HELICOBACTER PYLORI INFECTION IN APPARENTLY HEALTHY SOUTH INDIAN CHILDREN

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Abstract

Helicobacter Pylori infection has been established as a major cause of chronic gastritis in adults, and it has been implicated in the genesis of gastric carcinomas and the development of gastric and duodenal ulcers. It is now postulated that nearly 90% of the adult population in developing countries may be affected with the infection since childhood. Earlier studies on Indians using serology and endoscopic biopsy have shown a high incidence of H. pylori infection in small numbers of patients. The ^{13}C-urea breath test, which is simple, specific and non-invasive, is also increasingly being used to determine the presence of Helicobacter pylori infection. Preliminary data from India has shown a high prevalence in the urban Indian environment, and there is an urgent need to quantify the prevalence of H. pylori infections on an epidemiological basis in both urban and rural settings. It is also important to study the possible impact of this infection on growth in children, particularly in environments with low sanitation and high crowding. In this paper, we outline a proposal to study the prevalence of Helicobacter pylori infections in children from the following different environments: urban middle socio-economic class, urban slum, rural middle socio-economic class and rural village.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Helicobacter Pylori (H pylori) infection is a major cause of chronic gastritis in adults [1,2]. It has been implicated in the genesis of gastric adenocarcinomas [3] and the development of gastric and duodenal ulcers [2,4]. There is also a putative link between H pylori infection and non-gastrointestinal tract disorders [5]. Although the infection is non-invasive, and confined to the stomach, it triggers a marked local inflammatory response as well as a systemic immune response [6,7], thereby producing effects elsewhere by altering levels of systemic inflammatory mediators. However, a recent review of the evidence linking H pylori infection to a variety of non-gastrointestinal disorders has shown that there is no conclusive, strong evidence that the H pylori infection is causal in coronary heart disease, cerebrovascular disease, hypertension etc [5]. Nevertheless, it is important to recognize the potential that H. pylori infections may have as a causal agent in these chronic diseases, particularly since the incidence of these diseases is increasing in developing countries such as India [8], where the environment predisposes to underlying sub clinical infection [9]. While the etiology of chronic diseases is multifactorial, it is possible that chronic infections may have a contributory role, which may be difficult to dissect out.

It is postulated that nearly 90% of the adult population in developing countries may be affected with H pylori infection since childhood [10,11]. Earlier studies on Indians [12-15] using serology and endoscopic biopsy have shown a similar high incidence of H pylori infection in small numbers of patients. While it may be possible to isolate H pylori non-invasively from dental plaques or fecal samples [15-16], the only definite means of detecting the organism per se, at present, is to perform a gastric biopsy or assess seroprevalence [17].

These tests are not practical as they are invasive, and cannot be used in field settings. In addition, seroprevalence does not exclude the possibility of a past infection in a non-infected person at the time of testing. Two non-invasive tests are available for testing active H pylori prevalence, the ^{13}C-urea breath test [18,19], and a non-invasive stool antigen based assay [20]. The latter measures the presence of H pylori antigen in stool samples and has been shown to correlate well with the former, breath test method.

The ^{13}C urea breath test [18,19], which is simple, specific and non-invasive, is also increasingly being used to determine the presence of H pylori infection, and is now considered to be the gold standard for comparing other methods of measuring H pylori infection. This breath test is based on the principle that the bacterium, which contains the enzyme urease, will hydrolyze an orally administered dose of ^{13}C-labelled urea. The $^{13}CO_2$, which is liberated will then enter the body CO_2 pool through the gastric mucosa, and eventually be excreted through the breath as $^{13}CO_2$. As there is no other urease containing organisms located in the upper gastrointestinal tract, the breakdown of the ^{13}C-labelled urea molecule within the first hour after oral administration would exclusively be due to H pylori urease activity. The resultant $^{13}CO_2$ released in the breath is usually measured as the increase in

$^{13}CO_2$ enrichment over the normally occurring (background) $^{13}CO_2$ level in the breath. This test is most useful in epidemiological situations, and, in addition, is useful for detecting present infection as well as the density of infection. In one study [18], which used H pylori culture as the gold standard, the sensitivity of the ^{13}C-urea breath test was 90% and the specificity 98.6%. The accuracy of the test was 94.8% with a positive predictive value of 98.2%.

