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GENERAL PREVALENCE OF HELICOBACTER PYLORI INFECTION IN DYSPEPTIC POPULATION OF ISLAMABAD, PAKISTAN

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Helicobacter pylori was known as campylobacter pyloridis in the beginning of the twentieth century. Doenges was the first to find this bacterium in the autopsy specimens of stomach using haematoxylin and eosin stains. In 1940 Freedburg and Baron carried out a study on 35 partial gastrectomy specimens and found spirochetes in 37% after a long search. A major breakthrough occurred with the advent of fibroptic biopsy technique permitting the biopsy of stomach. Then in 1975 Steer and Colin Jones observed gram negative bacilli in 80% of patients with gastric ulcer. The bacterium was closely associated with the surface epithelium, both within and between pits. These microorganisms were poorly stained by haematoxylin and eosin stains but could be seen easily with Warthin Starry silver stain. Later on, a heavy growth of campylobacter like organism was found on non-selective culture media and so the first culture of helicobacter pylori was achieved in April 1982. Since then eight other helicobacter species have been found, one in man and the remainder in other animals including birds. The organism may remain silent for years or produce serious gastric disorders in the beginning. Many studies have been conducted on asymptomatic healthy individuals but its presence in dyspeptic patients has always been ignored and dyspepsia has been treated as a result of over acid production This study was conducted to determine the prevalence of helicobacter pylori [hp] infection in dyspeptic population of Islamabad using ^{13}C urea breath test, and to find the possible role of water in bug transmission. We have also tried to assess the type of gastric pathology resulted by bacterial colonization in stomach. A total of 278 individuals were studied. Out of these 115 who had serious complaints/symptoms were sent for endoscopy to get the antral biopsy sample. Breath samples of dyspeptic individuals were sent to Isotope Application Division, PINSTECH for $^{13}C/^{12}C$ isotope ratio analysis using Mass Spectrometer. Water drinking habits of patients were recorded to assess the possible role of drinking water in bacterial transmission to human stomachs. Analysis of data obtained by mass spectrometry showed an overall prevalence of 66.5%. Majority of our subjects used unboiled water. Therefore, use of unboiled drinking water could be the most possible cause of this infection. Chronic antral gastritis was the predominant endoscopic pathology seen in infective patients. We, therefore, conclude that prevalence of H.pylori infection is high in Islamabad region mainly due to the use of contaminated drinking water.

Keywords: Helicobacter pylori, ^{13}Curea breath test, Dyspepsia, Prevalence.

1. Introduction

For the last century the concept of 'no acid no ulcer' propagated by Scharwz has dominated our understanding of the pathogenesis of peptic ulcer disease. Therefore, all attempts at treatment have been towards reduction of acid insults to the mucosa of esophagus, stomach and duodenum [1]. In 1983 Marshall and Warren introduced a new player in the game which was bacterium helicobacter pylori [2], Since then helicobacter pylori is the only reported gastric pathogen world wide [3].

The rate of acquisition of infection varies remarkably between and within populations. It is greater in the developing world than in industrialized countries [4]. It is present in almost all individuals exposed to it during childhood with poor hygiene, but the prevalence mainly increases with age [5]. Helicobacter pylori is a remarkable microorganism because of its ability to readily colonize a major proportion of human population worldwide and to persist successfully for long periods probably decades in a hostile environment.

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At the same time it interacts with the host immune system in such a way as to permit long-term survival.

The presence of helicobacter pylori in the gastric mucosa has been related to multiple diseases including gastric ulcer, duodenal ulcer and gastric adenocarcinoma, the second leading cause of cancer death worldwide [6]. It has now been classified as a grade-1 carcinogen [6] H. pylori has also been implicated in gastric mucosa associated lymphoid tissue (MALT) lymphoma [7].

Fundamental question that how the organism is transmitted to a new host remains unanswered [7]. Several modes of transmission have been proposed including oro-oral, faeco-oral, gastro-oral, and gastro-gastric routes [8]. Poor socioeconomic conditions, household living conditions and domestic overcrowding are also closely associated with the acquisition of infection [9]. Use of contaminated water for drinking purposes and cooking is a known mode of transmission [10].

