Haematological response to iron supplementation is reduced in children with asymptomatic *Helicobacter pylori* infection

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We evaluated the adverse effect of asymptomatic *Helicobacter pylori* infection in children on the response to Fe supplementation. One hundred and sixty-nine children aged 1–10 years from the urban poor community underwent a [^{13}C]urea breath test for *H. pylori* and haematological tests at admission and after 8 weeks. Both *H. pylori*-positive and -negative children were randomly assigned to receive ferrous fumarate syrup (20 mg elemental Fe twice daily) or placebo for 8 weeks and a single dose of vitamin A (33,000 μg). Admission findings were compared between *H. pylori*-positive and -negative children. Response to Fe was compared between Fe-supplemented *H. pylori*-positive and -negative children. Seventy-nine per cent of the children were aged 1–5 years and half of them were boys. In eighty-five *H. pylori*-positive and eighty-four *H. pylori*-negative children, the differences in mean Hb (112 (SD 12·6) *v.* 113 (SD 12·0) g/l), haematocrit (34 (SD 3·5) *v.* 35 (SD 3·2) %) and ferritin (23·8 *v.* 21·0 μg/l) were similar. After 8 weeks of Fe supplementation, mean Hb was 5·3 g/l more (95 % CI 1·59, 9·0) and haematocrit was 1·4 % more (95 % CI 0·2, 2·6) in *H. pylori*-negative (*n* 44) compared with *H. pylori*-positive (*n* 42) children. Mean ferritin was similar at admission and improved in both *H. pylori*-positive and -negative children. Asymptomatic *H. pylori* infection was not associated with higher rates of anaemia or Fe deficiency in children, but had a significant adverse effect on response to Fe therapy. However, this result is based on exploratory analysis and needs confirmation.

Helicobacter pylori: **Anaemia: Children: Iron supplementation: Iron status**

The prevalence of *Helicobacter pylori* infection is very high in children of developing countries and in many of them it occurs in early infancy (Mahalanabis *et al.* 1996; Bardhan, 1997; Sarker *et al.* 1997). The vast majority of these children do not present with any overt symptoms and signs attributable to this infection, which is known to cause gastritis and altered gastric acid secretion (El-Omar *et al.* 1997; Gutierrez *et al.* 1997; Sarker *et al.* 2004). Infection with *H. pylori* has been incriminated as a cause of anaemia refractory to treatment with Fe supplements (Peach *et al.* 1998; Annibale *et al.* 1999; Choe *et al.* 1999, 2000, 2003*a*; Konno *et al.* 2000; Ashorn *et al.* 2001) and several case reports have shown that its eradication improves the anaemia (Choe *et al.* 1999, 2000; Konno *et al.* 2000; Ashorn *et al.* 2001; Sugiyama *et al.* 2002; Sarker *et al.* 2004). Lactoferrin is known to provide Fe for *H. pylori* in gastric mucosa. In an elegant study Choe *et al.* (2003*a*) showed that adolescents with *H. pylori*-associated gastritis coexisting with anaemia had very high levels of lactoferrin in the gastric mucosa, and eradication of *H. pylori* in them was associated with a significant fall of lactoferrin level in gastric mucosa along with a rise in Hb concentration. The authors suggested that the phenomenon of sequestration of lactoferrin in the gastric mucosa in subjects with *H. pylori* gastritis and

Fe-deficiency anaemia might lead to a better understanding of the mechanism of anaemia in *H. pylori* gastritis. Recently Sarker *et al.* (2004) showed that gastric acid secretion is significantly reduced in *H. pylori*-infected anaemic young children and its eradication restores gastric acid secretion. Absorption of Fe from two Fe salts, namely ferrous sulfate (water-soluble) and ferrous fumarate (water-insoluble but soluble in weak acids), in asymptomatic children aged 2–5-years infected with *H. pylori* was, however, not significantly different from that in children free of *H. pylori* infection (Sarker *et al.* 2004).

