Study of Genetic Diversity, dupA gene, and Association with Previous Illness, and Smoking in Peptic Ulcer Disease

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Abstract: In this paper, we studied about genetic diversity, dupa gene, and association with previous illness, and smoking in peptic ulcer disease. We also studied Pathogenesis of H. Pylori, smoking and Peptic Ulcer Disease. The Study was conducted at Sadar Hospital, Muzaffarpur which is located at North Bihar in India. In 2011 census, this figure for Muzaffarpur District was at 4.60 percent of Bihar population.

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

I. INTRODUCTION

In 1999, strain J99 was sequenced which was isolated from an American patient with a duodenal ulcer (Alm et al., 1999). Compared to strain 26695, it has a slightly smaller circular chromosome (1643831). The overall genomic organization, gene order and predicted proteomes of the sequenced strains are very similar. The predicted open reading frames are less in strain J99, amounting to1495.In 2006, a chronic atrophic gastritis H. pylori strain, HPAG1, was sequenced (Oh et al., 2006). It was isolated from an 80year-old female patient who was enrolled in a Swedish case-control study of gastric cancer (Enroth et al., 2000). The genome of HPAG1 (1596366 bp) is the smallest 12 in the three sequenced strains. A total of 1536 open reading frames were predicted. H. pylori strain G27 was sequenced recently (Baltrus et al., 2009). It was originally isolated from an Italian patient (Covacci et al., 1993) and has been used widely in H. pylori research. This strain is naturally transformable (Censini et al., 1996), capable of delivering CagA into epithelial cells in culture (El-Etr et al., 2004, Guillemin et al., 2002), and capable of adapting to variable environments (Amieva et al., 2003). The G27 genome has a similar size to the other three sequenced strains. It is 1652983 bp long with a GC content of 38.9% and 1515 open reading frames were predicted.

II. THE GENETIC DIVERSITY

H. pylori is one of the most genetically diverse of the bacterial species, with any given isolate easily distinguished from most other isolates by simple PCR based DNA fingerprinting or limited DNA sequencing (Akopyanz et al., 1992, Logan et al., 1996). This extraordinary diversity is due to variously differences among strains in gene content and gene rearrangement, to numerous point mutations within individual genes, and to a rich history of recombination between strains (Achtman et al., 1999). Some of this diversity may be adaptive, affecting variously the chance of successful transmission in a physiologically diverse human population, the chance that an infection will persist and the chance that persistence will lead to overt diseases. Any two independent clinical isolates are usually readily distinguished by DNA fingerprinting or sequencing of one or two housekeeping genes; strains also differ in types of virulence genes that they carry; and different genotype clusters predominate in different parts of the world (e.g., East Asia vs. Western Europe) (Linz et al., 2007, Wirth et al., 2005, Kersulyte et al., 2002, 2004) Several strains of H. pylori and a number of related bacteria have been fully sequenced so far; in 1997, H pylori strain 26695 was firstly sequenced (Tomb et al., 1997). It was isolated from an English patient with chronic gastritis. The chromosome of strain 26695 is circular and composed of 1667867 base pairs. The average GC content is approximately 39%. In the initial annotation, it has 1590 open reading frames that are possibly protein-coding (Tomb et al., 1997), in addition to the RNA coding genes (2 copies of 16S rRNA and 23S rRNA genes, 36 tRNA genes).

Later analysis of the genome sequence suggested a smaller number of ORFs in strain 26695 (Alm et al., 1999). The H. pylori Shi470 genome has also been sequenced by Washington University Medical School. It is 1.61 Million bp long and contains approximately 1609 predicted genes. This small genome size reflects a limited metabolic repertoire and biosynthetic capacity, consistent with the specialization of H. pylori for growth in unique gastric niche (Dong et al., 2009).

2.1. dupA Status from Indian Population

In our study, 38 (31 DU and 7 NUD) clinical strains were positive for jhp0917-0918 by PCR and dot-blot hybridization. Using sequencing study it was revealed that 35 strains had insertion of C and three strains had no insertion of T/C like J99. During analysis by RTPCR, it was found that 28 strains out of 35 were positive in the

RT-PCR. On the basis of sequencing and RT-PCR findings, we confirmed that 28 (23 duodenal ulcer and 5 NUD) strains were dupA positive and 7 (5 duodenal ulcer and 2 NUD) strains were negative for dupA. Finally, our study showed that the prevalence of dupA in duodenal ulcer patients 23/83 (27.7%) was significantly higher than the NUD 5/57 (8.7%). [P=0.001, OR= 6.49, 95% CI=1.71-28.94] (Table 1).

