PINSTECH 212

PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN SCHOOL GOING CHILDREN OF BHARA KAHU AREA, ISLAMABAD

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Abstract

Most *Helicobacter pylori* (*H. pylori*) infected individuals remain asymptomatic, but the presence of *H. pylori* is a risk factor for the development of peptic ulcer disease and gastric adenocarcinoma. Despite the fact of high prevalence of *H. pylori* infection around the world, data about its prevalence in children in Pakistan is scanty. Our study was the first epidemiologic study in Pakistan designed to assess *H. pylori* prevalence in a school based population of children without gastrointestinal symptoms.

The children were enrolled from three schools in the suburbs of Islamabad and their anthropometric data were noted. The non-invasive urea breath test was applied to find the prevalence of *H. pylori* infection. Overall, 72.3% of apparently healthy children were harboring the *H. pylori* bacterium and the prevalence was 69% in 3-6 years, 71% in 7-8 years, 79% in 9-10 years, 76% in 11-12 years and 55% in 13-16 years of age. The prevalence decreased in the higher age group significantly, while gender was not a risk factor for acquiring this infection as the prevalence of infection was not significantly different in males and females (74.0% vs. 70.3%, p=0.41). The lower prevalence in higher age group might be explained by change in degree of contact, Increasing antibody production with increasing age or improvement in sanitary habits of children as compared to younger children.

CONTENTS

1 INTRODUCTION

For many years, the human stomach was considered to be an inhospitable acidic environment in which bacteria could not grow. This view changed following the isolation of a novel gram-negative bacterial species, designated *H. pylori*, from the human stomach in the early 1980s [1]. In the absence of antimicrobial therapy, *H. pylori* can persist in the human stomach for many decades or potentially for the entire lifetime of the host.

In addition to predisposing children to gastrointestinal diseases, *H. pylori* has been observed in association with other clinically significant disorders, including growth retardation and anemia [2, 3].

Despite the fact of high prevalence of *H. pylori* infection around the world, data about its prevalence in Pakistan is scanty and derived mostly from hospital based studies of symptomatic adults [4, 5]. Very little data is available about its prevalence in children in Pakistan mainly due to the reason that invasive procedures on an apparently healthy child are not ethically approved. The availability of non-invasive ^{13}C urea breath test (UBT) made it possible to carry out the present study to determine the prevalence of *H. pylori* infection in normal school going children in the suburbs of Islamabad (Pakistan).

2 MATERIALS AND METHODS

2.1 Study Site and Subjects

Bhara Kahu area in the suburbs of Islamabad was selected as the study site and the study protocol was approved by the Advanced Studies Research Board of The PMAS Arid Agriculture University. The area was visited and three schools having both boy and girl students were chosen. The Principals of the schools were

approached and were briefed about the study design and research methodology. After they agreed, a day was fixed as enrollment day and all the children present on that day were enrolled subject to the inclusion and exclusion criteria. Each child was provided with a consent form to be signed by his/her parent or guardian (attached as Annex-1). The consent form explained methodology of the study and risks if any involved. Parents were encouraged to come to school on the enrollment day and discuss with the research team any concern they might have about the study methodology.

2.2 Inclusion Criteria

The children of both sexes having no severe illness and falling in the desired age range i.e., 3 to 16 years were included.

2.3 Exclusion Criteria

Children younger than 3 and older than 16 years of age and children on medication were excluded from the study.

2.4 Data Collection

On the enrollment day, consent forms were collected back. Date of birth of the eligible children was provided by the school staff and the height and weight were measured at the school. Height was recorded to the nearest centimeter and the weight to the nearest 0.5 kilogram using a pre-calibrated analogue scale. Each child was assigned a unique identification (ID) number. A day was fixed as the test day after consulting the school staff and the children were advised to come with an empty stomach on that day. A note to similar effect was also sent with the child for the parents.