Fe-deficiency anaemia remains a major nutritional problem in developing countries, particularly in children and women of childbearing age, and is an important cause of morbidity. In India, the National Family Health Survey II conducted in 1998/99 documented that about 74 % children aged between 6 and 35 months were anaemic (Kapil, 2003).

We hypothesized that asymptomatic *H. pylori* infection in children would be associated with Fe-deficiency anaemia and that its presence would also adversely affect the response to Fe supplementation of children in developing countries. To address these primary research questions we conducted exploratory and subgroup analysis within a randomized, double-blind,

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placebo-controlled trial of daily Fe supplementation along with an initial single large dose of vitamin A in children and evaluated the role of *H. pylori* infection on response to Fe supplementation by measuring Hb, haematocrit and ferritin values. Analysis comparing the response to Fe supplements in children with asymptomatic *H. pylori* infection and children without such infection (i.e. a subgroup in the trial) was pre-specified in the study design. We shall report separately the results of the randomized controlled trial of Fe supplementation with ferrous fumarate in underprivileged urban children in India, which was a secondary objective of the study.

Methods

Study population and sample selection

Children aged 1 to 10 years, of either sex, were included in the study. Additional inclusion criteria were no antibiotics during the past month, no sign/symptoms of infection, no gastrointestinal complaints such as diarrhoea, staying within a reasonable distance of the clinic (<3 km) and parents willing to give consent. Children attending the well baby clinic at the Infectious Diseases and Beliaghata General Hospital, Kolkata, India were approached for participation in the study. This is a large general hospital run by the government where treatment is free or subsidized and serves the urban poor. The medical officer (S.M.) of the clinic invited parents of the children in order of their registration and the first two eligible ones were recruited for the day. Written consent was obtained from the parents. The recruitment took place between June 1999 and October 2000. Baseline history and clinical examination results on the children enrolled were recorded on a pre-tested data collection form. These included age, sex, weight, height, mid-arm circumference and selected socio-economic indicators. The tests done at admission to the study included determinations of Hb, haematocrit, ferritin, serum Fe and total Fe-binding capacity, and a [^{13}C]urea breath test to detect *H. pylori* infection. The blood tests were repeated after 8 weeks of intervention. The study was approved by the Ethical Review Committees of the Society for Applied Studies, Kolkata, India and the University of Alabama at Birmingham, USA.

Randomization and treatments

A master randomization chart was prepared using permuted blocks of random numbers of variable block length (2, 4 and 6) by a person not directly involved in the conduct of the study. The sequence in which block lengths would appear was ascertained using a random number table. This randomization was incorporated into the serially numbered bottles of Fe syrup or placebo syrup, so that the serial numbers of a set of bottles corresponded to the patient's serial numbers. The randomization code was kept sealed centrally and was provided to the investigators only after they submitted the data sets on completion of the study. The children after recruitment received the supplement from the numbered bottles corresponding to their serial number, prior to any knowledge about whether the child was *H. pylori*-positive or -negative, because breath samples for the [^{13}C]urea breath test were analysed in batch mode and the results were available only after completing the treatment protocol. Children were randomly assigned to receive Fe syrup daily or a placebo (identical in colour, consistency and taste) for 8 weeks. In addition, all children received at the start of Fe therapy a single large dose (33,000 μg) of vitamin A. Subclinical vitamin A deficiency is common in this population and a single dose of vitamin A was given to all children at the start to improve response to Fe supplementation. Children were provided with four bottles of Fe syrup or placebo along with a plastic measure and their mothers were instructed to administer 5 ml syrup twice daily. Each ml of syrup contained 4 mg elemental Fe as ferrous fumarate. Therefore, all children in the intervention group would receive a daily dose of 40 mg elemental Fe (four times the RDA (Food and Nutition Board, National Research Council 1989) for 1–10-year-olds). Children received the first dose of the syrup at the clinic under the supervision of the health worker before being sent home with an appointment to return to the clinic after 4 weeks. At 4 weeks, parents were asked about syrup intake and side-effects if any, and a second set of four bottles was given for the second 4 weeks with an appointment to return at 8 weeks for final evaluation.

