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A Cross Sectional Study to Determine Risk Factors Associated with Prevalence of *H. pylori* Infection in Salivary Samples of Acid Peptic Disease Patients in Western India

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Abstract: The present study was designed to determine the prevalence of virulent *H. pylori* in the salivary samples of acid peptic disease patients and role of various risk factors associated with *H. pylori* infection by application of polymerase chain reaction. Salivary samples of 160 confirmed acid peptic disease patients were used to determine the prevalence of *H. pylori*. DNA was extracted from the samples using phenol chloroform CTAB method was used to amplify 16 s rRNA, HSP 60, cag E and cag T using *H. pylori* specific primers. Information regarding the risk factors was determined using a questionnaire in local language. The prevalence of virulent *H. pylori* in the patients belonging to category of males, age groups of (18-30), (61-75), smokers, rural inhabitants, raw water consumers, consumers of outside cooked food, having low socioeconomic status, having outdoor sanitary practices, bearing low clean water index and high crowding index was: (82%), (96.66%), (90%), (91.5%), (92.59%), (97.10%), (87.77%), (92.10%), (91.11%), (97.33%) and (95.95%), respectively and was significantly higher than the other groups ($p<0.0001$). Age, gender and NSAID use were not associated and alcohol consumption was negatively associated with *H. pylori* infection status. Successful amplification of cag E and T in the salivary samples of significant number of acid peptic disease patients elucidates that *H. pylori* is present in a virulent state in the oral cavity of the patients and measures need to be employed to eliminate the risk factors. Prevalence of virulent *H. pylori* is closely associated with environmental risk factors.

Key words: *H. pylori*, risk factors, acid peptic disease

INTRODUCTION

Helicobacter pylori (*H. pylori*)is a human pathogen spiral, microaerophilic, gram negative, panmictic ε proteobacterium reigning the gastric milieu of around 60% population in the world (McColl, 1999; Das and Paul, 2007). It is primarily associated with the transformation of gastric lesions into life threatening carcinomas and lymphomas (Kamangar *et al*., 2007). Various studies have elucidated that *H. pylori* resides in the stomach and oral cavity of asymptomatic and symptomatic patients (Ahmed *et al*., 2007; Tiwari *et al*., 2005).

Various factors associated with infection are age, ethnicity, gender, geography, urban and rural residence, water and food source, frequency of NSAID consumption, smoking, alcohol, socioeconomic status, clean water index, crowding index etc. (Ahmed *et al*., 2007; Mishra *et al*., 2008; Nurgalieva *et al*., 2002).

The virulence factors are expressed only in the patients of various gastro duodenal diseases. HSP 60 and 16s r RNA genes are present in highly conserved regions of *H. pylori* genome and have been used to detect *H. pylori* in salivary samples of asymptomatic subjects as well as subjects suffering from acid peptic diseases (Tiwari *et al*., 2005; Mishra *et al*., 2008). Virulent *H. pylori* is closely associated with the disease state (Tiwari *et al*., 2005). Its virulence lies in the pathogenic machinery of the three genes cag A, E and T which act as the syringe and needle to inject multimeric carcinogenic cytotoxins into the gastric cells (Blaser *et al*., 1995).

Cag E and T genes are associated with virulence and have been identified as important components of cag pathogenecity island (Tiwari *et al*., 2005). They have been depicted as bio markers to determine the virulent infection in patients of non ulcer dyspepsia and gastric carcinoma (Tiwari *et al*., 2005). Hence, saliva can be used as a

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reproducible sample to detect the presence and virulence of *H. pylori* in patients of various gastrointestinal diseases. Prevalence of *H. pylori* in the patients of acid peptic disease has not been investigated in patient population in western India. It is of prime importance to elucidate the prevalence of *H. pylori* and also investigate the factors related with the infection status in this patient population. Hence, the present investigation was designed to detect the prevalence of *H. pylori* using 16s r RNA and HSP 60 genes while virulence was determined using cag E and T as the biomarkers by application of polymerase chain reaction in the salivary samples of patients of acid peptic disease. Thereafter, confounding factors were correlated with the virulent infection status of *H. pylori*.

MATERIALS AND METHODS

All the chemicals for DNA extraction were procured from S. D. Fine chemicals, India. The reagents for PCR, gel preparation and visualization were purchased from Biolinx, Pune India. The forward and reverse primer for 16S rRNA, HSP 60, cag E, cag T genes were synthesized at Sci Fi Biologicals, Pune India. Gel electrophoresis unit (Bangalore genie, Bangalore) was used to perform gel electrophoresis and Gel documentation unit (Alpha Innotech Inc. USA) was used to visualize and capture the gel image.

