Attachment 1. (FACE SHEET) NSA-000032 Library CO Date 10.03.97

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr S.A. Sarker Trainee Investigator (if any)

Application No. 97-004 Supporting Agency (if Non-ICDDR,B)

Title of Study Is Helicobacter pylori infection ... anemia in children in Bangladesh?

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

(c) Minors or persons under guardianship (Yes) No

2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects. Yes (No)
   (d) Discomfort to subjects Yes (No)
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)

3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) (Yes) No
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body Fluids (Yes) No

Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used (Yes) No
   (c) Physical risks Yes No NA
   (d) Sensitive questions Yes No NA
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No NA

(if subjects are minors) Yes No

6. Will precautions be taken to protect anonymity of subjects (Yes) No

7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ✓ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
   2. Examples of the type of specific questions to be asked in the sensitive areas.
   3. An indication as to when the questionnaire will be presented to the Cttee. for review.

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator Trainee

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Is *Helicobacter pylori* infection, a cause or treatment failure of iron deficiency anemia in children in Bangladesh?

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Total Project cost: US$ 146,248

Starting date: As soon as possible

Date of completion: Two years after starting

Signature of the Division Director, CSD

Summary

Helicobacter pylori is recognized as a major gastrointestinal pathogen in developing countries and up to 60% of children less than five years are infected with this microorganism. The organism is strongly associated with chronic gastritis and peptic ulcer disease in children and adults. The progressions of gastritis into atrophy often leads to decreased gastric acid output, which is a well-known risk factor for anemia. Gastric acid is essential to increase the bioavailability and absorption of non-heme dietary iron, the most important source of iron in developing countries. Numerous reports exist to suggest iron malabsorption secondary to low gastric acid output is a problem in developing world countries. We now hypothesizing that the poor bioavailability of iron in those countries might be related to *H. pylori* -induced low gastric acid output. It has been further observed that iron deficiency anemia (IDA) is resistant to iron therapy particularly in developing countries. We propose to investigate the role of *H. pylori* infection as a cause of anemia and treatment failure of iron supplementation in Bangladesh. A prospective, randomized, double-blind, placebo-controlled field trial is proposed among four groups (65 each) of *H. Pylori* infected children of 2-5 years of age with iron deficiency anemia. The children will be assigned to one of the four therapies: antibiotics (for *H. Pylori* eradication) plus iron therapy, iron therapy alone, antibiotic therapy alone or placebo. Combination of three markers (hemoglobin, Hb; serum ferritin, SF; serum transferrin receptors, STfR) and urea breath test (UBT) will be used for detection of IDA and *H. Pylori* infection respectively and will be performed before and at 1 and 3 months after the therapy. We furthermore propose a complementary study in an additional group of 20 children with *H. Pylori* infection with IDA to assess iron absorption with a simple oral iron absorption test using 1 mg/kg elemental iron before and after therapy. The incidence of *H. Pylori* infection and IDA and values for iron stature and absorption will be compared before and after therapy. The results of the study are expected to have implication for the treatment and prevention of iron deficiency anemia in developing countries.

A. Hypothesis

1. H. pylori infection in infants and young children causes gastritis and reduced gastric acid output

2. Reduced iron absorption related to *H. pylori* infection is a cause of iron deficiency anemia in children

3. *H. pylori* infection is a cause of treatment failure of iron supplementation

B. Objectives of the study

1. To determine if *H. Pylori* infection is causally associated with iron deficiency anemia in children

2. To determine the effect of *H. Pylori* infection on iron absorption

3. To determine if *H. Pylori* infection is a cause of treatment (iron supplementation) failure of iron deficiency anemia

C. Project Background

Helicobacter pylori is one of the most common bacterial infections with a majority of many populations in the developing world being infected with this organism. [1,2,3,4] The organism is the causative agent of chronic antral gastritis and primary duodenal ulcer disease in children and adults. Evidence now exists to indicate an important relationship between *H. pylori* infection and recurrent enteric infection, malnutrition, and malabsorption resulting in growth faltering in children in the developing world countries.[5]