The optimal diagnostic approach in patients with dyspepsia is still controversial. Upper endoscopy is frequently performed as the primary diagnostic test, but it is costly and in most patients no underlying disease can be identified. It has been suggested that a strategy based on non invasive testing for H. pylori could be more cost effective. Such a strategy could either imply the referral of only H. pylori positive patients for endoscopy (test and scope strategy) or subjecting H. pylori patients to anti H. pylori treatment (test and treat strategy). Whatever approach is chosen, its efficacy is highly dependent on the accuracy of the test used to diagnose infection [11].

A non-invasive quick and reliable method is ^{13}C urea breath test which is very well validated [3] and tolerated by the patient. It is in fact an attractive alternative to invasive diagnostic tests, and is a field technique and can be carried out practically any where without fear of contamination. The initial sampling is simple and no expert clinician is required.

This study was done to evaluate the prevalence of infection in dyspeptic population of Islamabad and to determine its association with gastro duodenal diseases. Role of drinking water in transmission of this infection was also needed to be evaluated.

2. Methodology

2.1. Sample collection

The employees of PINSTECH, NEW Labs, PIEAS etc. residing at Nilore Colony and also at neighbouring villages like Nilore, Chirah, Tumair (small towns in Islamabad) were selected. A total 278 patients of this vicinity were found to have upper gastrointestinal symptons during their visit at PINSTECH Complex Hospital. Out of these, 115 patients who had serious complaints were sent for endoscopy to get single antral biopsy sample. Haematoxylin and eosin stains were used to see the histological changes in biopsy samples. ^{13}C urea breath test was performed on each patient on the same day. The breath samples were sent to Isotope Application Division, PINSTECH for analysis on mass spectrometer. Patients were divided into seven groups within an interval of 10 years (from 15 to 80 years age). Water drinking habits of each group were enquired and recorded to relate these habits with H. pylori infection. Pregnant and lactating mothers, pediatric population, patients on antibiotics, those on H_2 receptor antagonists for more than a week were excluded from the study.

2.2. Sample preparation

The ^{13}C urea breath test was performed in the following way. The procedure involved collecting one baseline breath sample from an overnight fasting subject followed by oral ingestion of 75-100 mg ^{13}C urea [Cambridge Isotope Laboratories, Boston MA, USA] which is a non-radioactive isotope dissolved in tap water alongwith 200 ml of Ensure [manufactured by M&R Laboratories, B.V. Zwolle, Division of Abbott Laboratories, USA] to slow gastric emptying. In the subsequent 10-30 minutes, the detection of isotope- labeled carbon dioxide in exhaled breath indicates that urea was split, this indicates that urease [the enzyme that H.pylori uses to metabolize urea] is present in the stomach and hence that H. pylori bacteria are present.

2.3. Mass spectrometric analysis

For the two different forms of urea, different instrumentation is required; carbon-14 is normally

Table 1. Percentage of patients positive for H. pylori infection obtained by applying ^{13}C Urea Breath Test (UBT).

Groups	Age	UBT Positive	UBT Negative	Total
Group 1	15 – 20 years	17 (68%)	08 (32%)	25
Group 2	21 – 30 years	37 (75.5%)	12 (24.5%)	49
Group 3	31 – 40 years	55 (63.2%)	32 (36.8%)	87
Group 4	41 – 50 years	44 (72.1%)	17 (27.9%)	61
Group 5	51 – 60 years	19 (59.4%)	13 (40.6%)	32
Group 6	61 – 70 years	11 (61.1%)	07 (38.9%)	18
Group 7	71 – 80 years	2 (33.3%)	4 (66.7%)	06
	Total	185 (66.5 %)	93 (33.5%)	278

Table 2. Water drink habits in UBT positive patients.

Groups	UBT Positive	Un-boiled water users	Boiled water users
Group 1	17	10 (58.9%)	7 (41.1%)
Group 2	37	22 (59.5%)	15 (40.5%)
Group 3	55	48 (87.3%)	7 (12.7%)
Group 4	44	38 (86.5%)	6 (13.5%)
Group 5	19	10 (52.6%)	9 (47.4%)
Group 6	11	9 (81.8%)	2 (18.2%)
Group 7	02	2 (100%)	0 (0%)
Total	185	139 (75.1%)	46 (24.9%)

measured by scintillation counter, carbon-13 by isotope ratio mass spectrometry (IRMS). For carbon-13, a baseline sample before taking urea is required for comparison with the post urea sample.