Recruitment of patients and sample collection: One hundred and sixty confirmed patients of acid peptic disease were recruited in the present study. The participation was voluntary and care was taken to explain the objectives of the study in local language to each patient before salivary sampling. All individuals signed an informed consent in local language or English in order to be included in the study. The patients of acid peptic disease were enrolled from the out patient departments of above mentioned hospitals located at various parts of Pune city. The patients were examined by gastroenterologists and only confirmed cases of acid peptic disease were included in the study. The study population consisted of men and women of more than 18 years of age. It was ascertained that none of the participants of the study had consumed proton pump inhibitors, H_2 blockers or antibiotics in the last one month of saliva sampling. Unstimulated saliva in the volume of 1.5 mL was collected in sterile eppendorf tube and stored at -80°C until processed. After collection, saliva was homogenized by vigorous shaking with the use of a vortex mixer and clarified by centrifugation (10,000 g, 4° C, 4 min).

Human ethics approval: The study protocol was approved by scientific and institutional human ethics committee and a formal written permission was obtained from the governing authorities of Bharati Hospital and research centre, Tirupati Hospital, Khenat Hospital, Agarwal Hospital, Dhekane Clinic for the recruitment of acid peptic disease patients from the outpatient departments of these hospitals. The study was carried out in strict adherence to laws and guidelines laid down by international health authorities and organizations (The Code of Ethics of the World Medical Association for experiments involving humans).

Collection of data: A questionnaire in local language was available for data collection that included gender, water source, information for calculation of clean water and crowding index. Clean Water Index (CWI) was calculated using previously reported method (Ahmed *et al*., 2007) and crowding index (CRI) was determined using (Nurgalieva *et al*., 2002). The details about processing of water in the household of each subject were determined using a suitable questionnaire regarding the practice of purification of potable water used for drinking in their household. Subjects were considered to consume processed water if the tap/well/river water was filtered using a house hold water purifier/automated purification system, or boiled or chlorinated whereas subjects who consumed raw/unfiltered/unboiled water were categorized as consuming unprocessed water. The socioeconomic status was evaluated using a validated questionnaire in local language (Aggarwal *et al*., 2005).

Preparation of genomic DNA for PCR: DNA isolation from salivary samples was performed according to phenol chloroform CTAB method (Tiwari *et al*., 2005). All the steps were performed in aseptic conditions to minimize contamination. DNA was preserved at -20°C until amplification was performed.

PCR amplification

Sensitivity assay: The detection limits of the PCR assay was determined by preparation of 10-fold serial dilution, from 50 ng to 1 fentogram of the isolated genomic DNA from *H. pylori* strain ATCC 26695 in sterile water for injection. An aliquot of each dilution was amplified by PCR and the amplicons visualized on 1.5% agarose gel stained with ethidium bromide. Sensitivity of this PCR assay was ascertained based on the maximum dilution of genomic DNA in which the primers were able to amplify their specific gene sequences.

Specificity assay: DNA isolated from an entirely sequenced *H. pylori* reference strain DNA (ATCC 26695) was used as a positive control. The specificities of the PCR method was evaluated for three different bacteria obtained from NCIM (National Centre for Industrial Microbes): *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2345, *Bacillus subtilis* NCIM 2063.

Amplification of genes of *H. pylori*: DNA isolated from the salivary sample of each individual was subjected to PCR thermal cycles using specific *H. pylori* primers to amplify 16 s rRNA gene to yield an amplicon of 534 bp (Tiwari *et al*., 2005) and another set of primers to amplify HSP 60 gene to yield an amplicon of 501 bp (Mishra *et al*., 2008). Cag E and T genes were amplified to determine the virulence in the *H. pylori* samples (Tiwari *et al*., 2005). DNA isolated from each subject was initially subjected to to two different PCR amplifications to determine the presence of HSP 60 and 16s r RNA genes and subsequently the aliquots from the same DNA was used to amplify cag E and T. The primer sequences and respective amplicon sizes have been mentioned in Table 1. At each amplification step, *H. pylori* DNA isolated from strain ATCC 26695 was used as a positive control, while sterile water for injection instead of DNA served as a negative control. The PCR products were analyzed by agarose gel electrophoresis unit (Bangalore Genei, India) and all the gel photographic registries were performed using a gel documentation system (Apha Innotech Inc. USA).

Statistical methods: The statistical analysis was carried out to examine the association between the various study variables with saliva PCR positivity for *H. pylori* using Fischer exact test. Statistical analyses was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), Odds ratio, 95% confidence interval of OR, Relative risk, 95% confidence interval of RR were determined.