Table 1: Prevalence of the dupA, cagA genes among the studied strains in India population

Gene	Total (n=140)	DU (n=83)	NUD (n=57)
dupA	28 (20%)	23 (27.7%)	5 (8.7%)
CagA	129 (92.1%)	76 (91.5%)	53 (92.9%)
vacAslml	98 (70%)	59 (71%)	39 (68.4%)

Prevalence and Association of dupA with clinical outcome H. pylori colonize the stomach of more than 50% of the world‟s population. However, why only a few percentages of infected subjects develop severe diseases like duodenal ulcer and gastric carcinoma is unknown. This discrepancy has been the source of considerable impetus to identify bacterial markers for diseases outcome. One possible reason for the varying outcomes of H. pylori infection relates to differences in the virulence of H. pylori strains in addition to host, environmental, and dietary factors. The genome comparison of the some completely sequenced H. pylori isolates provided important information regarding the genetic heterogeneity of this microorganism (Alm et al., 1999). Around 6% of the coding sequences of the strains are genome specific. Half of these strain-specific genes are located in the plasticity region (Yamoka et al., 2008)). The strain-specific genes are likely to play a role in the pathogenesis of H. pylori related diseases.

However, reports on the clinical predictive value of putative virulence factor status and diseases outcome are controversial, at least in all ethnic groups (Chiarini et al., 2008; Dossumbekova et al., 2006; Douraghi et al., 2008; Erzin et al., 2006; Gomes et al., 2008; Ho¨cker & Hohenberger, 2003; Lu et al., 2005a). Recent studies have proposed the possibility of using genetic markers in the plasticity zone as indicators of pathogenecity for H. pylori infection, in spite of a lack of credible knowledge regarding the functions of the putatively encoded proteins in this cluster. It seems that these determinants may play a key role in determining the virulence capacity of H. pylori strains either directly or by encoding factors that may lead to varying clinical outcomes. The association between some of the ORFs in the plasticity zone and various disease categories has been previously reported. For instance, Occhialini et al. (2000) found that two single ORFs (jhp0940 and jhp0947) were more prevalent in strains isolated from patients with gastric adenocarcinoma in Costa Rica. However, Santos et al. (2003) showed the association between jhp0947 and DU as well as GC in Brazilian patients. This was once more confirmed for jhp0947 and jhp0949 genes in DU patients from the Netherlands (de Jonge et al., 2004).

We found that dupA gene was 6.5 times more prevalent in duodenal ulcer patients than non-ulcer patients among Indian population. Hence, dupA gene was significantly associated (P=0.001) with DU. Associations between the presence of dupA and H. pyloridiseases varies around the world (Nguyen et al., 2009, Argent et al., 2007, Gomes et al., 2008, Hussein et al., 2010). Several issues starting from geographical variations to study procedures have to be considered. In some studies, only one set of primer pairs for jhp0917 and jhp0918 was used (Lu et al., 2005; Zhang et al., 2008; Douraghi et al., 2008; Pacheco et al., 2008). Our study showed that some dot blot positive strains for jhp0917-jhp0918 failed to 93 provide any amplicon by initial set of primers. But later on, additional different set of primers yielded amplicon of the same strains. Hence, use of multiple primer pairs is recommended for detection of the dupA gene in future studies. Besides that, sequence based analysis showed that 7.8 % (3/38) jhp0917-jhp0918 positive strains did not have any insertion of C or T after position 1385 in the 3‟region of jhp0917 indicating that they are not forming the dupA.

This report is inconsistent with previous reports in other populations, which indicated that all clinical isolates possessed a continuous dupA gene (Douraghi et al., 2008; Schmidt et al., 2008; Gomes et al., 2008). Moreover, in our study not a single strain was detected with the insertion of T after position 1385 of jhp917 that means all our dupA positive strains have insertion of C only which was inconsistent with the finding of lu et al., (2005) Semiquantitative RT-PCR analysis showed that 20% (7/35) of the dupA positive strains did not show any dupA transcript. This contradicted the findings of Nguyen et al. (2009) that dupA was always expressed. The reason for the absence of dupA transcript is not clear yet, but it might be due to absence of dupA promoter region or some mismatch at ribosomal binding site of dupA promoter due to which ribosomal protein was unable to bind with promoter. Indeed there is need of more study on promoter analysis of dupA gene. dupA gene expression is similar to expression of the blood group antigen binding adhesins (BabA) that is not always correlated with babA gene expression (Yamoka et al., 2008).

Randomly, six dupA transcript positive strains (I-77, I-114, 127(1a), I-87, 217(4b) and san77) were taken for the quantitative PCR analysis. Expression level of strain I-77 was very low as compared to other five strains tested that‟s why we have calculated fold change of remaining five strains against strain I-77. The expression of dupA transcript of strain I-114, 127(1a), I-87, 217(4b) and san77 was 4, 10.8, 11.6, 10.4, and 8 fold higher than strain I-77 respectively, which showed that expression level of dupA transcript varied from strain to strain. The

reason of this different expression level might be the copy no. of dupA transcript present in the strains that might vary from strain to strain (Boonjakuakul et al., 2005). A recent systematic review study demonstrated the importance of the presence of the dupA gene for duodenal ulcer, especially in Asian countries (Shiota et al. 2010). Arachchi et al. (2007) showed that dupA gene was present in 37.5% and 22.8% of DU and Functional Dyspepsia patients, respectively from North India, but in our study dupA gene was present in 27.7% and 8.7% of DU and NUD patients, respectively.

