Study of Isolation and Identification of Helicobacter Pylori in Ulcer Patient

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Abstract: *In this paper, we studied about Isolation and Identification of Helicobacter Pylori in Ulcer Patient. The Study was conducted at Sadar Hospital, Muzaffarpur which is located at North Bihar in India. Muzaffarpur is located at $26^0$7'NM $85^0$24'E and occupies 3,181 square Kilometer (1,228 sq mil). The city lies in a highly active seismic zone of India. In the disastrous earthquake on 15 January 1934, much of the town suffered severe damage and many lives were lost. It has an average elevation of 47 meters (154 feet). This saucer shaped, low-centered town lies on the great Indo-Gangetic plains of Bihar, over Himalayan silt and sand brought by the glacier-fed and rain-fed meandering rivers of the Himalayas.*

Keywords: Ulcer, Gastric, Mucosal, duodenum, Esophagus, Disease.

1. Introduction

The city has a water-table just 20 ft. below ground level. According to the 2011 census Muzaffarpur district has a population of 4,778,610, roughly equal to the nation of of Singapore or the US state of Alabama. This gives it a ranking of 24th in India (out of a total of 640). The district has a population density of 1,506 inhabitants per square kilometer (3,900/sq mi). Its population growth rate over the decade 2001-2011 was 27.54%. Muzaffarpur has a sex ratio of 898 females for every 100 males. Males constituted 52.68% (2,517,500) of the population and females 47.31% (2,261,110). Muzaffarpur had a literacy rate of 65.68%, close to the national average of 74%. Male literacy was 73.61% and females literacy was 56.82%.

Helicobacter pylori is a gram-negative, spiral-shape, microaerophilic organism that infects more than 50% of the world's population (Brown, 2000). It was first isolated by Barry Marshal and Robin warren, in 1982 and they proposed a role of bacterial infection in the pathogenesis of gastroduodenal diseases, which triggered an avalanche of research intended to prove or disprove their theory (Marshall and Warren, 1984). Now it is proved by epidemiological studies that H. pylori is associated with gastroduodenal diseases like gastritis, peptic ulcer, gastric cancer, non-ulcer dyspepsia and gastric adenocarcinoma. Infection with H. pylori plays an important role in development of peptic ulcer disease, distal gastric carcinoma and gastric mucosa-associated lymphoid tissue lymphoma (Megraud & Lamouliatte, 1992; Parsonnet et al., 1991; Wotherspoon et al., 1991). In India, around 65- 70% populations are infected with the H. pylori (Graham et al., 1991; Singh et al., 2002). It usually acquired in childhood and when left untreated generally persists for the host's lifetime (Taylor et al., 1995, Blaser, 1994). The infection remains latent in the majority of the infected population, with only approximately 20% of infected individuals developing severe diseases. H. pylori infection is more prevalent in developing countries, and its incidence is decreasing in western countries (Czinn et al., 2005). Environmental factors include a high salt intake, seasonal diets and nitrate consumption (Graham et al., 2009). Several bacterial virulence genes such as vacA, cagA, babA and oipA of H. pylori have been investigated to understand their association with gastroduodenal diseases (Covacci et al., 1993; Atherton et al., 1995; Yamaoka et al., 1999, 2000, 2002; Argent et al., 2007).

Cytotoxin-associated gene (cagA) was the first reported gene that varies in H. pylori strains and considered as a marker for the presence of the cag Pathogenecity Island which include a number of other genes associated with increased virulence (Broutet et al., 2001; Censini et al., 1996; Rahman et al., 2003). However, none of the mentioned virulence factors have demonstrated discriminating roles in the development of peptic ulcer versus 2 Gastric Cancer. H Pylori populations are extremely diverse genetically, owing to point mutation, substitution, insertion or deletion in their genome. H. pylori genome is highly diverse and the genomes of around fifty H. pylori strains have been completely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,550 genes (Tomb et al., 1997).The genome analysis between 26695 and J99 shows that the sequence variation is significantly greater at the nucleotide level than at the amino acid level. The fact that many of the nucleotide differences are silent with respect to the protein sequences suggests that there is a strong selective pressure for functional conservation at the protein level (Doig et al., 1995). In addition to the cag-PAI, comparison of whole genome of two unrelated H. pylori (J99 and 26695) (Alm et al., 1999; Tomb et al., 1997), indicated presence of a hypervariable region called „plasticity zone" with low G+C content along with strain specific open reading frames (ORFs). This plasticity region is 45 kb long, continuous in strain J99 and 68 kb discontinuous in strain 26695. As compared to 38 ORFs of the plasticity zone (jhp0914-jhp0951) in strain J99, 33 were absent in strain 26695 (Yamaoka., 2008; Pacheco et al., 2008; Yakoob et al., 2010; Kersulyte et al., 2003; Occhialini et al., 2000).