Table 1: Primer sequences and respective amplicon sizes of *H. pylori* specific genes

Primer	Sequence	Amplicon size (bp)
16S rRNA-F	5'-TAAGAGATCAGCCTATGTCC-3'	534
16S rRNA-R	5'-TCCCACGCTTTAAGCGCAAT-3'	
HSP1	5'-AAGGCATGCAATTTGATAGAGGCT-3'	
HSP2	5'-CTTTTTTCTCTTTCATTTCCACTT-3'	
HSPN1	5'-TTGATAGAGGCTACCTCTCC-3'	501
HSPN2	5'-TGTCATAATCGCTTGTCGTGC-3'	
CagE-F	5'-GCGATTGTTATTGTGCTTGTAG-3'	329
CagE-R	5'-GAAGTGGTTAAAAAATCAATGCCCC-3'	
cagT- F	5'-ATGAAAGTGAGAGCAAGTGT-3'	301
cagT- R	5'-TCACTTACCACTGAGCAAAC-3'	

RESULTS

The DNA isolated from all the samples were amplified to get a 534 and 500 base pair fragments corresponding to 16s r RNA and HSP 60 genes of *H. pylori*. To determine the samples possessing virulent *H. pylori*, DNA isolated from identical samples subjected to different PCR amplifications to yield amplicons of 301 and 329 base pairs corresponding to cag E and T genes respectively (Fig. 1).

Non virulent infection: Prevalence of non virulent infection in the patients of acid peptic disease patients was determined by the amplification of HSP 60 and 16s r RNA genes in two separate PCR reactions using two aliquots of the same DNA template. Both the genes were successfully amplified depicting non virulent *H. pylori* in all the 160 patients of acid peptic disease. The prevalence of infection in male and female subjects was found to be equal to (100%) (Table 2).

Determination of relation of virulence with risk factors: Prevalence of virulent infection was determined by the amplification of cag E and T genes in two different PCR reactions using two aliquots of the same DNA template.

Table 2: Prevalence of non virulent *H. pylori* infection in the patients of acid peptic

Variables	No.of subjects	HP positive
Gender		
Male	100 (62.5%)	100 (100.0%)
Female	60 (37.5%)	60 (100.0%)

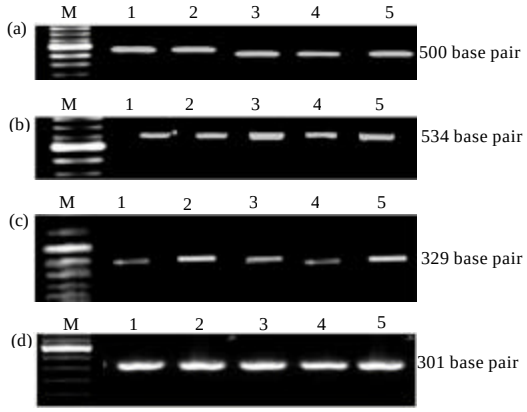

Fig. 1(a-d): Successful amplification of HSP 60 (500 base pairs), 16s rRNA (534 bp), cag E (329 bp) and cag T (301 bp), respectively using the DNA isolated from salivary samples of acid peptic disease patients

Table 3: Statistical analysis between groups carried out using Fischer exact test