It is also possible that H pylori infection may impact on the growth of children. For instance, it has been shown that the height-for-age is lower in H pylori infected children, compared to uninfected children [19]. In addition, 11% of a sample of 11-year-old children in Scotland had evidence of H pylori infection from the age of 7 years, and these children grew less than their uninfected counterparts, regardless of gender [21]. On the other hand, in a survey of 569 rural Bangladeshi children, there were no correlations between nutritional indexes and prevalence of H pylori infection [22]. It seems possible that H pylori infection may be a marker for poor socio-economic status and its consequence on growth performance, rather than a causal agent for poor growth per se. Therefore, this kind of analysis may be difficult in samples where a large proportion of the children is expected to be undernourished for a number of unrelated reasons. It is also difficult to establish a priori, that a measured growth defect is due to the H pylori infection exclusively. In all probability, a number of co-factors related to the socio-economic status of the individual will also contribute to the growth deficit.

H pylori can cause malnutrition in a variety of ways, including decreased food intake due to dyspepsia, or due to defective digestion/ absorption. However, these are likely to be seen in-patients with symptoms. In infants less than a year old, there was a significant relation between urine acid output and H pylori infection, during weaning [23]. Those infants with sustained infection grew less well than those without, and it was speculated that the early infection with H pylori in infancy could be event leading to reduced gastric acid output, followed by enteric infections and the sequelae of recurrent diarrhea and growth failure [24]. In contrast to these Gambian infants however, higher rates of H pylori infection did not appear to be a risk factor for diarrhea in Nicaraguan children [25]. Further, the incidence of diarrheal disease in H pylori infected children showed no association between the presence of infection and increased risk of diarrhea in infants and children up to 2 years of age [26]. Therefore, there is conflicting evidence with regard to intestinal sequelae such as diarrhea, in children. Another mechanism by which H pylori infection can cause malnutrition in symptom free children, is in terms of a general systemic acute phase response [27], which could in turn, lead to chronic malnutrition [28,29].

However, a recent study on a 100 patients testing positive for H. pylori infection showed no alteration of acute phase markers after successful eradication of the infection with antibiotics [30]. It is also not known if H. pylori could specifically reduce the absorption of nutrients, and therefore lead to malnutrition. It is known that children with H pylori infection show progressive deterioration of their gastric mucosa [31]. Therefore, it is possible that with long standing infection, there may be deterioration in the digestive or absorptive capacity of the intestine. The present study aims to address this issue by the use of stable isotope labeled protein, carbohydrate and fat, and the measurement of breath $^{13}CO_2$ production.

We have earlier studied 50 children (29 male and 21 female) from a lower socio-economic stratum in Bangalore [32]. The children were aged between 6-16 years, and had no significant previous medical history. The high prevalence of 82% confirmed the generally high prevalence of H. pylori infection in Indians, which was predicted by earlier serological studies. The male children had an 86.2% positive rate, while 76.2% of the females were positive.

The state of Karnataka, in which the city of Bangalore is located, and where the proposed urban and rural studies will be carried out, shows that 45 and 42% of the urban and rural population is below the poverty line, with an infant mortality of 73 per 1000 live births. In terms of access to safe drinking water, 90% of urban households had this, compared to only 73% of rural households. These data, which are taken from Dreze and Sen [33], along with our preliminary breath test data showing a high prevalence in the Indian environment [31], suggest an urgent need to quantify the prevalence of H pylori infections on an epidemiological basis and to study the possible impact of this infection on growth in children. This particularly needs to be done in environments with low sanitation and high crowding.

The aims of the proposed study are:

a) To measure the prevalence of H pylori infection in apparently healthy children between the ages of 6 to 10 years, from low and middle socio-economic classes in Bangalore City, and from rural areas around Bangalore.

b) To further stratify these prevalences on the basis of age and sex groups.
c) To correlate the prevalence of H Pylori in these children with a number of socio-economic indexes.
d) To assess if H pylori infection is associated with growth deficits.

2. METHODS

The study will be conducted in 600 children (300 urban and 300 rural), both male and female, evenly distributed between the ages 6 and 10 years. In the urban sample, 150 children will be studied from three schools that cater to middle socio-economic class (SES) families and the remaining 150 from three other schools that are subsidized by the Indian Government and cater to low SES children. The socio-economic status will be assessed by using a standard scale for Indian conditions (Kuppuswamy Scale, Manasayan, New Delhi, India). After obtaining permission from the school authorities to carry out this study on their children, meetings will be arranged with the parents of these children to obtain their consent for participation of their child in the study. Information related to the age of the child, housing conditions, family income, educational status and occupation of both parents will be also be obtained from the parents during these interviews. The above information will be checked for correctness from the school records. In addition, we will also obtain information on morbidity related to diarrhea and any major illness in the children in the past year from the parents.

We will also repeat this study in a rural setting.