Prevalence of *H. pylori* infection based on seroepidemiological studies in developing countries and in immigrant populations are given below:

Author, year (ref.)	Country	No. in study	Population	Age range (years)	Prevalence (%)
Graham *et al.* 1991[6]	India	238	Outpatients (minor illnesses)	0->60	81
Al-Moagel *et al.* 1990[7]	Saudi Arabia	490	Healthy volunteers	0->60	72
Mègraud *et al.* 1989[8]	Vietnam	365	Blood donors (paid)	0->60	53
	Ivory Cost	374	Serum survey	0->60	73
	Algeria	277	Blood donors outpatients	0-59	79
Mitchell *et al.* 1988[9]	Papua New Guinea	157	Serum survey	10-62	56
Dwyer *et al.* 1988[10]	El Salvador	75	Australian immigrants	20-60	40
	Ethiopia	74	Australian immigrants	20-49	43
	Vietnam	190	Australian immigrants	20->60	18
Perez-Perez *et al.*1990[11]	Thailand	89	Serums survey	0-75	<10(ages 0-5) >75(age >30)
Li *et al.* 1991[12]	China	89	Healthy volunteers	15-22	49

Adapted from Talley et al 1993[13]

Recent interest has focussed on determining the precise relationship between gastric acid secretion and *H. pylori* infection.[14] Longitudinal population-based studies suggest that chronic superficial gastritis following *H. pylori* infection ultimately progresses to chronic atrophic gastritis,[15] which has marked effects on gastric physiology and function.[16,17,18] Acid output, pepsinogen, and intrinsic factor linearly decrease with increasing atrophy of oxyntic glands in the corpus mucosa. Thus, in the developing world countries where the prevalence of *H. pylori* infection is very high, adverse consequences of reduced gastric acid output including increased susceptibility to enteric infections, and iron deficiency anemia might also be attributed to *H. pylori*-induced hypochlorhydria.

Low gastric acid output state and achlorhydria in particular have been found to be associated with iron deficiency anemia.[19,20] There are numerous reports of iron malabsorption

secondary to low gastric acid output in countries where the incidence of *H. pylori* infection and associated chronic gastritis in these same communities is also very high.[1] The poor bioavailability of iron might be related to low gastric acid, the role of which is well recognized in iron absorption. The possibility that *H. pylori* induced low gastric acid output is related to the increased prevalence of iron deficiency anemia in the developing world countries cannot be excluded.

Prevalence of iron deficiency anemia

Iron deficiency anemia (IDA) is common worldwide affecting millions of people, and the most common cause of anemia is iron deficiency.[21] Pregnant women and young children are at greatest risk. The consequences of IDA are particular severe in children and include abnormalities of immune function, increased risk of infections, poor growth, and potentially irreversible deficits of cognition and motor function in infants. [22]The identification and treatment of iron deficiency anemia isareherefore one of the highest health care priorities in developing worlds. In Asia, the prevalence of iron deficiency anemia is relatively high in most countries, although reliable national data is lacking in many countries. The percentage of infants and young children found to be anemic has been reported to be 74-82% in Bangladesh,[23] 34-69% in India,[24] 37-73% in Indonesia, [25] and 42-47% in the Philippines.[26]

The cause of anemia in the developing world has not been thoroughly studied. Inadequate and poor quality diets together with parasitism and other infections are often cited as the immediate causes; the role of poor absorption of iron as a cause of iron deficiency anemia has not been adequately studied. However, there are reports of malabsorption of iron associated with low gastric acid output in these countries,[19,20] and the incidence of *H. pylori* infection and associated chronic gastritis in these same communities are also very high. [1] It is therefore possible, and perhaps likely, that poor bioavailability of iron might be related to low gastric acid, the role of which in iron absorption is well recognized. It has been observed in a preliminary study that *H. pylori* infection in children is associated with anemia as assessed by measurement of packed cell volume in a group of infected children aged 4-24 m compared to control (Bardhan PK, unpublished observation). We believe it is important that the role of *Helicobacter pylori as* a potential cause of iron deficiency anemia among children be investigated.