Variables	No. of subjects	Vir HP positive (128)	Vir HP negative (22)	p-value	Odd ratio	95% CI of OR	RR	95% CI of RR
Gender								
Male	0.100	82 (82%)	22 (22%)	0.0641	0.32410	0.1052-0.9985	0.8570	0.7534-0.9749
Female	0.50	46 (22%)	4 (8%)	Referent				
Age								
18-30	0.30	29 (96.66%)	1 (3.33%)	Referent				
31-45	0.40	31 (77.5%)	9 (22.5%)	0.0358	0.11880	0.01415-0.9971	0.8017	0.6698-0.9596
46-60	0.50	41 (82%)	9 (18%)	0.0809	0.15710	0.01885-1.309	0.8483	0.7331-0.9815
61-75	0.30	27 (90%)	3 (10%)	0.6120	0.31030	0.03039-3.170	0.9310	0.8122-1.067
Alcohol								
Current	0.20	9 (45%)	11 (55%)	<0.0001	0.03188	0.007465-0.1361	0.4675	0.2874-0.7605
Former	0.80	77 (96.25%)	3 (3.75%)	Referent				
Never	0.50	42 (84%)	8 (16%)	0.0218	0.20450	0.05149-0.8126	0.8727	0.7675-0.9924
Smoking								
Current	0.87	80 (91.95%)	7 (8.05%)	Referent				
Former	0.10	7 (70%)	3 (30%)	0.0650	0.20420	0.04299-0.96960	0.7613	0.5049-1.148
Never	0.53	41 (77.35%)	12 (22.64%)	0.0211	0.29900	0.1094-0.8172	0.8413	0.7180-0.9857
NSAIDs use								
Yes	0.63	55 (87.30%)	8 (14.54%)	Referent				
No	0.87	73 (83.90%)	14 (16.04%)	0.6443	1.31800	0.5167 -3.364	1.040	0.9121-1.187
Area								
Rural	0.54	50 (92.59%)	4 (7.4%)	Referent		0.1108-1.084		0.7766-0.9916
Urban	0.96	78 (81.25%)	18 (18.75%)	0.0905	0.34670		0.8775	
Drinking water								
Raw	0.69	67 (97.10%)	2 (2.89%)	Referent				
Processed	0.81	61 (75.30%)	20 (24.69%)	0.0001	0.09104	0.02042-0.4059	0.7756	0.6802-0.8843
Eating habit								
Home	0.60	49 (81.66%)	11 (18.33%)	0.3494	0.62030	0.2500-1.539	0.9304	0.8068-1.073
outside	0.90	79 (87.77%)	11 (12.22%)	Referent				
Socioeconomic status								
Low	0.90	85 (94.44%)	5 (5.55%)	Referent				
Medium	0.38	35 (92.10%)	3 (7.89%)	0.6939	0.68630	0.1555-3.029	0.9752	0.8774-1.084
High	0.22	8 (36.36%)	14 (63.64%)	<0.0001	0.03361	0.009606-0.1176	0.3850	0.2210-0.6708
Sanitation practices								
Outdoor	0.45	41 (91.11%)	4 (8.88%)	Referent				
Indoor	0.105	87 (82.85%)	18 (17.14%)	0.2191	0.47150	0.1500-1.483	0.9094	0.8017-1.032
CWI								
High	0.29	4 (13.79%)	25 (86.20%)	<0.0001	0.22810	39.34-1323	7.057	2.838-17.55
Middle	0.56	51 (91.07%)	4 (7.13%)	0.2411	0.34930	0.06161-1.98	0.9527	0.8768-1.035
Lower	0.75	73 (97.33%)	2 (2.67%)	Referent				
Crowding index (CRI)								
High	0.99	95 (95.95%)	4 (4.05%)	Referent				
Middle	0.35	31 (88.57%)	4 (11.43%)	0.2053	0.32630	0.07698-1.383	0.9230	0.8140-1.047
Low	0.07	2 (28.57%)	5 (71.43%)	<0.0001	0.01684	0.002466-0.1150	0.2977	0.09220-0.9615

The values in paranthesis indicate the percentage of the number of subjects. The numbers of virulent *H. pylori* positive individuals indicate the successful amplification of cag E and cag T genes, The number of *H. pylori* positive individuals indicate the successful amplification of 16s r RNA and HSP 60 genes

Relation of virulence with risk factors: The results indicated in Table 3 indicate that, in male and female subjects was found to be equal to (82) and (22%), respectively. The p value was equal to 0.05 when male patients were considered as referent. The prevalence in age groups of (18-30), (31-45), (46-60), (61-75) was found to be equal to 96.66, 77.5, 82 and 90%, respectively. The p-value was equal to 0.0358, 0.0809 and 0.612 in the age groups 31-45, 46-60 and 61-75 when patients of age group 18-30 were considered as referents. The prevalence of virulent infection in patients with respect to consumption of alcohol was (45%) current, (96.25%) former and (84%) never. The p value was equal to <0.0001, 0.0218 in patients who were current alcoholics and never consumed alcohol when former alcoholics were considered as referent. The prevalence of virulent infection was found to be equal to 92.10, 94.44 and 36.36% in the patients who were current former and never smokers, respectively. The p-value was equal to 0.0650 and 0.0211 in patients who were former smokers and never smoked when former smokers were considered as referent. The prevalence of virulent infection was found to be equal to (87.30%) and (83.90%) in the NSAID users and non users, respectively. The p-value was equal to 0.6443 in non NSAID users when NSAID users were considered as referent. The prevalence of virulent infection was found to be equal to (92.59%) and (81.25%) in the people staying in rural and urban areas, respectively. The p-value was equal to 0.0905 in urban inhabitants when rural inhabitants were considered as referents. The prevalence of virulent infection was

found to be equal to (81.66%) and (87.77%) in the people home and outside food. The p-value was equal to 0.0001 in patients consuming raw drinking water when patients consuming processed drinking water were considered as referent. The p-value was equal to 0.3494 in the patients consuming outside cooked food when patients consuming home cooked food were considered as referent. The prevalence of virulent infection in patients of low, middle and high socioeconomic status was 94.44, 92.10 and 36.36%, respectively. The p-value was equal 0.6939 and <0.0001 in patients of low and high socioeconomic status when patients of medium socioeconomic status were considered as referent. The prevalence in the subjects using outdoor sanitation practices was 91.11% whereas in the subjects using indoor sanitation practices it was 82.85%. The p-value was equal 0.2191 in patients having indor sanitation practices when patients having outdoor sanitation practices were considered as referent. The prevalence of infection in the subjects who belong to low, medium or high CWI was found to be equal to (13.79%), (91.07%) and (97.33%), respectively. The p-value was equal <0.0001and 0.2411 in patients of high and middle CWI when patients of lower CWI were considered as referents. The prevalence of infection in the subjects who belong to high, medium and low CRI was found to be equal to 95.95, 88.57 and 28.57%, respectively. The p-value was equal <0.0001 and 0.2053 in patients of middle and low CRI when patients of high CRI were considered as referent.