Random allocation to two groups resulted in the following four groups: (i) *H. pylori*-positive children treated daily with Fe syrup; (ii) *H. pylori*-positive children treated daily with placebo syrup; (iii) *H. pylori*-negative children treated daily with Fe syrup; and (iv) *H. pylori*-negative children treated daily with placebo syrup.

Follow-up procedures

Children were asked to return to the clinic after 4 weeks or earlier if they felt the need. If they missed the appointment at 4 weeks, a home visit was made to ascertain the reason and to encourage them to come. An appointment for a second visit at 8 weeks was given and if they failed, a home visit was made to encourage them to return.

Sample size

For comparison between *H. pylori*-positive and -negative children and to detect a difference of 5 g/l in Hb between the two groups at admission, seventy-five children would be needed in each group based on an expected mean Hb level of 110 g/l with sd of 11 g/l (at 80 % power and 95 % confidence). For evaluating the difference in response to Fe supplementation between *H. pylori*-positive and *H. pylori*-negative children with an expected difference in mean Hb of 8 g/l, we would need forty *H. pylori*-positive and forty *H. pylori*-negative children in the Fe-supplemented group. Using a conservative estimate that 50 % would be positive for *H. pylori*, we would need to recruit a total of 160 children for the subgroup analysis. We added 5 % more to this figure to compensate for loss to follow-up.

Statistical considerations and data analysis

We planned to make comparisons at two levels. At enrolment, the haematological indices were compared between *H. pylori*-positive and -negative children. For evaluation of the role of *H. pylori* infection on the response to Fe supplementation, we compared the haematological indices in the Fe-supplemented *H. pylori*-positive children with those in the Fe-supplemented *H. pylori*-negative children (i.e. groups (i) and (iii)) at admission and after 8 weeks of supplementation. This subgroup would constitute about half of the children enrolled in the trial. This analysis plan was pre-specified and is reflected in the sample size calculation given above.

EpiInfo version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA and WHO, Geneva, Switzerland) was used for entering and editing data. To evaluate the association of *H. pylori* infection with Fe deficiency and/or anaemia, all children recruited into the trial were divided into two groups according to *H. pylori* infection status. Their baseline features were summarized and compared. Their haematological indices at admission were compared by one-way ANOVA, a non-parametric test or the χ^2 test. Where relevant, use of appropriate regression models (multiple linear regression and logistic regression) was planned to adjust for covariates.

To evaluate the effect of *H. pylori* infection on the response to Fe we used multiple linear regression models. As a first step all children in the trial were included in the model and the interaction between Fe treatment and *H. pylori* infection was evaluated along with other explanatory and/or confounding factors. Separate models were fitted with Hb, haematocrit or ferritin values at 8 weeks after supplementation. Treatment effect was modelled with the interaction term between treatment and *H. pylori* status along with other baseline features such as sex and age. We then directly compared the subgroups of children with and without *H. pylori* infection treated with Fe supplement to evaluate the role of *H. pylori* infection on the treatment outcome. Regression models were used on this subgroup for evaluating treatment effect on Hb, haematocrit and ferritin with *H. pylori* status, sex and age as covariates. These models provided estimates for the role of *H. pylori* infection on the response to Fe supplementation. For regression models we used Stata version 7.0 software (Stata Corporation, College Station, TX, USA).