Importance of gastric acid in iron absorption

Gastric acid is known to be an important intraluminal factor necessary for optimal nonheme iron absorption. [27] Gastric acidity has effects iron absorption in several ways. Ingested inorganic (Fe^{++}) solubilized and ionized by the gastric secretions, reduced to the ferrous (Fe^{+++}) form, and chelated. Substances that form low-molecular iron chelates such as ascorbic acid, among others, promote iron absorption. Normal gastric secretions contain a stabilizing factor that slows the precipitation of ferric at alkaline pH of the small bowel. The impaired iron absorption observed in achlorhydric or gastrectomised people is probably related to decreased solubilization and chelation of ferric iron food. [28,29] The divalent

(ferrous) form of iron is more readily absorbed than the trivalent (ferric) form because of low solubility of ferric hydroxides and phosphates at the alkaline pH of intestinal fluid. Healthy individuals normally absorb 5 to 10% of dietary iron, and those who are iron-deficient absorb up to 10 to 20%. Two defects in iron absorption are observed after partial or total gastrectomy. First, absorption of dietary, non-heme iron is subnormal.[30] Second, the normally enhanced absorption that usually accompanies iron deficiency does not take place.[31],[32] Therefore, children or adults with atrophic gastritis and achlorhydria and who become iron deficient are not able to increase the uptake of iron as much as individuals with normal gastric function. Gastric acid is also critical for effective digestion of dietary proteins, which may inhibit iron absorption by binding iron in an insoluble form[33]. Finally gastric acid alters the physicochemical characteristics of iron complexes formed with dietary constituents.[34] Reduced absorption of dietary iron has been observed in patients receiving a long term H_2 receptor antagonist.[35] Iron malabsorption in these subjects was related to inhibition of gastric acid secretion by the long term use of H_2- receptor blocker.

Gastrointestinal function including gastric acid secretion was studied in children and adults with iron deficiency anemia. Niemann and his colleagues studied 14 iron-deficient infants and children and observed profound suppression of gastric acid secretion in a majority of the subjects.[36] Ghosh reported reduced gastric acid secretion in all the anemic children compared to healthy controls.[37] In 17 adult patients with iron deficiency anemia, a high incidence of decreased acid secretion was also noted.[38] Reduction of acid secretion including histamine-fast achlorhydria, has been reported in patients with iron deficiency anemia in India.[39],[40] The values for acid output were significantly lower in patients with iron deficiency compared to those in control subjects. Histological abnormality of the gastric mucosa suggestive of chronic gastritis was demonstrated in 61% of the patients. The incidence of chronic gastritis in patients with iron deficiency anemia was also high (75%).[41] It is not known whether such abnormalities precede iron deficiency anemia or not. An early acquisition of *H. pylori* infection in children in developing countries may indicate that *H. pylori* related chronic gastritis might precede iron deficiency anemia in those countries.

Whereas the effect of reduced iron bioavailability on hemoglobin levels and other hemopoietic indices has been recognized for a long time, the association between iron deficiency with or without anemia, with immunocompetence, cognition and, physical work capacity, thermogenesis and other functions have recently been documented. A number of organs and systems show variable changes in structure and function, often before any decrease of iron status occurs. This is not surprising because iron is an integral component or an essential cofactor for several enzymes that play important roles in metabolic processes and cell proliferation; these include aconitase, catalase, cytochrome C, cytochrome C reductase, cytochrome oxidase, monoaminoxidase, peroxidase, tyrosine hydroxylase and xanthine oxidase.[42],[43] These enzymes are involved in a number of key pathways such as DNA synthesis, mitochondrial electron transport, catecholamine metabolism, neurotransmitter levels and other function. It is not surprising then, that iron deficiency anemia results in variety of functional abnormalities.

The prevalence of *H. Pylori* infection in Bangladesh is also very high.[44] Preliminary results demonstrated an increased prevalence of anemia in *H. Pylori* infected children compared to non-infected controls (Bardhan PK, personal communication). There are also reports of non responders to oral iron therapy in children in developed countries[45],[46] and in adults in Bangladesh (S. Rahaman: Ph.D. thesis work, University of Brussels: personal communication). The possibility that treatment failure in anemia is related to *H. pylori* - related poor absorption of iron is therefore particularly important.