The odds ratio and 95% CI of odds ratio in the population of virulent infection with respect to gender was (0.3241, 0.1052-0.9985) in males when females subjects were considered as referent. The odds ratio and 95% CI of odds ratio in the population of asymptomatic subjects with respect to age was as follows: (0.1188, 0.01415-0.9971) 31-45 years, (0.1571, 0.01885-1.309) 46-60 years, (0.3103, 0.03039-3.170) 61-75 years, when subjects of age group 18-30 years were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to alcohol consumption was as follows: (0.1188, 0.01415-0.9971) 31-45 years, (0.1571, 0.01885-1.309) 46-60 years, (0.3103, 0.03039-3.170) 61-75, years when subjects of age group 18-30 years were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to alcohol consumption was as follows: (0.03188, 0.007465-0.1361) current, (0.2045, 0.05149-0.8126) never, when former alcoholics were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to smoking was as follows: (0.2042, 0.04299-0.9696) former, (0.2990, 0.1094-0.8172) never, when current smokers were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to NSAID consumption was as follows: (1.318, 0.5167 -3.364) non NSAID users, when NSAID users were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to residence was as follows: (1.318, 0.5167 -3.364) urban dwellers, when rural dwellers were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to drinking water was as follows: (0.09104, 0.02042-0.4059)unprocessed water consumers, when processed water consumers were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to eating habit was as follows: (0.6203, 0.2500-1.539) home cooked food consumers, when people consuming outside cooked food were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to socioeconomic status was as follows: (0.6863, 0.1555-3.029) patients of medium socioeconomic status, (0.03361, 0.009-0.117) patients of high socioeconomic status, when patients of low socioeconomic status were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to sanitation practices was as follows: (0.4715, 0.1500-1.483) people having indoor sanitation practice, when people having outdoor sanitation practices were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to CWI was as follows: (0.2281, 39.34-1323) patients of high CWI status (0.3493, 0.06161-1.980) patients of medium CWI status when people from low CWI status were considered as referent. The odds ratio and 95% CI of odds ratio in the acid peptic disease patients with respect to CRI was as follows: (0.3263, 0.07698-1.383) patients of middle CRI status (0.01684, 0.002466-0.1150) patients of low CRI status when people from low CRI status were considered as referent.

The relative risk and 95% CI of odds ratio in the population of virulent infection with respect to gender was (0.8570, 0.7534-0.9749) in males when females subjects were considered as referent. The relative risk and 95% CI of relative risk in the population of asymptomatic subjects with respect to age was as follows: (0.8017, 0.6698-0.9596) 31-45 years, (0.8483, 0.7331-0.9815) 46-60 years, (0.9310, 0.8122-1.067) 61-75 years when subjects of age group 18-30 years were considered as referent. The relative risk and 95% CI of relative risk in acid peptic disease patients with respect to age was as follows: (0.8017, 0.6698-0.9596) 31-45 years, (0.8483, 0.7331-0.9815) 46-60 years, (0.9310, 0.8122-1.067) 61-75 years, when subjects of age group 18-30 years were considered as

referent. The relative risk and 95% CI of odds ratio in the acid peptic disease patients with respect to alcohol consumption was as follows: (0.4675, 0.2874-0.7605) current, (0.8727, 0.7675-0.9924) never, when former alcoholics were considered as referent. The relative risk and 95% CI of odds ratio in the acid peptic disease patients with respect to smoking was as follows: (0.7613,0.5049-1.148)former,(0.8413,0.7180-0.9857)never, when current smokers were considered as referent. The relative risk and 95% CI of relative risk in the acid peptic disease patients with respect to NSAID consumption was as follows: (1.040, 0.9121-1.187) non NSAID users, when NSAID users were considered as referent. The relative risk and 95% CI of relative risk in the acid peptic disease patients with respect to residence was as follows: (1.040, 0.9121-1.187) urban dwellers, when rural dwellers were considered as referent. The relative risk and 95% CI of relative risk in the acid peptic disease patients with respect to drinking water was as follows: (0.7756, 0.6802-0.8843) consumers of processed water, when processed water consumers were considered as referent. The relative risk and 95% CI of relative risk in the acid peptic disease patients with respect to eating habit was as follows: (0.9304, 0.8068-1.073) people consuming home cooked food, when people consuming outside cooked food were considered as referent. The risk ratio and 95% CI of risk ratio in the acid peptic disease patients with respect to socioeconomic status was as follows: (0.9752, 0.8774-1.084) patients of medium socioeconomic status, (0.3850, 0.2210-0.6708) patients of high socioeconomic status, when patients of low socioeconomic status were considered as referent. The relative risk and 95% CI of relative risk in the acid peptic disease patients with respect to sanitation practices was as follows: (0.9094, 0.8017-1.032) having indoor sanitary practices, when people having outdoor sanitation practices were considered as referent. The risk ratio and 95% CI of risk ratio in the acid peptic disease patients with respect to CWI was as follows: (0.9752, 0.8774-1.084) patients of high CWI status (0.3850, 0.2210-0.6708) patients of medium CWI status when people from low CWI status were considered as referent. The risk ratio and 95% CI of risk ratio in the acid peptic disease patients with respect to CRI was as follows: (0.9230, 0.8140-1.047) patients of middle CRI (0.2977, 0.09220-0.9615) patients of low CRI status when people from CRI status were considered as referent.