Laboratory analyses

[^{13}C]Urea breath test. The [^{13}C]urea breath test has high specificity and sensitivity and is widely accepted as a non-invasive technique for diagnosing current *H. pylori* infection (Logan *et al.* 1991; Vandenplas *et al.* 1991). [^{13}C]Urea is degraded rapidly in the presence of *H. pylori* urease to $^{13}CO_2$ and NH_3, and the former is excreted in the breath. A baseline breath sample was collected. The child then drank about 100 ml milk, followed by a test dose of [^{13}C]urea (99 % [^{13}C]urea; Sigma, St. Louis, MO, USA) at a dose of 30 mg for children less than 24 months old and 40 mg for children older than 24 months, dissolved in 50 ml sterile water. After 30 min the second breath sample was collected. Older children who were able to cooperate were asked to blow air through a tube into a plastic bag with an attached needle. Vacutainer tubes were filled using the attached needle. For younger children, not able to cooperate, a facemask with a one-way outlet valve was used to collect breath directly into a Vacutainer tube. Samples were collected in duplicate. Breath samples were analysed for ^{13}C of respiratory CO_2 by GC–isotope ratio MS at St. John's Medical College, Bangalore, India. Breath samples with an excess over baseline enrichment of $^{13}C > \Delta 5.0‰$ were regarded positive for *H. pylori* infection. The [^{13}C]urea breath test has been shown to be 100 % sensitive and 92 % specific and is considered a reference standard for the diagnosis of *H. pylori* infection in children (Rowland *et al.* 1997).

Haematological tests. Venous blood samples (2·5 ml) were drawn; 0·5 ml was taken into an EDTA-treated tube and analysed for Hb using the cyanmethaemoglobin (colorimetric) method (Randox® kit; Randox Laboratories, San Diego, CA, USA) and the remaining 2 ml was taken into a plain tube and treated to obtain serum. Serum Fe and total Fe-binding capacity were determined using a colorimetric method (Randox® kit; Randox Laboratories). For ferritin, a solid-phase immunoradiometric assay based on monoclonal and polyclonal anti-ferritin antibodies was used (Coat-A-Count Ferritin IRMA kit; Diagnostics Products Corporation, Los Angeles, CA, USA). The radioactivity is measured using a gamma counter and the concentration of ferritin is obtained by comparing the counts per minute with those obtained for the set of calibrators provided. The calibrators contain ferritin in terms of the WHO Second International Reference Preparation of Ferritin for Immunoassay, number 80/578 (2nd IS 80/578).

Results

Two hundred and forty-one children were assessed for eligibility and 176 children were recruited in the study (Fig. 1). Seven withdrew before the [^{13}C]urea breath test for the diagnosis of *H. pylori* infection could be done. Of the 169 children, eighty-five were positive and eighty-four were negative for *H. pylori* infection by the [^{13}C]urea breath test (Table 1). The two groups were similar in terms of age, sex and anthropometric indicators such as weight-for-age, weight-for-height and height-for-age SD scores compared with the National Center for Health Statistics (Dibley *et al.* 1987) reference data, and mid-arm circumference. The proportion of illiterate mothers was marginally higher in the *H. pylori*-positive children.

Haematological indices for *H. pylori*-positive and -negative children at admission are compared in Table 2. Mean Hb, haematocrit, serum Fe, total and unsaturated Fe-binding capacity, the proportions with low ($< 12 \mu$g/l) serum ferritin and the geometric mean serum ferritin concentrations were not significantly different between the two groups. About 40 % of the children were anaemic as defined by Hb < 110 g/l.

Eighty-three children were randomized to the placebo group, of whom forty-three were positive and forty negative for *H. pylori*. Haematological indices at admission and after 8 weeks of supplementation with placebo were similar between the *H. pylori*-positive and -negative groups, and between admission and 8-week values in each group (data not shown).

In three separate regression models on all children in the trial we evaluated the effect of the interaction between *H. pylori* infection and Fe treatment on Hb concentration, haematocrit and ferritin after 8 weeks of supplementation (Table 3). A trend for a negative interaction was noted for Hb concentration (-4.5; 95 % CI 2·15, -11.15) and haematocrit (-1.2; 95 % CI 0·73, -3.13). A positive interaction was seen for ferritin concentration (ratio of geometric means $= 1.67$; 95 % CI 1·02, 2·75).