We hypothesize that *H. pylori* infection in children is a cause of iron deficiency anemia and is also a cause of treatment failure of an iron supplementation program in developing countries.

D. Experimental Design and Proposed Methodology

We propose to complementary study:

D.1. A prospective, double-blind, placebo-controlled field trial in two hundred sixty children of a peri-urban community near Dhaka City is proposed.

D.2. The assessment of iron absorption before and after anti-infective therapy in 20 children with *H. Pylori* infection and iron deficiency anemia

D.1 Field trial

D.1.1 Description of the study population

Children will be recruited from Nandipara, a peri-urban community situated 7 miles northeast of Dhaka City. This community has a population of 3,500 in an area of approximately 2.5 square miles. Among the residents, 70% of males are classified as day laborers, 20% are rickshaw pullers, and 5% are carpenters or service holders. Fifteen percent of women are day laborers and 85% are housewives. The average family size is 4.5 members. The slum has a municipal water supply for drinking and cooking and most families live in poorly constructed houses. The great majority of houses are mud walled with thatched dry leaves and bamboo or tin roofs. The population less than five years of age is about 750. The prevalence of *H. pylori* infection in less than five children in this community is 50%.[44] A weekly clinic has been run by the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B) for minor case of illnesses in the population since 1986.

All children in the community will initially be screened for iron deficiency anemia and *H. pylori* infection. After explanation of the study and the tests, informed consent will be obtained from the parents or legal guardians. Demographic information including name, date of birth, sex, weight, height, economic status of the parents, nature of feeding, and previous medication in the past 1 month will be recorded.

Screening for iron deficiency anemia

50 μl capillary blood samples obtained by finger stick will be assessed for hemoglobin (Hgb.) concentration. Measurement of hemoglobin concentration is the most useful test in screening iron deficiency anemia since it directly reflects the quantity of most abundant essential iron compound in the body. Children with hemoglobin concentration below 110g/l will be considered as anemic.[47] In children with low Hgb. identified through the initial screening, the diagnosis of iron deficiency anemia will be confirmed by venepuncture Hgb., serum ferritin concentration,[48] and serum transferrin receptor(sTfR).[49] Hemoglobin concentration can be transiently depressed due to inflammation or infection, and in such a situation does not indicate pathological anemia. Of all the biochemical tests for assessing iron status, a low SF is the most specific test for iron deficiency. The major disadvantage of SF is that it is readily elevated in response to any infection or inflammatory condition. The impact of infection on SF is often described as a "reticulo-endothelial block" which reduces the release of iron from phagocytic cells breaking down hemoglobin so reducing the serum iron concentration and causing ferritin iron to accumulate intracellularly.[50] One major advantage of sTfR is that unlike SF, sTfR is not significantly affected by infection or the inflammatory process. However, sTfR levels are increased in situations of increased RBC turnover such as hemolytic anemia, i.e., thalassemia, etc. A uniform cutoff value of >8.5 mg/l indicates an elevated sTfR and therefore iron deficiency or hemolytic anaemia.

Table. Laboratory indicators of iron deficiency and iron deficiency anemia

Indicator	Iron depletion	Iron deficiency anemia
Hb	↔	↓
SF	↓	↓
sTfR	↑	↑

IDA, iron deficiency anemia; Hb, hemoglobin; SF, serum ferritin; sTfR, transferrin receptor.

Measurement of serum transferrin receptors also becomes a promising new test for the evaluation of iron status particularly in epidemiological assessment of anemia. Performed in conjunction with serum ferritin measurements, the serum transferrin receptor serves to distinguish true iron deficiency from the anemia from the anemia of chronic diseases or inflammatory diseases and, therefore, offers a major advantage in establishing the true prevalence of iron deficiency anemia in population studies.[49] The combination of low hemoglobin, a low serum ferritin, and an elevated serum transferrin receptor are diagnostic of iron deficiency anemia and will be made [51] when the following criteria are met: transferrin receptor > 8.3 mg/l, serum ferritin concentration < 12ng/ml, and hemoglobin concentration(< 11 gm/dl).