DISCUSSION

Ever since its discovery in 1985, *H. pylori* has been synonymous with acid peptic diseases and has now acquired the *Prima donna* status as the principle gut pathogen responsible for a number of gastrointestinal maladies including gastric carcinoma and MALT (mucosa associated lymphoid tissue) lymphoma (McColl, 1999; Das and Paul, 2007). Epidemiological status of *H. pylori* in symptomatic and asymptomatic subjects has been reported to be governed and modulated by various bacterial, host and environmental factors. The prevalence has decreased in the developed countries where the transmission factors have been identified and eliminated resulting in eradication of *H. pylori* which is not in the developing countries. In the developing countries, its transmission has been implicated to be oral-oral or fecal oral route of transmission. The factors affecting the prevalence in acid peptic disease have been poorly understood (Graham *et al*., 1991). Presence of *H. pylori* is closely related with clinical symptoms of acid peptic disease (Tiwari *et al*., 2005). An array of risk factors like age, gender, socioeconomic status, consumption of outside cooked food, unprocessed water, irrational consumption of NSAIDs, consumption of smoking and alcohol, sanitary conditions, clean water index, crowding index etc have been investigated to be associated with symptomatology of acid peptic disease. However, these studies were carried out in southern India using salivary and biopsy samples of patients suffering from various gastrointestinal diseases (Ahmed *et al*., 2007; Tiwari *et al*., 2005). A similar study in northern India was carried out using the salivary samples of asymptomatic subjects (Mishra *et al*., 2008). Our study was designed to evaluate the role of virulent *H. pylori* present in the salivary samples of acid peptic disease patients in a representative western Indian population of acid peptic disease patients.

16 s r RNA and HSP 60 have been used extensively as biomarkers to detect the presence of *H. pylori* in the salivary samples (Ahmed *et al*., 2007; Mishra *et al*., 2008) Presence of salivary Cag E and T have been closely associated with various gastric diseases (Tiwari *et al*., 2005). Hence in this study, we used these biomarkers to determine the presence of *H. pylori* in the salivary samples of the acid peptic disease patients and its association with the various sociodemographic and environmental risk factors.

Gender has been investigated as a factor deciding the infection status but no conclusive evidences have been drawn. Our study shows a higher proportion of male subjects harbored *H. pylori*. The underlying reason needs to be investigated by designing further investigations. Age has been investigated as an important determinant of the prevalence of infection status. The present investigation elucidated that the patients belonging to lower age groups (18-30) and very high age group (60-75) had a higher prevalence of *H. pylori*. This could be explained by the fact that *H. pylori* is acquired in the childhood and adolescence due to fecal oral transmission

and in the old age the decreasing immunity and other co morbid diseases increase the vulnerability of the individuals. Alcohol consumption led to reduced *H. pylori* prevalence in the saliva depicting its localized anti microbial activity. These findings are in accordance with the findings of various epidemiologists in the western countries (Gao *et al*., 2010). Smoking has been the prima donna risk factor involved in *H. pylori* infection. Smoking has a wide variety of detrimental effects on the gastric mucosa and hence allows *H. pylori* to exist in the gastrointestinal lining. Indiscriminate use of NSAID consumption has been co related with increased prevalence of *H. pylori* (Ogihara *et al*., 2000; Sharma *et al*., 2006). NSAIDs are known to reduce the gastric mucosal secretion by down regulation of arachidonic pathway thus providing suitable environment for *H. pylori* to reside (Gisbert *et al*., 2004). However, in this study such observations were not recorded. This seems to have occurred due to the inadequate or incomplete self reporting by the subjects. Our observations are in stark contrast to the previous reports and further studies would unravel the reasons behind such anomaly.

A higher percentage of the subjects residing in the villages or the rural dwellings were found to possess *H. pylori* in comparison to the urban inhabitants showing a discernable vulnerability of the rural residents towards this organism. Similar findings were reported by other authors (Brown *et al*., 2002; Cheng *et al*., 2009; Aguemon *et al*., 2005) and the role of geographic and demographic factors in *H. pylori* transmission cannot be denied.