Among the Fe-supplemented subgroup, forty-two children were positive and forty-four were negative for *H. pylori*. Haematological indices at admission and after 8 weeks of supplementation are shown in Table 4. Two in the *H. pylori*-negative group defaulted at the final evaluation at 8 weeks. Eight children (five in the *H. pylori*-positive and three in the *H. pylori*-negative group) did not receive Fe syrup for the second 4 weeks but returned for final evaluation at 8 weeks. These eight children were included in the analysis. Upon daily Fe supplementation the children with *H. pylori* infection had no significant increase in mean blood Hb concentration, whereas the *H. pylori*-negative children had a significant rise, with a mean increase in Hb of 5·3 g/l (95 % CI 1·59, 9·00; $P \leq 0.001$) compared with baseline values. Similarly, the *H. pylori*-negative children had a significant rise in haematocrit values after Fe supplementation compared with
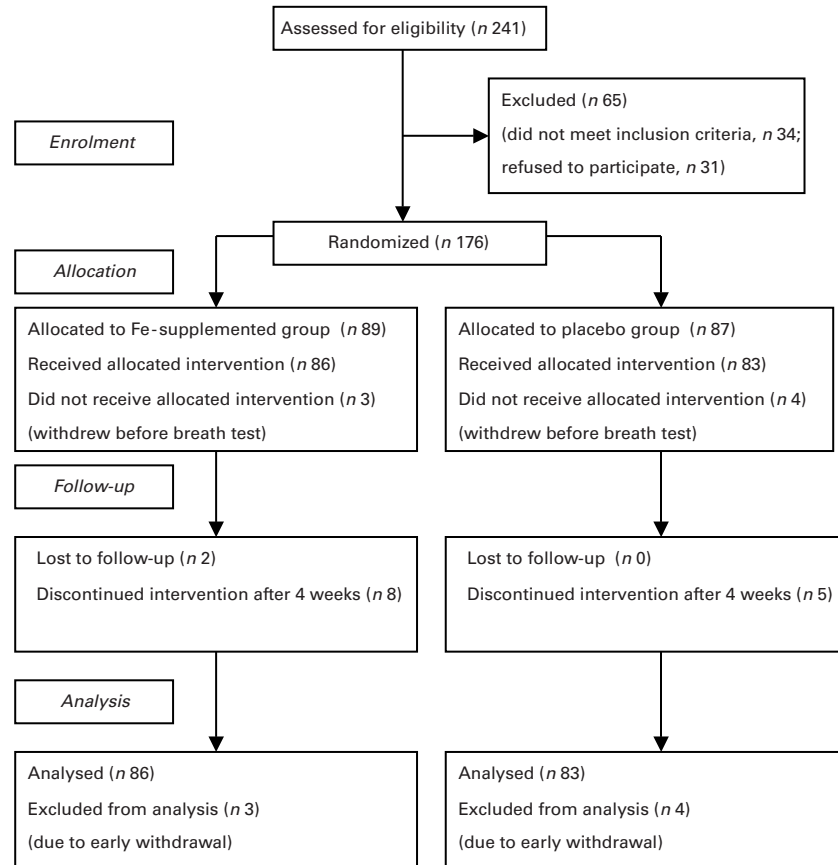
Fig. 1. Flow diagram showing subject recruitment.

admission values of 1·34 % (95 % CI 0·07, 2·62), but there was no significant increase in the *H. pylori*-positive children. After 8 weeks of Fe supplementation the proportion of children with Hb < 110 g/l was nearly double in the *H. pylori*-positive children compared with the *H. pylori*-negative ones (relative risk = 1·9; 95 % CI 1·04, 3·48; *P* = 0·029). Furthermore, after 8 weeks of Fe supplementation, Hb and haematocrit values were higher in the *H. pylori*-negative children than in -positive ones (Table 4). There was no significant difference in serum ferritin values between the two groups after Fe supplementation. However, Fe supplementation resulted in a significant fall in the proportion of children with serum ferritin < 12 μg/l in both groups compared with admission values. Moreover, the proportion with low ferritin levels (i.e. < 12 μg/l) was substantially reduced as a whole after Fe supplementation (i.e. in *H. pylori*-positive and -negative children taken together) compared with the two placebo groups combined (relative risk = 15; 95 % CI 2, 653; *P* = 0·002; data not shown). Using regression models on all children in the trial we could also show that the geometric mean ferritin level after 8 weeks of supplementation was 54 % higher in the Fe-supplemented group than in the placebo group (ratio of geometric means = 1·54; 95 % CI 1·20, 1·97; *P* = 0·001). There was a trend for total (*P* = 0·076) and unsaturated (*P* = 0·087) Fe-binding capacity to be higher in *H. pylori*-positive children after Fe supplementation compared with *H. pylori*-negative ones. No significant difference was noted in serum Fe values either at admission or after Fe supplementation. We used separate regression models with Hb and haematocrit as dependent variables and *H. pylori*