2.5 Urea Breath Test

Urea Breath Test (UBT) was carried out by the method previously described by Graham *et al*. (1987) [6] and validated in local population with some modifications by Bilal *et al*. (1996) [7]. The basic principle of the test is that ingested solution of isotope-labeled urea will be rapidly hydrolyzed by the expressed urease of *H. pylori*. The released ^{13}C labeled carbon dioxide ($^{13}CO_2$) is absorbed across the mucus layer of the gastric mucosa, and hence, via the systemic circulation, excreted in the expired air. The presence of ^{13}C carbon dioxide in the exhaled breath sample is an indication of active *H. pylori* infection.

Fifty milligrams of ^{13}C labeled urea (Cambridge Isotope Laboratories, Boston, USA, 99% atom ^{13}C) was weighed into plastic containers and the glass tubes for the UBT were labeled with the ID numbers and arranged sequentially. There were four tubes for each subject, two for baseline breath sample before the dose and two for breath sample 30 minutes after the dose. On the test day, the children were divided into five groups as there were five trained persons who could administer and supervise the test. Each subject was handed the tubes and the straws and were demonstrated how to take the test. On the instruction of the supervisor, the children blew into the two baseline tubes, closed the cap securely and kept aside. Next the subjects were instructed to drink the ^{13}C labeled urea dose which was prepared in orange juice. The supervisors made sure that the subjects drank all the liquid. Empty containers were collected and the children were instructed to sit during whole process. After 30 minutes, the subjects blew into the second pair of tubes and the test was completed. Breath sample tubes were collected, arranged and packed for transportation to the laboratory.

2.6 Analysis of Breath Samples

The breath samples were stored at room temperature in the laboratory until analyzed. Analysis was done on a BreathMAT^{plus} mass spectrometer (Thermo Finnigan, Germany) at a facility established by the Pakistan Atomic Energy Commission (PAEC) at Nuclear Medicine, Oncology and Radiotherapy Institute (NORI), Islamabad. Each sample was analyzed for the $^{13}C/^{12}C$ ratio and the results were expressed as $\delta^{13}C‰$ vs. PDB. The PDB stands for Pee Dee Belemnite formation, which is an international standard for carbon isotope ratios. Because the differences in ratios between the sample and standard are very small, they are expressed as parts per thousand or 'per mil' (‰) deviation from the standard. The difference between the $\delta^{13}C$ values in post and pre dose samples was recorded. The difference of more than 5 per mil (>5‰) was taken as positive result [8].

2.7 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) release 10.0, standard version, copyright, SPSS Inc. 1999 was used for data analysis. Results were expressed as mean ± standard deviation, number (percentage), odds ratio (OR) and 95% confidence interval (95% CI) for odds ratio. Univariate analysis was performed by using the Pearson chi-square and Fisher's exact test wherever appropriate. When there were more than two categories, the category having the lowest *H. pylori* infection prevalence was taken as the reference category for the intergroup comparisons. A *p* value less than 0.05 was considered statistically significant.

3 RESULTS AND DISCUSSION

A total of 418 children were enrolled initially. Twelve children had inadequate breath sample or undetermined UBT results and were excluded. The houses could not be located or the data were not complete for six other children and these were also excluded. In the end we had complete data of 400 subjects, out of this 208 were males (52%) and 192 were females (48%). Age of the subjects ranged from 3.1 to 16.0 years (mean 9.0±2.59 years). The age of male subjects ranged from 3.2 to 15.6 (mean 9.0±2.49 years) and female subjects ranged from 3.1 to 16.0 (mean 9.0±2.70 years).

3.1 Urea Breath Test

In the present study, the UBT was well accepted by the children of 3 to 16 years of age. The only problem faced with children especially younger than five was that they needed particular instructions and demonstrations on how to blow through the straw instead of sucking, that they are generally used to. ^{13}C-UBT is an accurate noninvasive tool for diagnosis of *H. pylori* infection in children and adolescents. It is considered the best method for epidemiological studies and it has been observed that the test is highly sensitive and specific for diagnosis of the *H. pylori* infection in children of all ages [9, 10, 11].