Screening of H. Pylori infected children

The anemic children will be screened for *H. Pylori* infection using a urea breath test (UBT) which is considered by many of the reference diagnostic test for *H. pylori* infection.[52] The test is a simple, noninvasive test that has been recommended as a gold standard for the detection of H. Pylori infection with a sensitivity of 96 % and specificity of 100%. [51] We have recently established the test at ICDDR, B.[44] In brief the procedures are as follows.

Urea Breath Test

A breath sample will be collected for base line $^{13}CO_2$ in a vacutainer following a fasting period of 2 hr. A volume of 100 ml of whole milk will then be allowed to be consumed by the children. Ten min later 40 mg of ^{13}C urea (99% ^{13}C, Tracer Technologies, Boston, Massachusetts) will be given after dissolving in 25 ml of water. After administration of the substrate, breath samples will again be collected in duplicates after 30 min. Samples will be collected through two-way pediatric mask with an attached non-return valve into a vacutainer and will be stored for shipment to the University of Basel, Switzerland, to measure the ^{13}C concentration of a respiratory CO_2 mass spectrometer. The excess over the baseline will be expressed as parts per thousand(delta $^0/_{00}$). A breath test in which the excess over a baseline is $5.0^0/_{00}$ at 30 min will be regarded as positive for *H. pylori* infection.

D.1.2 Selection of Study children

Iron deficiency anemic children with weight for age >60% of National Center for Health Statistics (NCHS), with no evidence of deficiency diseases or systemic infection and whose parents give informed consent will be enrolled in this study.

Exclusion criteria

The children with following problems will be excluded.

- Acute infection or apparent inflammatory process
- Signs of vitamins deficiency
- Severe anemia (Hemoglobin <70 G/l)
- Severe malnutrition (marasmus, marasmic kwasiorkar or kwasiorkar)
- Presence of hook worms and /or *Giardia lamblia* (cyst or vegetative form) in a stool microscopic examination
- Presence of fat in a stool microscopic examination
- Presence of occult blood in a stool as demonstrated by Guaiac test
- History of taking antibiotics or any drugs for any cause in the preceding month

D.1.3 Procedure

A detailed medical history will be recorded. Body weight and height will be measured upon enrolment into the study. Naked weights will be obtained to the nearest 10 g using a balance scale (Gebrüder Soehnle, Murrhardt, Germany) and the mean of three consecutive measurements recorded. Recumbent length will be measured in children less than two years of age using a slide board infantometer (Harpenden, St. Albans, England). In older children, standing height is to be determined using a locally constructed instrument in which a metal tape measure is extended between a footplate and head bar that has an attached fluid level to ensure a horizontal position. The mean of two consecutive height measurements to the nearest 0.1 cm is recorded as the observed value. Measurements are compared to the standards according to the National Center for Health Statistic data and the nutritional status assessed by Z-score.[54],[55] Percent of median equivalents of standard deviation units will be determined and categorized according to the scheme of Waterlow.[56]

All children will be subjected to physical examination by one of the investigators. The children will be assigned to one of the four groups according to randomization:

- Anti Hp alone group
- Fe^{+} alone group
- Anti -Hp group + Fe^{+} Group
- Placebo group

Anti Hp therapy: Clarithromycin 15 mg/kg/day in two divided doses plus omeprazole 20 mg single dose for 14 days.[57],[58]

Iron therapy: An iron dose of 3 mg/kg/day (or about 30 mg/day) as ferrous sulphate as advocated by Dallman[41] will be fed in a single dose in the morning in one half an hour before food. The iron therapy will be given for 90 days.

Placebo Glucose syrup

D.1.3.1 Supplementation

The intervention syrup(s) will be fed daily in a single dose in morning in an empty stomach (Half an hour before food). The children in the placebo group will receive same amounts of glucose syrup. All therapies will be kept in identical dark brown containers and will be fed by a health worker who will be kept unaware about the nature of the study and of the contents inside the bottle. Twenty four-hour food intakes will be recorded on a precoded form by dietary recall.