2. Taxonomy of the Helicobacter

Warren and Marshall isolated one organism that resembled Campylobacter in several respects, including curved morphology, growth on rich media under microaerophilic conditions, failure to ferment glucose and a G + C content of 34%. It was therefore first referred to as "pyloric Campylobacter" (pylorus, Greek, gatekeeper or one who looks both ways) and validated as Campylobacter pyloridis in 1985 (Anonymous, 1985). However, pyloridis is an incorrect Latin term and, in 1987, the species name was changed to pylori. Finally, in 1989, when it became clear that H. pylori were not a member of the genus Campylobacter, the genus name was changed to Helicobacter to reflect its distinct functional and enzymatic properties (Goodwin et al., 1990). Later on, electron micrographs showed multiple sheathed unipolar flagella of the bacterium, in contrast to the single bipolar unsheathed flagellum typical of Campylobacter spp (Goodwin et al., 1985). Fatty acids and major protein bands were also significantly different from those of Campylobacter species (Goodwin et al., 1985). It was renamed Helicobacter pylori (H. pylori) on the basis of 16S rRNA gene sequence, the first member of the new genus Helicobacter (Goodwin et al., 1989). The name "Helicobacter" was based on the helical shape of the bacterium and the word "pylori" was used because the bacterium was commonly isolated from the pylorus of the stomach (Goodwin et al., 1989). H. pylori belong to the 'S-type' using the classification based on their amino acid compositions determined from the complete genome and was independent of gram staining (Sorimachi et al., 2004).

Table 1: Scientific Classification

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Epsilon Proteobacteria
Order:	Campylobacterales
Family:	Helicobacteraceae
Genus:	Helicobacter
Species:	Pylori
Binomian Name:	Helicobacter pylori

3. Respiration and Metabolism

H. pylori is a microaerophilic bacterium that is, it requires less amount of oxygen, 5% as compared to atmospheric oxygen, this is because H. pylori use oxygen as a terminal electron acceptor. The elevated level of CO2 required for growth of H. pylori in vitro may be due in part to activity of the enzyme acetyl coenzyme A carboxylase (Burns et al., 1995). H. pylori exhibit a narrow host and target organ range, but infection is usually life long. This suggests strong adaptation to its natural habitat, the mucus layer overlying the gastric epithelial cells. As a consequence, H. pylori lacks

several of the biosynthetic pathways 7 commonly found in less specialized bacteria, such as many enteric bacteria (Alm et al., 1999, Berg et al., 1997, Doig et al., 1999, Marais et al., 1999, Tomb et al., 1997). It has been inferred from genomic comparisons and metabolic studies that H. pylori has a stripped-down metabolic route with very few redundancies and lacks biosynthetic pathways for some amino acids. As a consequence, H. pylori can be grown only in chemically defined medium with the additional amino acids arginine, histidine, isoleucine, leucine, methionine, phenylalanine and valine, and some strains also require alanine and or serine (Nedenskov et al., 1994, Reynolds et al., 1994). H. pylori can catabolize glucose, and both genomic and biochemical information indicates that other sugars cannot be catabolized by H. pylori (Berg et al., 1997, Doig et al.,1999, Marais et al., 1999, Nedenskov et al., 1994) H. pylori possess specific Dglucose transporters; some characteristics of the glucose transport system appear to be unique (Mendz et al., 1995). H. pylori has urea cycle, which may serve as an effective mechanism to extrude excess nitrogen from bacterial cells (Mendz et al., 1996). The Entner-Doudoroff pathway has been demonstrated in H. pylori (Mendz et al., 1994). Fumarate reductase is an essential component of the metabolism of H. pylori and as such constitutes a possible target for therapeutic intervention (Mendz et al., 1995a).