Within rural areas, there is also a gradation of living standards, partly depending on the proximity of village or small towns to highways. Therefore, a total of 300 children between the ages of 6 and 10 years will be studied in two settings: 150 children from a small town school within a rural district, which will be referred to as "semi-rural", and 150 children from a "truly" rural village school, which is remote and far from highways. Male and female children will be equally distributed in the sample. As in the urban study, information about the age of the child, housing conditions and water sources, family income, educational status and occupation of both parents will be also be obtained. Particular attention will be paid to water sources as well as housing conditions.

Following this, at a time convenient for both the school authorities and the investigators, the study protocol will be carried out in the school premises. The children will be at least 4 hours post breakfast at the time the measurement starts (zero time). After a brief medical examination and the measurement of the body weight and height to nearest 100 g and 0.1 cm respectively, a baseline breath sample will be collected in duplicate in sealed vacutainers using a standard procedure. The breath samples will be collected in a modified urine collection bag and transferred by needle into vacutainers. The subjects will then ingest a dose (~ 50 mg) of ^{13}C-urea dissolved in 100 ml of water along with a fat rich meal to delay gastric emptying. Breath samples will be collected in duplicate at 30 minutes post ingestion of the isotope. The children will be kept in a seated position throughout the experiment to maintain a steady production of CO_2. This procedure has been earlier validated in children, where the breath test had a sensitivity of 97.92% and a specificity of 97.96%, when compared against histology and the rapid urease test, in 115 children [34].

Experimental protocol

Time (minutes)	-5	0	30
Meal	+		
Dose ^{13}C-urea	+		
Breath	+		+

The breath samples will be analyzed in duplicate for the ratio of $^{13}CO_2/^{12}CO_2$ using an isotope ratio mass spectrometer within 24 hours. The samples will be analyzed by continuous flow mass spectrometry (Europa Scientific, UK), which incorporates separation of the sample by gas chromatography followed by isotope ratio measurements. The enrichment of the samples is measured

against the enrichment of a standard gas mixture, which was earlier calibrated against PDB. The coefficient of variation of the system is < 0.01% (APE of background enrichment samples). A positive response will be judged by a cut-off increase in enrichment of 3.5 per mil.

3. PRELIMINARY RESULTS

There are some methodological issues that deserve consideration when performing the ^{13}C-urea breath test. The contribution of the test meal to the background $^{13}CO_2$ is an important issue in the interpretation of data from secondary metabolite experiments. This is because most dietary plant sugars have ^{13}C in varying quantities.

For instance, CO_2 fixation by the C4 pathway results in grain (maize) with a delta value of – 11 per mil (more enriched), whereas fixation by the C3 pathway yields grain (rice, wheat) with a delta value of –27 per mil (less enriched). Therefore, test meals with rice and wheat are less likely to raise the background enrichment of breath $^{13}CO_2$ appearance. Of particular interest in the Indian situation is the use of cane sugar (a part of many dietary preparations), which can raise the background enrichment of breath by a peak value of about 6 per mil, three hours after its oral ingestion.

In our preliminary experiments, the increase in enrichment in $^{13}CO_2$, in the first hour after ingestion of cane sugar, was about 3 per mil. While this scale of contribution of ^{13}C from test meal origin could interfere with interpretation of the breath test, it is important to note that this is the maximum dietary contribution possible. In mixed diet meals, with low carbohydrate and high fat contents, this contribution could be minimized. Another solution to this problem is to decrease or omit the test meal altogether [18]. However, it has been shown that this approach reduces the sensitivity of the breath test as gastric emptying is not retarded, and the resultant levels of $^{13}CO_2$ from the ^{13}C urea (in positive subjects) could be actually reduced, as the dose of ^{13}C urea is emptied from the stomach at a faster rate. Therefore, it is important to keep the limitation of the dietary contribution of $^{13}CO_2$ in mind when carrying out this test.

An earlier pilot study by us in this area has shown a high incidence of H pylori infection in children form low socioeconomic classes [32]. We extended this study in the same area to 342 children from the age group of 5-18 years (Kurpad AV & Dore SP, unpublished), and the distribution of H pylori infection in these children is shown in Table I.

TABLE I. H. PYLORI INFECTION IN CHILDREN AGE GROUP 5-18 YEARS.

'n'	Age Group (years)	Male (% positive)	Female (% positive)
48	5-6	71.4	72.0
55	7-8	91.3	80.6
47	9-10	82.6	75.0
50	11-12	88.5	83.3
47	13-14	91.7	91.3
48	15-16	96.3	71.4
47	17-18	80.0	77.3
Mean		**86.0**	**78.7**

This data was obtained in individuals from a poor area, with water supply restricted to a street tap, and shared toilets between houses.

4. FUTURE PLANS

The research plan for the year 2000-2001 has been detailed above, in the methods section, for urban and rural studies.

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