated with an elevated risk of recurrent *H. pylori* infection.

## 5. CONCLUSION

It was concluded that homB gene was most frequent in *H. pylori* infected population. Peptic ulcer disease and gastritis are markedly associated with dupA and iceA genes, while homB gene infected patients are at high risk of gastric cancer. Further studies are required to know the mechanism of homB gene and gastric cancer.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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