4. Isolation and Identification of Helicobacter Pylori

In this paper, a total of 345 subjects of both genders (aged between 20 and 65 years) were enrolled with upper gastrointestinal disorder underwent endoscopy at the Sadar Hosppital, Muzaffarpur, Bihar, Institute of Post Graduate Medical Education and Research, Kolkata, and St. John's Medical College Hospital, Bangalore, India during the year 2018 to 2020. Out of 125 subjects attending the endoscopy in the sadar hospital Muzaffarpur, bihar, H. pylori strains were cultured from 80 subjects giving an isolation rate of 64 % (Table 2), 145 subjects, attending the endoscopy units in the Gastroenterology department at Institute of Post Graduate Medical Education and Research, Kolkata, H. pylori strains were cultured from 109 subjects giving an isolation rate of 75.17% (Table 2). Among the 75 subjects attending the endoscopy unit in the Gastroenterology Department at St. John's Medical College Hospital, Bangalore, H. pylori strains were isolated from 30 subjects giving an isolation rate of 40% (Table 2). All the H. pylori strains were identified by their typical colony morphology as they appear like transparent water droplets on Brain Heart Infusion Agar plates after 64 incubation for 3 to 7 days at 37°C in a microaerophilic condition of 10% CO_2, 5% O_2 and 85% N_2 in a double gas incubator. H. pylori colonies were further confirmed based on the appearance on Gram staining and positive reaction in urease, catalase and oxidase, along with a urease (UreBF/UreBR) PCR.

Table 2: Isolation of H. pylori strains from different parts of India

Place	No. of Subjects Enrolled	Positive for H. Pylori	% of Isolation
Bihar (Sadar Hospital Muzaffarpur)	125	80	64
West Bengal (P.G. Hospital)	145	109	75.17
Bengalooru (St John’s Medical College)	75	30	40

4.1. Association between helicobacter pylori infection and peptic ulcers.

Table 3: Helicobacter Pylori infection and peptic ulcers Chi-Square Tests

	Value	df	Asymp. Sig. (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-sided)
Pearson Chi-square	12.130	1	.000		
Continuity	10.025	1	.002		
Likelihood Ratio				.001	.001
Fisher's Exact Test	11.827	1	.001		
Linear-by-Linear	40				
Association					
N of Valid cases"					

4.2. Association between family history of peptic ulcers and peptic ulcer disease

Table 4: Family history of peptic ulcers and peptic ulcer diseases

	Value	df	Asymp. Sig. (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-sided)

Pearson Chi-square	.000	1	1.000		
Continuity	.000	1	1.000		
Correction	.000	1	1.000		
Likelihood Ratio				1.000	.624
Fisher's Exact Test	.000	1	1.000		
Linear-by-Linear	40		1.000		
Association					
N of Valid cases"					

4.3. A Summary on factors associated with peptic ulcers

Table 5: Summary on factors associated with peptic ulcers

Factors associated with peptic ulcers	**chi square value**
Helicobacter Infection	0.001
Family history of peptic ulcers	0.624
History of previous illnesses	0.624
Tobacco smoking	0.038
Usage of nsaid drugs	0.371
Alcohol Consumption	0.041
Intake of hot and Spicy Foods	0.642
Stress related condition	0.374

The table above show a cross tabulation between different factors and peptic ulcers by using a chi square test to determine the main factors that are associated with peptic ulcers among the peptic ulcer patients. According to the chi square test, the level of significance is set at < 0.05 meaning any value more than this has no association with peptic ulcers. From the summary table above the associated factors were; Infection with H. Pylori infection, alcohol consumption and tobacco smoking. Among the factors H. Pylori infection showed the strongest association with peptic ulcer diseases.

5. Conclusions

Complete dupA sequence was performed from 10 Indian H. pylori strains. Phylogenetic analysis based on complete dupA sequence showed that Indian strains clustered with the East Asian strains and European strains formed a separate group. This is the first known genetic element of Indian H. pylori which intermingled with the East Asian strains but differed with the European strains. Our result showed that prevalence of virB8, virB9 and virB10 were more in DU patients than NUD but neither vir genes (virB8, virB9, virB10, virB11, virD4 and virD2) nor dupA cluster were significantly associated with clinical outcome (P =0.05).

This study revealed that dupA gene existed in two allelic forms: long type of 2.5kb and short type of 1.8 kb. The distribution of both alleles was equal in our population which contradicts the findings of the other research groups. In our population, intact dupA gene without frame shift mutation was more prevalent in DU than NUD, consistent with the finding of other studies.

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