Role of drinking water has been thoroughly studied and consumption of unprocessed water has been deduced as the underlying cause of transmission of *H. pylori* by previous investigators (Malaty *et al*., 2003; Kawaguchi *et al*., 2009). A study carried out on southern Indian subjects demonstrated that low CWI was associated with *H. pylori* infection (Ahmed *et al*., 2007) Similar observations were evident in our study and it is evident that water plays a crucial role in the transmission of *H. pylori*. A possible mechanism of water borne infection is the hypothesized co-existence of *H. pylori* inside the yeast (*Candida albicans*) (Salmanian *et al*., 2008). Various studies have been carried out by other authors where presence of *H. pylori* DNA in water samples has been identified, bacterial coccoid forms in water samples have been observed and some survival studies depicting the capacity of the bacterium to reside and resuscitate in contaminated water have been studied (Hulten *et al*., 1996; Horiuchi *et al*., 2001; Mazari-Hiriart *et al*., 2001a; Mazari-Hiriart *et al*., 2001b; Fujimura *et al*., 2004; Cellini *et al*., 2004; Queralt *et al*., 2005). Our findings provide credence to these reports and indicate to a similar route of transmission in India. *H. pylori* has been proven to reside in the biofilms (a slimy matrix of bacterial film) in wells, rivers, urban water distribution pipelines and is unaffected by the water purification systems (Moreno *et al*., 2007; Baker *et al*., 2002; Johnson *et al*., 1997). *H. pylori* being a micraerophillic bacteria, resides in the biofilm (Giao *et al*., 2008; Giao *et al*., 2010). *H. pylori* DNA has been isolated from well water (Karita *et al*., 2003) which may have its origin in the bacteria residing in the biofilm. In India, a major population of rural population uses raw well water for drinking and hence oral cavity of subjects is a suitable niche for the bacterium. The viability, culturability is partially lost with chlorination or boiling at high temperature (Shahamat *et al*., 1993). But measures for complete elimination of bacterium from water have not yet been formulated. Consumption of under cooked, raw, or unprocessed food has been extensively studied and reported to have an effect on the prevalence of *H. pylori* and *H. pylori* has been reported to survive under low temperatures in ready to eat food (Poms and Tatini, 2001; Bohmler *et al*., 1996; Jiang and Doyle, 2002; Gomes and De Martinis, 2004a; Gomes and De Martinis, 2004b; Quaglia *et al*., 2008; Fujimura *et al*., 2002; Dore *et al*., 1999; Dore *et al*., 2000). This seems to be due to the fact that in a developing country like India, optimum hygienic standards are not maintained in the preparation of edibles. Usage of *H. pylori* infected water or vegetables or inadequate processing of other food articles eg. dairy products may be an answer to this finding. *H. pylori* has been found to exist in a viable but non culturable form (VBNC) in vegetables, dairy products especially milk (Fujimura *et al*., 2002; Dore *et al*., 1999; Dore *et al*., 2000; Goodman *et al*., 1996; Hopkins *et al*., 1993; Chen *et al*., 2005; Mazari-Hiriart *et al*., 2008). Growth of *H. pylori* is unlikely in most food products, but it has the ability to survive in a low acid, high moisture environment for long periods, in refrigerated conditions (Bohmler *et al*., 1996). Proof of the ability of *H. pylori* to survive in common foods supports the hypothesis that primary contamination of a food product (animal reservoir) or secondary contamination due to inappropriate handling (human reservoir) can be a vehicle for *H. pylori* transmission (Brown *et al*., 2001; Dimola and Caruso, 1999). Animals included in the human food-chain, like the pigs sheep etc. have been considered as possible reservoirs for this bacterium (Webb *et al*., 1996; Begue *et al*., 1998; Van Duynhoven and de Jonge, 2001). As referred *H. pylori* can be found inside yeasts (Shahamat *et al*., 1993). Yeasts are present in the human

oral cavity and different foods. Thus measurements regarding control of yeast content of foods might be the important to prevent the transmission of *H. pylori* (Salmanian *et al*., 2008). Poor sanitary practices during food preparation might also be involved in vertical transmission, with water and food acting as vehicles of transmission. Hence, transmission through food appears as a major transmission pathway leading to *H. pylori* infection It has been proven that *H. pylori* can survive in the water samples upto one hundred days and cause gastritis (Mizoguchi *et al*., 1999). Moreover, *H. pylori* survives in a temperature dependent manner in the water samples (Moreno *et al*., 2007). Another startling facet is *H. pylori* can be isolated from spring and sea water from the samples containing zooplanktons only. If the planktons are absent then *H. pylori* could not be isolated from the sample (Percival and Thomas, 2009). However, the matching of the genotypes between human and food isolates would be the concrete proof of transmission through food. Also differentiating between VBNC, dead, coccoid forms of bacteria is of prime importance to determine the route of transmission.

