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Full Length Article

Prevalence of *Helicobacter pylori* Infection in Asymptomatic Children of Islamabad Suburbs (Pakistan)

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ABSTRACT

This study was carried out to assess the prevalence of *Helicobacter pylori* in a school based population of children without gastrointestinal symptoms. The children were enrolled from three schools in the suburbs of Islamabad (Pakistan) and their anthropometric data were noted. To this end, non-invasive ^{13}C urea breath test (UBT) was used. Overall, 72.3% of apparently healthy children were harboring the *H. pylori* bacterium and the prevalence was 70.3% in girls and 74.0% in boys, while the difference in the genders was not significant. When analyzed according to age, the prevalence was 73.6% in 3-8 years age group, 74.4% in 8-12 years age group and 60.4% in children 12-16 years of age. In conclusion, high prevalence of *H. pylori* infection warrants further studies to identify the environmental risk factors.

Key Words: *Helicobacter pylori*; Children; ^{13}C urea breath test; Pakistan

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 (Marshall & Warren, 1984). 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 (Choe *et al*., 2000; Bravo *et al*., 2003).

Despite the fact of high prevalence of *H. pylori* infection around the world, data about its prevalence in Pakistan are scanty and derived mostly from hospital based studies of symptomatic adults (Kazi *et al*., 1990; Abbas *et al*., 1998). Very little data are 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 rates of *H. pylori* infection in normal school going children in the suburbs of Islamabad (Pakistan).

MATERIALS AND METHODS

Study site. Bhara Kahu area in the suburbs of Islamabad was selected as the study site. It has a population of around 30,000 with low to medium socioeconomic status. Some people do small government jobs, while majority are manual workers such as shop keepers, daily wages laborers etc. The area was visited and three schools having both boy and girl students were chosen.

Subject selection. 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. 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.

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.

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

Data collection. On the enrollment day, consent forms were collected back and dates of birth of the eligible children were noted from the school record. Each child was assigned

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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.

Urea breath test (UBT). This test was carried out by the method initially described by Graham *et al.* (1987) and validated in local population with some modifications by Bilal *et al*. (1996). 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 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 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 min after the dose. Each subject was handed over 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 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 min, 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.

Analysis of breath samples. The breath samples were stored at room temperature in the laboratory until analyzed. Analysis was done on a Breath MAT 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) Hospital, Islamabad. Each sample was analyzed for the $^{13}C/^{12}C$ ratio and 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 (Bilal *et al*., 2007).

Statistical analysis. The Statistical Package for Social Sciences (SPSS) release 10.0, standard version was used for data analysis. 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. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Out of a total number of 400 subjects, 208 were male (52%) and 192 were female (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).

Fig. 1. Prevalence of *H. pylori* infection in the study population

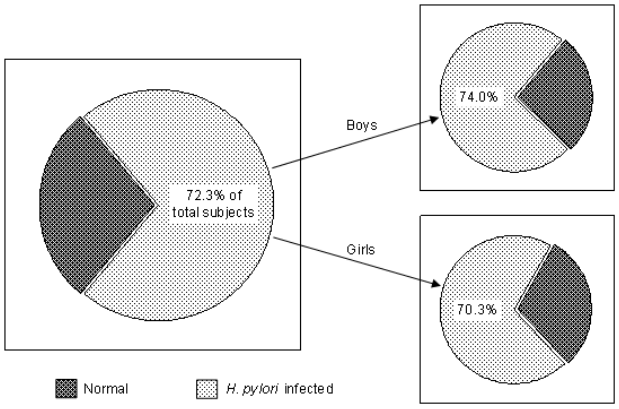
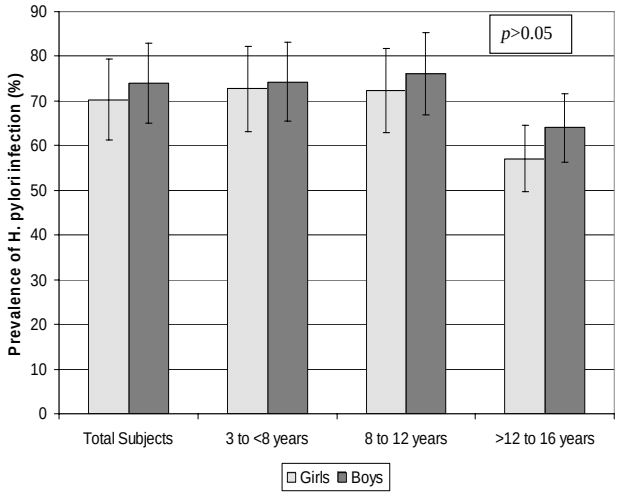


Fig. 2. Prevalence of *H. pylori* infection in children of different age groups



Urea breath test. ^{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 infection in children of all ages. The sensitivity, specificity and positive and negative predictive values for the ^{13}C-UBT in children aged 1 to 18 years of age were reported as 93.8, 99.1, 97.8 and 98.0%, respectively (Cardinali *et al*., 2003), while Niv *et al.* (2003) have shown that the test could be performed in children of all ages. 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.

Age and prevalence of *H. pylori* infection. Out of 400 children, 289 were positive for *H. pylori* infection, which makes 72.3% (Fig. 1). These were apparently healthy school going children with no gastro-intestinal (GI) symptoms. The overall prevalence of *H. pylori* infection recorded in the current study is consistent with that reported in India (Dore *et al*., 1997). The levels of *H. pylori* infection reported in this study are higher when compared to reported prevalence levels in earlier studies from this region (Qureshi *et al*., 1999; Memon & Ejaz, 2000), which may be due to different methods of detection (serology & endoscopy) in their investigations. A recent study from Islamabad, Pakistan revealed that 72% symptomatic children aged 5 to 12 years were positive for *H. pylori* infection (Hafeez *et al*., 2007). These children were evaluated through histological examination. It was quite interesting to note that the prevalence rate in symptomatic children as reported by Hafeez *et al*. (2007) was quite similar to that reported in the present study for asymptomatic children.

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 (Ertem *et al*., 2003). In our study, the prevalence was 73.6% in 3-8 years age group, 74.4% in 8-12 years age group and 60.4% in children 12-16 years of age (Fig. 2). *H. pylori* prevalence tended to be lower in children more than 12 years of age as compared to children 3 to 12 years of age, which substantiated the results of Alborzi *et al.* (2006) where the 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-years-old group. One possible reason for this pattern may be that increasing antibody production with increasing age may have led to the decline of the prevalence rate in the teen age group (Alborzi *et al*., 2006). The other reasons may be auto-curability (spontaneous elimination) in higher ages and better attention to health issues in older children (Rothenbacher *et al*., 2002). 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 (Rowland, 2000).

Gender and prevalence of *H. pylori* infection. The prevalence of *H. pylori* infection was higher in male subjects as compared to female (Fig. 1 & 2). There was no significant difference between boys and girls in the prevalence of *H. pylori* infection, which is consistent with the results of Sathar *et al.* (1997), but in contrast to Yang *et al.* (2003) who reported that the rate of *H. pylori* infection was higher in male than in female children. The reason for this is not clear but Kaltenthaler *et al.* (1995) had suggested that this might relate to young boys having poorer hygiene than young girls.

CONCLUSION

High prevalence of *H. pylori* infection warrants further studies to identify the environmental risk factors and treatment/preventive options.

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