The children will be followed for three months after the end of supplementation. Urea breath test will be repeated at the end and at 1 and 3 months after the supplementation. Venous

blood (500 µl) will be taken for determination of SF, STFR and Hgb concentration at the end and after 1 and 3 months of supplementation. Children in the placebo group will be given a three-month course of supervised iron supplementation at the end of study period.

D.1.3.2 Assessment of growth and growth velocity

Measurement will include weight, height, mid upper arm circumference, triceps skin fold and knemometry. Height increments are such that measurements at least 8 weeks apart are required before height velocity can be accurately assessed. Portable knemometry by experienced operators permits far more accurate measurement of knee-heal length, enabling meaningful length velocity assessment over shorter time periods.[59]

Naked weights will be obtained to nearest 10 g using electronic digital scale (Seca model 770, Sweden) standardized with 1 kg standard weight prior to each weighing. Recumbent length will be determined with a locally constructed instrument using the pressure technique in which a metal tape measure is extended between a footplate and head bar. The mean of the two consecutive measurements of the nearest 0.5 cm will be recorded as the observed value. All measurements will be subsequently compared to the standard Of National Centre for Health Statistics data and the nutritional status will be assessed by Z score.[60] Percent of median equivalents of standard deviation units will be determined and categorized according to the system of Waterlow.[61] Body mass indexes (BMI) (weight/cm2), triceps skin folds thickness and mid arm circumference will also be assessed. BMI results will be compared to the standardized curves of the National Health and Nutrition examination study while skinfolds thickness and midarm circumference compared to the standards of Kerlberg, et al. [62] The measurements of all indices will be recorded before, at 1 month and 3 month of supplementation.

D.1.4 Sample size calculation

The sample size is calculated on the basis of (i) prevalence of *H. Pylori* infection, (ii) prevalence of non response to iron therapy.

Sample size calculation based on the prevalence on H. Pylori infection

Considering the prevalence of *H. Pylori* infection 50% and iron deficiency anemia 70%.[44] we anticipate *H. Pylori* related anemia in 50% of infants and children in this community. We expect a reduction in prevalence of *H. Pylori* related iron deficiency anemia after therapy with antibiotic plus iron therapies 25%. To detect a difference of this magnitude at 5% level and at 80% power the sample size is calculated as follows:

$N = [P1(100-P1) + P2(100-P2)]*8/(P1-P2)^2$

Where P1 = prevalence of *H. Pylori* related anemia

P2 = prevalence of *H. Pylori* related anemia after therapy

$= [50(50) + 25(75)] * 8 / (25)^2 = 56$ in each group

Based on a previous study in this community we anticipate a drop out rates of approximately 15% (Akramuzzaman SM, personal communication), the necessary sample size will be 65 children in each group and the total sample of children will be 260.

Calculation based on prevalence of non-responder to iron therapy

Observation from an ongoing study with an oral iron supplementation program in anaemic patients being conducted in Dinajpur (a northern district of Bangladesh) suggests that 70% of the iron supplemented group do not respond (S. Rahman, personal communication). The prevalence of *H. Pylori* infection in the proposed study population is approximately 50%, and we expect that after oral iron supplementation the non responders will be 40% less among the *H. pylori* negative group, compared to the *H. pylori* positive group. To detect a difference of this magnitude at 5% level and at 80% power the sample size is calculated as follows:

$$N = [P1(100-P1) + P2(100-P2)]*8/(P1-P2)^2$$

Where P1= prevalence of anemia after iron supplementation in *H. pylori* infected children

P2= prevalence of anemia after iron supplementation in H. pylori non-infected children

$$=[(70)(30) + (42)(58)] * 8 / (35)^2$$

=46 in each group

With 15% drop out rates, the number of children will be 53 in each group, i.e., total samples of 212 children.

We will take the larger sample size, i.e., 260 children for this study.