3.2 Prevalence of *H. pylori* Infection

Out of 400 children, 289 were positive for *H. pylori* infection which makes 72.3%. These were apparently healthy school going children with no gastrointestinal symptoms. The overall prevalence of *H. pylori* infection is in line what has been reported previously from other developing countries. For example, Dore *et al*. (1997) reported a high prevalence of *H. pylori* infection i.e., 82% in Indian school children using ^{13}C UBT [12].

Similarly, few studies carried out in Pakistan showed varying degree of *H. pylori* infection. In a study done by Qureshi *et al*. (1999) [13] the serology was used to investigate the seroprevalence of *H. pylori* infection in apparently healthy children. In this study, it was found that in the 5-10 years age group, 40% were positive. Further age grouping showed that the prevalence increased from 34.3% at 5-7 years to 62.5% in those aged 8-10 years. In another study from Karachi, Memon and Eiaz (2000) [14] reported the prevalence of *H. pylori* infection in symptomatic children being 41.4% based on endoscopy method. Aziz *et al*. (2007) [15] studied apparently healthy children from Karachi using serology and found the prevalence of *H. pylori* infection being 11%, 18% and 16% in 3-6, 7-10 and >10 years of age. The levels of *H. pylori* infection reported in this study are unusually low when compared to reported prevalence levels in earlier studies from this region [13, 14]. A recent study from Islamabad, Pakistan by Hafeez et al. (2007) revealed that 72% symptomatic children aged 5 to 12 years, evaluated through histological examination were positive for *H. pylori* infection [16]. It was quite interesting to note that the prevalence rate in symptomatic children as reported by Hafeez *et al*. (2007) [16] was quite similar to what is reported in present study for asymptomatic children.

In the present study, the prevalence of *H. pylori* infection was slightly higher in male subjects as compared to female as presented in Table 1.

It was seen that 74.0% of male subjects (154 out of 208) had a positive UBT while 70.3% of female subjects were positive by UBT (135 out of 192). We found that there was no significant difference between boys and girls in the prevalence of *H. pylori* infection. Similarly, Sathar *et al.* (1997) [17] reported that there was no significant difference in the prevalence of *H. pylori* infection between boys (68%) and girls (64%).

Table: 1 Prevalence of *H. pylori* infection in school going children of Bhara Kahu, Islamabad according to the gender

Variable	UBT + (%)	UBT − (%)	Total	*P* (OR) 95% CI
Gender				
Male	154 (74.0)	54 (26.0)	208	NS*
Female	135 (70.3)	57 (29.7)	192	
Total	289 (72.3)	111 (27.7)	400	

*NS = non significant

Several epidemiological reports have shown that the rate of *H. pylori* infection increases significantly with age, with more than 80% of children being infected by the age of 10 years [18, 19]. In our study the prevalence was 69% in 3-6 years, 71% in 7-8 years, 79% in 9-10 years, 76% in 11-12 years and 55% in 13-16 years of age (the data is shown in Figure 1). It was seen that the prevalence slightly increased with increasing age and then decreased in the 13-16 years of age group. A significant difference in *H. pylori* prevalence was observed when children aged 3 to 12 years were compared with children more than 12 years of age (74% vs. 55%, OR=2.35, 95% CI 1.21-4.57, p=0.010). These findings are supported by the study of Alborzi *et al.* (2006) [20] who evaluated the prevalence and age distribution of *H. pylori* infection in children in Shiraz (a city in the south of Iran). The