The difference between the pre- and post- urea measurements is used to determine infection. This value is compared to a cut off value. Results below the value are assumed to be negative those above positive. The cut off value itself is determined by comparing the results of patients with two or more different detection methods. The value is chosen that gives the best combination of sensitivity and specificity.

A number of factors can affect the final results. The test measures active H.pylori infection. If antibiotics are depressing the amount of bacterium present or the stomach conditions are less acidic than normal, the amount of urease present in stomach may be affected leading to biased results.

Accordingly the test should only be performed 14 days after stopping acid reducing medication or

antibiotics. Some clinicians believe that a reservoir of H. pylori in dental plaques can affect the result. All these limitations were taken into consideration while carrying out this study.

3. Results and Discussion

Results of mass spectrometric analyses of air breath samples from dyspeptic subjects are shown in Table 1. A total of 278 subjects were examined during this study period. 185 (66.5 %) subjects were UBT positive and 93 (33.5%) were negative for H. pylori infection.

Data on drinking habits of UBT positive subjects is shown in Table 2. 139 out of 185 UBT positive subjects (75.1%) used unboiled water in their routine life. Hence majority of UBT positive subjects used contaminated water, which could be contaminated with bacterium. This suggests that contaminated water is the most possible mode of infection in our subjects.

Data on drinking habits of UBT negative subjects is shown in Table 3. Here 54 out of 93 subjects (58.1%) were found to use boiled water.

Table 3. Water drink habits in UBT negative patients.

Groups	UBT negative	Boiled water drinkers	Un-boiled water drinkers
Group 1	8	3 (37.5%)	5 (62.5%)
Group 2	12	3 (25%)	9 (75%)
Group 3	32	22 (68.8%)	10 (31.2%)
Group 4	17	11 (64.7%)	6 (35.3%)
Group 5	13	9 (69.2%)	4 (30.8%)
Group 6	7	5 (71.4%)	2 (28.6%)
Group 7	4	1 (25%)	3 (75%)
Total	93	54 (58.1%)	39 (41.9%)

Water supplied to Islamabad from Simli dam and Rawal dam was analyzed for feacal contamination and found highly contaminated and unfit for drinking purposes as shown in Table 4.

The endoscopic findings of 115 subjects who underwent endoscopic investigations are given in Table 5. Chronic antral gastritis was the most frequent endoscopic finding (59.1%). Therefore, chronic antral gastritis was predominant in dyspepsia. All patients with no detectable lesion on endoscopy were also UBT positive. This confirms that UBT is very helpful in early detection of this infection even with normal stomach.

Table 4. Analysis and culture of water samples collected from Rawalpindi and Islamabad

Parameter (Ref Value)	Islamabad	Rawalpindi
PH (6.5-6.8)	7.0	7.0
Ammonia (upto 1.0 ppm)	0.5 ppm	0.6 ppm
Chloride (200-300mg/L)	225mg/L	250mg/L
Hardness (17-28mg/L)	10mg/L	24mg/L
Culture report		
Pseudomonas species (count should be less than 10 in 100ml of water)	Not detected	More than 100 counts /100ml
E.Coli and other Coli form of bacteria (No E.Coli count in 100 ml of any water sample	More than 180	16 counts /ml

Table 5. The endoscopic findings of dyspeptic patients

Endoscopic Finding	Total	Percentage
Ch. Antral gastritis	68	59.1 %
Duodenal ulcer	21	18.3 %
Pan gastritis	10	8.69 %
Duodenitis	5	4.34 %
Gastric ulcer	4	3.47 %
Reflux esophagitis	2	1.73 %
Normal	5	4.34 %
Total	115	

3.1. Our results versus other studies in Pakistan

This dyspeptic population from Nilore, Islamabad has very high prevalence (66.5%) of H. pylori infection. This suggests that Helicobacter pylori is the main cause of dyspepsia than high acid production. The prevalence of disease in our population is comparable with other studies. H. Qureshi and W. Ahmad in their study on Helicobacter pylori clearance and its eradication

found the infection rate about 83% in adult patients undergoing upper GI endoscopy for dyspeptic symptoms [12]. Mohsin et al. in Lahore had found 43.6% prevalence in dyspeptic population [13], whereas Shahana in Karachi had observed 21-60% prevalence rate among dyspeptic subjects [14]. This variation in prevalence rates within country may be due to water environment and the intrinsic properties of diagnostic methods and sampling techniques used by researchers.