infection along with admission value of Hb or haematocrit as independent variable to estimate the adverse effect of *H. pylori* infection in the subgroup of children who received Fe supplements (data not shown). The results were closely similar to unadjusted analyses (Table 4). The presence of *H. pylori* infection was associated with a reduction of 5·27 g/l in Hb (95 % CI 1·31,

Table 1. Baseline characteristics of *Helicobacter pylori*-positive and -negative children as determined by the [^{13}C]urea breath test

(Values are means and standard deviations or *n* (%) unless stated otherwise)

Characteristic	*H. pylori*-positive (*n* 85)		*H. pylori*-negative (*n* 84)	
	Mean	SD	Mean	SD
Age (months)*	42·0 (29, 60)		37·5 (25·5, 57·5)	
Age group (years)				
1–2	13 (15·3)		19 (22·6)	
> 2–3	24 (28·2)		20 (23·8)	
> 3–5	27 (31·8)		31 (36·9)	
> 5	21 (24·7)		14 (16·7)	
Boys	43 (50·6)		42 (50·0)	
Weight-for-age *Z* score†	− 1·82	1·00	− 1·93	0·92
Weight-for-height *Z* score†	− 1·40	0·95	− 1·43	0·76
Height-for-age *Z* score†	− 1·20	1·21	− 1·38	1·30
Mid-arm circumference (cm)	14·3	1·48	13·9	1·19
Illiterate mother	35 (41·2)		23 (27·4)	

* Median (quartiles).
† Compared with the National Center for Health Statistics reference data.

Table 2. Admission laboratory findings of *Helicobacter pylori*-positive and -negative children

(Values are means and standard deviations or *n* (%) unless stated otherwise)

	H. pylori-positive (*n* 85)		*H. pylori*-negative (*n* 84)		
	Mean	SD	Mean	SD	95 % CI
Hb (g/l)	112	12·6	113	12·0	− 4·74, 2·74
Haematocrit (%)	34·0	3·5	34·5	3·2	− 1·52, 0·52
Ferritin (μg/l)*	23·8		21·0		0·87, 1·47†
Ferritin level <12 μg/l	16/81 (19·8)		18/76 (23·7)		0·46, 1·51†
Serum Fe (μg/dl)	67·7	33·4	69·1	33·0	− 11·48, 8·68
Unsaturated Fe-binding capacity (units)	256·8	79·8	248·5	84·6	− 16·67, 33·27
Total Fe-binding capacity (units)	325·1	83·4	312·7	84·9	− 13·16, 37·96
Hb (g/l)					
≤ 100	9 (10·6)		8 (9·5)		
> 100 to <110	25 (29·4)		23 (27·4)		
≥ 110	51 (60·0)		53 (63·1)		

* Geometric mean.
† Ratio mode.
None of the differences between the two groups were significant (ANOVA or χ^2 test). Analysis adjusted for sex and age showed no significant difference between the two groups (regression models).

Table 3. Effect of interaction between *Helicobacter pylori* infection and treatment with Fe supplements on Hb, haematocrit and serum ferritin after 8 weeks of treatment for all children in the trial

(Values are mean effect with 95 % CI in parentheses)

	Effect on		
Variable*	Hb (g/l)	Haematocrit (%)	Ferritin (μg/l)†
Interaction (between treatment and *H. pylori*)‡	− 4·50 (2·15, − 11·15)	− 1·20 (0·73, − 3·13)	1·67 (1·02, 2·75)
P value	0·18	0·22	0·04

* Separate multiple linear regression models were fitted for Hb, haematocrit and ferritin values after 8 weeks of supplementation (dependent variables) on all children in the trial. Other variables in the models are treatment with Fe, *H. pylori* status, sex, age and admission values of Hb or haematocrit or ferritin (log-transformed).
† Ferritin was modelled after log transformation and therefore the effect and confidence interval are in ratio mode.
‡ *H. pylori*: present = 1, absent = 0; treatment with Fe: yes = 1, no = 0.