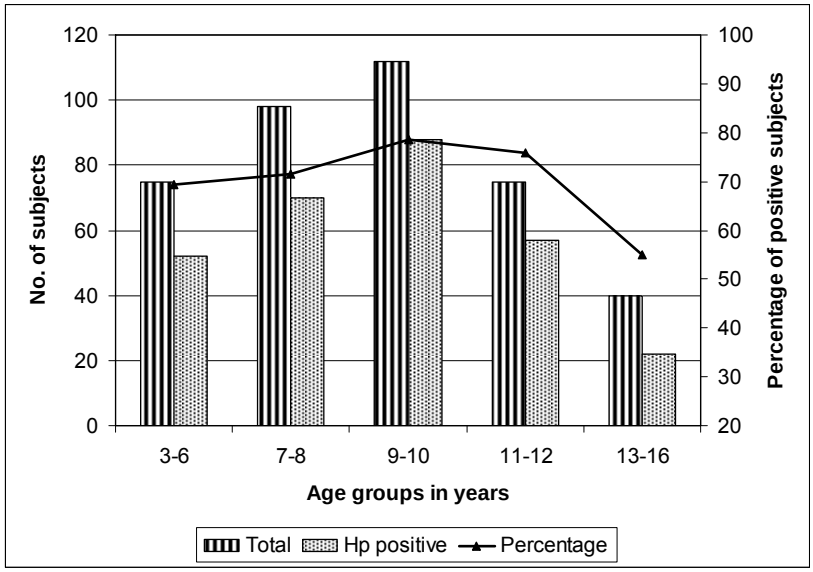

Figure 1: Prevalence of *H. pylori* infection in school going children of Bhara Kahu, Islamabad

prevalence rates were 82%, 98%, 88%, 89%, and 57% in age groups of 9 months, and 2, 6, 10, and 15 years, respectively. There were no significant differences between the prevalence of *H. pylori* infection in the first 4 age groups, but there was a significant decrease in the 15-year-old group. One possible reason for this pattern may be that increasing antibody production with increasing age may lead to the decline of the prevalence rate in the teen age group [20]. The other reasons may be auto-curability (spontaneous elimination) in higher ages and better attention to health issues in older children [21]. Improvement in sanitary habits with increasing age may be an explanation but the change in degree of contact between family members as children grow up may also be important in reducing the exposure to infection [22].

The data was further categorized in different age groups according to gender. The prevalence of *H. pylori* infection in boys was 74%, 70%, 84%, 70% and 63% in 3-6, 7-8, 9-10, 11-12 and 13-16 years age groups respectively (data presented in Figure 2). Although, the prevalence was high in the 9-10 years age group, the difference was not statistically significant (p=0.256). The data for the prevalence of *H. pylori* infection in different age groups of girls is shown in Figure 3. It was seen that the prevalence of *H. pylori* infection in girls was 65%, 73%, 73%, 83% and 48% in 3-6, 7-8, 9-10, 11-12 and 13-16 years age groups,

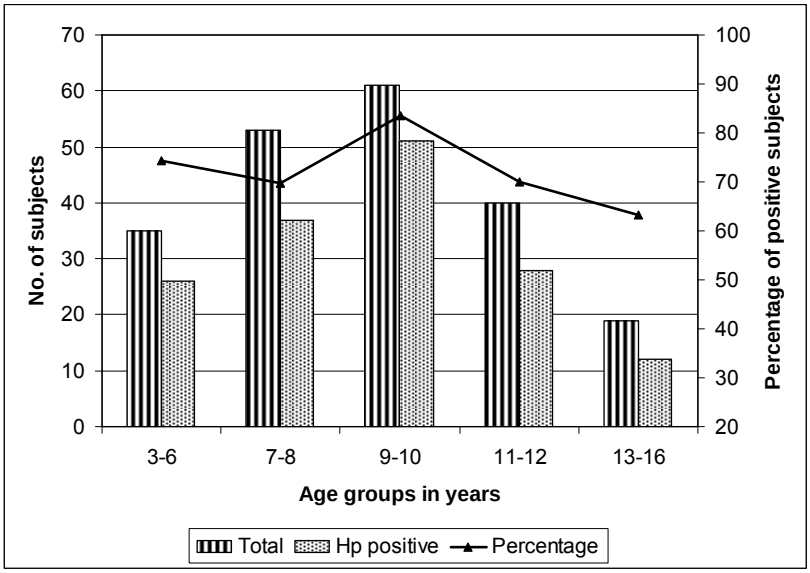

Figure 2: Prevalence of *H. pylori* infection in school going male children of Bhara Kahu, Islamabad