3.2. Our results verses global prevalence

The isolation rate of H.pylori from different parts of the world in dyspeptic population is quiet variable, being more in underdeveloped countries as compared to developed countries .Investigators from other parts of the world including Brazil, Korea, Japan, Canada, Turkey have reported H.pylori incidence in the range of 31-78% [15]. The prevalence of H. pylori determined by endoscopy among British immigrant symptomatic Indian community was 52% as compared to 43% in white population [16].

The factors for the difference in prevalence between ethnic groups or races may include hygiene, environmental contamination, water contamination, standards of living, sanitation practices and socioeconomic status [9]. Studies have shown that Helicobacter pylori seroprevalance is inversely associated with levels of income and education

3.3. Helicobacter pylori and gastro duodenal disorders

Several reports have described an association of H. pylori with gastric or duodenal ulcer, non-ulcer dyspepsia and gastritis [17]. Direct evidence of a pathogenic role of H. pylori was obtained by self-inoculation experiments or by inadvertent iatrogenic transmission followed by acute gastritis and in some volunteers, the subsequent development of chronic gastritis associated with persistent infection [18]. The most common endoscopic gastric pathology in our population was Ch. antral gastritis (59%). In the study done by Shahana et al. in Karachi, the highest incidence was recorded in gastritis patients and gastro duodenal ulcers patients, as compared to endoscopically normal patients with typical symptoms of disease [14]. Mohsin Aftab in his happen study on dyspeptic patients visiting Jinnah Hospital, Lahore found 42 out of 78 patients having Ch. gastritis [13].

3.4. Water as a source of H. pylori

One of the possible route of transmission in our study was contaminated drinking water. Although the water supplied to the twin cities of Rawalpindi and Islamabad is chlorinated but according to Johnson et al. [19], Helicobacter pylori is more resistant to chlorine than E. Coli, suggesting that Helicobacter pylori is able to survive for prolonged periods of time in chlorinated drinking water supplies. In the study done by Shahana in Karachi most of the study population came from areas with poor hygiene, had close contact with household animals, had improper water supply and sewage disposal system and consumed unboiled water [14]. Besides the racial and genetic factors, one major route of transmission is faeco-oral [13, 14, 19] the dissemination of viable Helicobacter pylori cells through the faeces [6] in water. A.P West and M.R. Millar have demonstrated that H.pylori could survive for prolonged periods in faeces and water [20]. In Peru, children whose homes had external water supplies were three times more likely to have H. pylori infection than those with internal supplies independent of socio-economic status [21]. The results of several community and environmental studies are consistent with the view that water is a significant mode of infection because the survival of H.pylori is enhanced with active colonization of the mixed heterotrophic species biofilms found in all drinking water storage and distribution systems [10].

3.5. Analysis and culture of water samples

H.pylori is difficult to detect by most culture techniques. Shahmat and co-workers have reported that ^{3}H thymidine –labeled forms of H.pylori could be recovered from water and induced to proliferate under special conditions [22]. Until H.pylori can be detected in environmental samples or in secretions, such as saliva or stool, transmission patterns will have to be deduced on the basis of indirect evidence such as that in our study. Fast growing commensal organisms in stool, saliva and contaminated water over grew the culture plates and made isolation of H. pylori impossible.

4. Conclusion

This study suggests that *Helicobacter pylori* is a cause of dyspepsia in our population. Contaminated drinking water is the main cause of transmission of this disease to human stomachs where the organism may remains silent or produces serious gastric diseases like chronic gastritis. ^{13}C urea breath test established at our hospital is a highly sensitive and specific test, suitable for reliable diagnosis of this disease and future research. Our research may not reflect the actual magnitude of this infection in our community. Considering the importance of this pathogen which causes active chronic gastritis leading to stomach cancer, further investigation should be carried out to develop an understanding as to how the organism colonizes and causes the disease. Hence there is a need to involve public health authorities, scientists and our medical community to develop effective treatment and control strategies.

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