The reason of this variation of dupA prevalence in India might be due to the fact that their study did not include the sequencing of intergenic region of jhp0917-918 to check the insertion of one nucleotide after position 1385 and RNA expression profile of dupA gene or might be related to the geographical genome variation of H. pylori as India is a 94 big country with lots of diversities. Some studies reported that dupA gene have single nucleotide polymorphism (SNP) that created a premature stop codon and may have considerable effects on protein expression or function (Gomes et al., 2008; Hussein et al., 2010; Queiroz et al., 2011; Moura et al., 2012). Moreover, Douraghi et al. (2008) reported that dupA was inversely associated with the histological feature dysplasia, a main premalignant and precancerous lesion associated with increased incidence of cancer in Iranian population. As a result, dupA gene may be applicable as a protective marker against GC development. But we were unable to study this hypothesis as we did not have samples from gastric cancer patients. However, a very recent study showed that the presence of a complete dupA cluster (type IV secretory system with vir genes around dupA) seemed to be important in DU development (Jung et al., 2012). The prevalence of dupA positivity differed significantly among the various regions around the world, with highest prevalence found in South Africa (84%) and Brazil (89%). Overall, there are distinct geographical variations in the prevalence of the dupA gene, and there appears to be an association between dupA and DU in some populations but not in others. Hussein speculated that differences in dupA prevalence between populations could be associated with the selected nature of the dyspeptic populations studied or there may be genetic differences between versions of the dupA gene in different strains, similar to the difference found in vacA alleles and cagA phosphorylation motifs (Hussein et al., 2010). Our results also indicate that dupA is highly variable over the region sequenced (1.8 kb) with a sequence variation in the range of 10.2%.

This indicates a high degree of variation comparable to other virulence genes. Similar findings reported by others would support the high degree of variation of this gene observed in our study. The high degree of sequence variation of dupA suggested that the acquisition might be an ancient event as dupA is located in the plasticity zone. In conclusion, infection with the dupA-positive H. pylori increased the risk for DU overall and this evidence was significant in Indian study. The gene dupA can be considered as an important biomarker for DU in Indian population. However, further studies are required to determine the functionality of dupA and its relationship with disease.

The discrepancy of dupA association with diseases outcome could be related to the limitation of PCR techniques for detecting the intact dupA gene or may be a consequence of the plasticity of H. pylori, which contributes to its genetic diversity and requires additional studies for a firm conclusion.

Table 2: Association between previous illnesses and peptic ulcers

	Value	**df**	**Asymp. Sig. (2-Sided)**	**Exact Sig. (2-Sided)**	**Exact Sig. (1-sided)**
Pearson Chi-square	.742	1	.389		
Continuity	.283	1	.595		
correction"	.745	1	.388		
Likelihood Ratio				.514	.298
Fisher's Exact Test	.723	1	.395		
Linear-by-Linear	39				
Association					
N of Valid cases"					

Table 3: Intake of hot and spicy foods and peptic ulcer disease

	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	.642
Fisher's Exact Test	.000	1	1.000		
Linear-by-Linear	40		1.000		
Association					
N of Valid cases"					

Table 4: Smoking and peptic ulcers

	Value	df	Asymp. Sig. (2-Sided)	Exact Sig. (2-Sided)	Exact Sig. (1-sided)
Pearson Chi-square	4.358	1	.037		
Continuity	3.104	1	.078		
Correction	4.445	1	.035		
Likelihood Ratio				.054	.038
Fisher's Exact Test	4.246	1	.039		
Linear-by-Linear	39				
Association					
N of Valid cases"					

Table above shows that 75.5% of peptic ulcer patients had a habit of taking NSAID drugs, 45% had a family history of peptic ulcers, 37% had a history of previous illness, 40% smoked tobacco, 70% consumed alcohol, 75% were affected by intake of hot and spicy food, 60% had suffered from stress related conditions and 52.5% had been infected with "H.pylori infection". This therefore indicates that the factor that played a high role in peptic ulcer condition in the patients was intake of NSAID drugs (75.5%).

III. CONCLUSIONS

Rate is not yet clear but probably either due to involvement of dupA gene in cell division of H. pylori as dupA gene has homology with FtsK gene of E. coli which involved in cell division or dupA gene down regulates the activity of cell division related gene cdrA (HP0066) of H. pylori. Finally, our study showed that infection with the dupA-positive H. pylori increased the risk for DU 6 times than the NUD in Indian population. So, the dupA can be considered as an important biomarker for DU at least in Indian population. The discrepancy of dupA association with diseases outcome in different geographical regions could be related to the limitation of PCR techniques for detecting the intact dupA gene or may be a consequence of the plasticity of H. pylori, which contributes to its genetic diversity.

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