respectively. A significant difference in *H. pylori* prevalence was observed when girls aged 3 to 12 years were compared with girls more than 12 years of age (73% vs. 48%, OR=2.99, 95% CI 1.19-7.51, *p*=0.016). It is interesting to see that the prevalence of *H. pylori* infection in girls of 13-16 years of age (48%) was significantly lower when compared with younger girls but this phenomenon was not present in boys. The reason for this is not clear but Kaltenthaler *et al.* (1995) [23] had suggested that this might relate to young boys having poorer hygiene than young girls or, it may be due to the reason that boys are more social as compared to the girls.

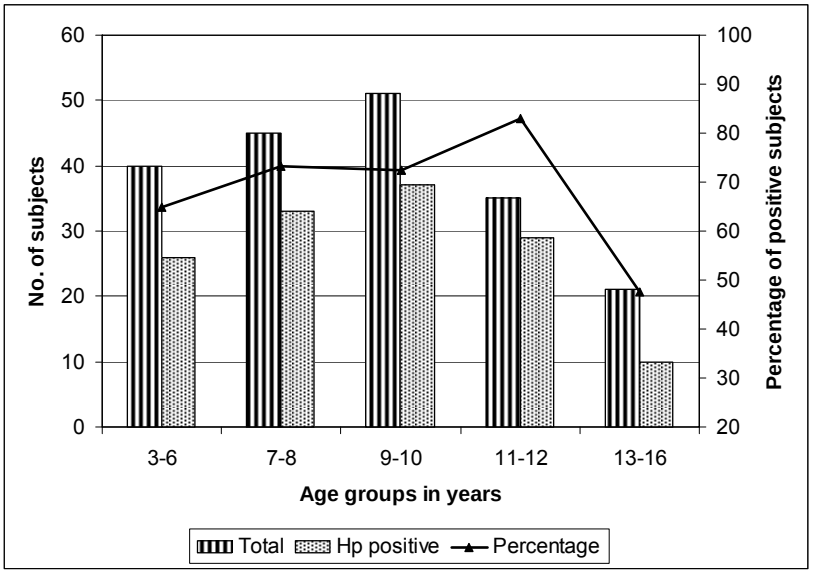

Figure 3: Prevalence of *H. pylori* infection in school going female children of Bhara Kahu, Islamabad

4 CONCLUSIONS

In conclusion, the prevalence of *H. pylori* infection is high in asymptomatic school going children. The infection probably starts in infanthood and remains with a child for life if not treated. Overall, the children in their teens had lower prevalence of *H. pylori* infection when compared with younger children. This phenomenon was more pronounced in the case of girls as compared to boys where the difference was not statistically significant. Better attention to personal care and hygiene in teen-aged girls could be a possible reason for lower prevalence of *H. pylori* infection.

5 RECOMMENDATIONS

This high prevalence of *H. pylori* infection warrants further studies to identify the environmental risk factors and treatment/preventive options. It would also be interesting to study the trend in adults as well as to find the point in the life of a child when the infection is first acquired.

6 ACKNOWLEDGEMENTS

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Annex-1

Volunteer Consent Performa

I have read/been explained in detail the purpose and rationale of the study my child is volunteering for. I understand that by participating in this study my child will not be exposed to any health hazards. I have had a chance to ask questions and have them answered. I understand that I am free to withdraw my child from study at any time, and also Investigators may decide to stop study any time if they desire.

Signature of the Parent/Guardian

Date:

Signature of the person obtaining consent

Signature of the Investigator