Table 4. Laboratory findings at enrolment and after 8 weeks of supplementation with iron and vitamin A

(Values are means and standard deviations or *n* (%) unless stated otherwise)

	Helicobacter pylori-positive (*n* 42)				*Helicobacter pylori*-negative (*n* 42)			
	Admission		8 weeks		Admission		8 weeks	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hb (g/l)	113·1	11·1	113·8‡	9·2	112·4	9·5	119·1‡	7·8
Haematocrit (%)	34·1	3·3	34·5§	2·9	34·4	2·9	35·9§	2·6
Ferritin (μg/l)*	26 (15, 38)		35 (28, 52)		21 (15, 30)		28 (24, 56)	
Ferritin <12 μg/l†	9/41 (22·0)		1/27 (3·7)		7/38 (18·4)		0/29 (0·0)	
Serum Fe (μg/dl)	62·0	26·9	82·1	36·3	71·8	36·2	71·5	27·5
Unsaturated Fe-binding capacity (units)	258·5	69·4	241·7‖	62·5	242·0	80·8	209·4‖	55·5
Total Fe-binding capacity (units)	324·8	65·1	321·6¶	68·7	308·2	84·8	287·8¶	46·8
Hb <110 g/l	13 (30·9)		20 (47·6)**		17 (40·5)		11 (26·2)**	

* Median (quartiles).
† *P*=0·043 for *H. pylori*-positive (relative risk = 5·9; 95 % CI 0·8, 44·2) and *P*=0·016 for *H. pylori*-negative children (Fisher's exact test, two-tailed) for comparison between admission and at 8 weeks.
‡ Mean difference = 5·3 (95 % CI 1·59, 9·00).
§ Mean difference = 1·4 (95 % CI 0·20, 2·60).
‖ Mean difference = 32·3 (95 % CI 6·64, 57·96).
¶ Mean difference = 33·8 (95 % CI 8·28, 59·32).
** RR=0·55, (95 % CI 0·30 to 1·00, *P*=0·041).
Differences among the groups at admission were not significant at the 5 % level.

9·23) and a reduction of 1·34 % in haematocrit (95 % CI 0·07, 2·62). *H. pylori* infection was associated with a non-significant increase in serum ferritin of 20 % (95 % CI − 17, 72).

Among children who received the placebo, forty-three were positive and forty were negative for *H. pylori* by the [^{13}C]urea breath test. Hb, haematocrit and serum ferritin values at 8 weeks were not significantly different from the admission values (data not shown). Likewise, serum Fe, total and unsaturated Fe-binding capacities at 8 weeks were also similar to admission values in both groups.

Discussion

The present study confirmed that the prevalence of *H. pylori* infection is high in children from among the urban poor in India. One important finding was that both Hb concentrations and haematocrit values after 8 weeks of Fe supplementation were adversely affected by the presence of *H. pylori* infection. A major new finding was the absence of any adverse effect of *H. pylori* infection on improvement in serum ferritin concentration, which increased substantially in the presence of *H. pylori* infection. Improvement in ferritin concentration following Fe supplementation was not associated therefore with an improvement in Hb and haematocrit values in *H. pylori*-infected children. However, we conducted an exploratory subgroup analysis in a randomized controlled trial and the findings should therefore be interpreted with caution.

In the present study we could not demonstrate an association between asymptomatic *H. pylori* infection in children with anaemia or Fe status. However, we note that about 40 % of the children had an initial Hb value less than 110 g/l and was similar in the two groups. Nearly 80 % of the children in this study were 1–5 years old whereas the association between *H. pylori* infection and anaemia and/or Fe deficiency has been reported largely in older subjects. We speculate that a longer duration for *H. pylori* infection may be needed to show a difference.