Socioeconomic status has been investigated to play a pivotal role in deciding the *H. pylori* status of an individual or a community. Low socioeconomic status has been proven to be a potential risk factor in the increased prevalence and transmission of *H. pylori* in asymptomatic subjects (Mishra *et al*., 2008; Ghosh and Bodhankar, 2011). The present investigation elucidates that lower socioeconomic status is an unequivocal risk factor for *H. pylori* prevalence in acid peptic disease patients. Sanitation practices have been investigated to play a major role in the transmission of *H. pylori* by various authors. It is closely related with CWI which serves as a scale to determine the nature of water being consumed by the individuals in a population. A greater proportion of patients who reported a low CWI and poor sanitation practices were found to possess of *H. pylori* in the oral cavity as compared to patients belonging to other categories. Both these factors were found to influence the prevalence of *H. pylori* in the present investigation and the study findings are in tune with those of previous epidemiological studies. CRI is a factor that has been implicated to maneuver the transmission of infection in the inmates of a household or siblings. High CRI in the household has been found to be a risk factor by previous workers and our findings show a similar trend (Nurgalieva *et al*., 2002).

The vertical transmission among family members and siblings is be possible in the subjects in a population in a urban setting where CRI is high.The presence of *H. pylori* in the salivary samples of acid peptic disease patients indicates that the bacterium migrates from its gastric niche to the oral cavity and hence provides a non invasive method to determine the infection status of the patient. Most of the physicians prescribe a triple drug regimen comprising of lansoprazole, tinidazole and clarithromycin to the patients of acid peptic disease without much investigation about the *H. pylori* status of the patient. This technique originally proposed by (Tiwari *et al*., 2005), is an excellent method to determine the non invasive infection status of *H. pylori* in the patients. The clinical significance of oral *H. pylori* has been a matter of debate among various authors. There are various schools of thoughts to the relevance of *H. pylori* in the oral cavity. It has been postulated that cag E and T positive *H. pylori* in the salivary samples of patients are closely associated with disease state (Tiwari *et al*., 2005). The present investigation shows that salivary cag E and T bear close relation with disease state.

H. pylori eradication is a major goal for the healthcare team due to its seismic disease causing potential. Measures need to be undertaken to completely eradicate this organism and also initiate measures to hinder the transmission pathways of *H. pylori* namely contaminated water, food and unhygienic sanitary practices. *H. pylori* has been reported to survive in the slimy biofilms as VBNC forms which acquire fulminant state once it gains access to a suitable niche in the host (Fujimura *et al*., 2002). Potable water may be infected if source of drinking is a community well which may serve for other household uses also. This water may be contaminated with *H. pylori* from the sewage lines or poor sanitary practices. In India, the rural population has lower CWI, have poor sanitary practices, use unprocessed water for consumption and belong to lower socioeconomic status. The urban population has high CRI and the water pipes bearing potable water run in close vicinity to sewage lines. This explains the causal relation between *H. pylori* infection in rural and urban population. Prevalence could be attributed to the existence of *H. pylori* in VBNC form in biofilms in water or in yeasts and disseminate from infected to non infected subjects. It could be hypothesized that, after being transmitted, when *H. pylori* embarks a suitable environment in the host, it may culminate acid peptic disease and express the virulence factors. In such a condition, it may migrate to oral cavity via gastro esophageal reflux and can be detected by PCR. Purification of potable water via filtering, chlorination, irradiation, reverse osmosis of potable water are probable measures that may hold the key to eradication of *H. pylori*. Advanced techniques need to be employed to detach *H. pylori* from the biofilm or remove it from yeasts which serve as the reservoir of *H. pylori*. All the risk

factors seem to be intertwined and exert a synergistic effect on the transmission of *H. pylori* in the patient population. A single risk factor cannot be identified and a plethora of factors seem to function in a closely knit pattern and orchestrate the spread the infection. This study provides credence to previous studies carried out on the asymptomatic subjects (Ahmed *et al*., 2007; Ghosh and Bodhankar, 2011) where a similar trend was visible in the asymptomatic subjects. However, in previous studies virulent genes were not amplified from the salivary samples of asymptomatic subjects. Present study provides a pioneer insight into the present state of *H. pylori* prevalence in the salivary samples acid peptic disease patients in western India and its association with various risk factors. Similar studies need to be carried out in other parts of the country to determine the status of *H. pylori* in other patient pools to enable us to draw a picture of the epidemiology of *H. pylori* in India and arrive at a consensus for its effective eradication.

CONCLUSION

Prevalence of virulent *H. pylori* in salivary samples of acid peptic disease patients is closely associated with environmental risk factors which need to be eliminated using suitable measures.

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