We can only speculate on the reasons for this difference. Several studies (largely in older children, teenagers and adults) have shown that anaemia associated with *H. pylori* infection (Peach *et al.* 1998; Annibale *et al.* 1999; Konno *et al.* 2000; Ashorn *et al.* 2001; Choe *et al.* 2003a,b) is resistant to Fe supplementation unless *H. pylori* is eradicated (Choe *et al.* 1999, 2000; Sugiyama *et al.* 2002; Sarker *et al.* 2004). We speculate that lack of response to Fe supplementation in *H. pylori*-positive children could be due to (i) the presence of an inflammatory state associated with *H. pylori* infection (Yip & Dallman, 1988; Crabtree, 1997; Hatz *et al.* 1997; Kirchner *et al.* 1997), or (ii) inadequate absorption in the presence of gastritis and/or reduced gastric acid secretion associated with *H. pylori infection*, or (iii) both. Improvement in ferritin status after Fe supplements in both *H. pylori*-positive and -negative children associated with a lack of improvement of anaemia in the *H. pylori*-positive children suggests that chronic infection with *H. pylori* may play a significant role. Unfortunately, we did not measure C-reactive protein or any other acute-phase reactant in our study children. Even though these children were asymptomatic, acute-phase response could have still occurred.

H. pylori contains an Fe-binding protein similar to ferritin and a system of Fe-repressive outer membrane proteins with ability to bind haem Fe (Jurado, 1997; Annibale *et al.* 2000). This raises the possibility of *H. pylori* competing with the host for Fe absorption, particularly for haem Fe.

We considered the role of infection and inflammation due to *H. pylori* in causing anaemia in the light of our findings and the reported improvement in anaemia after eradication of *H. pylori* by combined therapy with antibiotics in 2–5-year-old children in Bangladesh (Sarker *et al.* 2004). However, the degree of systemic inflammatory response associated with *H. pylori* infection has not been evaluated. Gastrointestinal blood loss associated with *H. pylori* infection as a cause of anaemia, as reported in one study (Kostaki *et al.* 2003), has not been confirmed (Sarker *et al.* 2004).

Using doubly labelled isotopes Sarker *et al.* (2004) directly measured Fe absorption from ferrous sulfate and ferrous fumarate in 2–5-year-old children in Bangladesh and showed that reduced gastric acid secretion associated with *H. pylori* infection did not significantly influence Fe absorption from these two salts. However, Fe absorption from ferrous fumarate was substantially lower in young children (2–5 years old) compared with ferrous sulfate (Sarker *et al.* 2004). Mean absorption of Fe from ferrous sulfate was more than triple that from ferrous fumarate. Eighty per cent of the children in the present study were 1–5 years old and we can only speculate whether use of a more efficiently absorbed Fe salt such as ferrous sulfate would have been effective in improving anaemia in *H. pylori*-infected asymptomatic children. The dose used was four times the RDA for 1–10-year-old children. The younger children in the study may have received a relatively high therapeutic dose of Fe for 8 weeks. However, we expect the bioavailability of Fe from ferrous fumarate to be low.

Refractory anaemia associated with *H. pylori* infection has been reported in adolescent girls (Choe *et al.* 2000). In the present study we did not find any association of sex with anaemia or *H. pylori* infection.

The problem of anaemia and Fe deficiency in children in developing countries is overwhelming. The adverse effect of *H. pylori* infection on response to Fe supplementation makes it more difficult to design an adequate control strategy in populations with a high rate of asymptomatic *H. pylori* infection. A programme of treatment for *H. pylori* eradication in children from a developing country would be expensive, unrealistic and has doubtful ethical justification. In light of these findings it is worthwhile to explore if the use of different forms of Fe and/or Fe-absorption enhancers such as ascorbic acid would reduce this adverse effect in *H. pylori*-infected children.

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