D.1.5 Randomization

A master randomization chart will be prepared by an appropriately trained person who is not connected with the study using random permuted block numbers taking a size of three. Bottles containing anti-Hp therapy, iron therapy or placebo will be identical in appearance and will be arranged in sequence that corresponds to the randomization chart and will be serially numbered. The serial number of the bottles will correspond to the serial number of the patients enrolled in the study. As one group of children would receive anti-Hp therapy and iron therapy simultaneously, there will be two bottles (bottles A or B) for each group of children to make the study double blind. For children belonging to anti-Hp plus Iron therapy, there will be one bottle containing anti-Hp therapy or and the other containing iron therapy. Children belonging to iron therapy or anti-Hp therapy alone, will be getting one bottle containing the medication, while the other will contain glucose syrup. As the duration of anti-Hp therapy is 14 days, the children belonging to this group will be provided glucose syrup for the rest of the month in order to make the duration of therapy equal among the four groups.

D.1.6 Laboratory test

Hb concentration will be determined by the cyanmethemoglobin method, serum ferritin will be determined by a radio immunometric assay.[63] Serum transferrin receptor will be measured by enzyme-linked immunosorbent assay (ELISA).[64] Stool will be tested for the presence of occult blood by the guaiac reaction (Occultotest, Ames Co., Elkhart, Inc., USA).

D.1.7 Withdrawal from the study

- The noncompliance of subject, either because the leaves the study area for other place before the end of the study or
- because the child requires unscheduled treatment for serious interim infection.

Treatment of withdrawal during analysis

Results of all randomized children will be included in the analysis of the study. Data from the patients withdrawn will be included up to the time of withdrawal. A supplemental analysis in which such patients are excluded will also be made.

D.1.8 Outcome variables

Major

- The incidence of *H. Pylori* infection following the therapy.
- The incidence of IDA in each group as primarily defined before and after the intervention
- Rise of serum hemoglobin and serum ferritin concentration or reduction of level of transferrin receptors.

Minor

- Change of anthropometric indices

At the end of the supplementation a repeat Urea breath test will be performed. One ml venous blood will be taken again for determination of hemoglobin, serum ferritin and transferrin receptor. If the values are still low iron supplementation with same doses of iron will be made for another 1 month.

D.1.9 Withdrawal from the study

-The noncompliance of subject, either because the leaves the study area for other place before the end of the study or

- because the child requires unscheduled treatment for serious interim infection.

D.2 Iron absorption study

The iron absorption study will be done in twenty additional children with positive urea breath test and iron deficiency anemia. The purpose of the study and the procedure of iron absorption study will be explained to the parents and the consent will be obtained. The children will be kept in the metabolic study ward of the Clinical Research and Service Centre of ICDDR, B. From 2:00 A.M. of the next day of admission, the children will be kept nothing per oral for 6 H. A volume of 150- 250 ml of intravenous fluid containing 5% dextrose will be infused during this period to support required energy. In the morning iron absorption test will be performed.

Procedure

Iron absorption tests will be performed as previously described and was found greater sensitivity as a screening test for iron malabsorption. [65] Briefly, after an overnight fast, iron will be given in a dose of 1.0 mg/kg body weight in the form of ferrous sulfate absorbed on polystyrene sulfonate. This preparation is commercially available (Liquifer-CR, Abbott Laboratories) and contains 10.5 mg of elemental iron and 50 mg of vitamin C/ml. Venous blood will be obtained before and 2 h after administration of the test dose. Serum iron will be determined. Children will be considered to have iron malabsorption if, for a given baseline serum iron concentration, the increase in serum is less than 220 μg/dl.[66]

E. Data analysis

Clinical characteristics such as age, weight, hemoglobin and serum ferritin concentration will be compared among the groups by one way analysis of variance (ANOVA). When significant F-ratio ($p<0.05$) is obtained, means will be compared by Student's t-test using a multiple comparison procedure. Outcome variables, e.g., value of serum iron, transferrin receptor and ferritin before and after treatment in each group will be compared using paired 't' test. The data with skewed distribution will be normalized by log transformation, and the 't' test will be applied on transformed data. Chi square (X^2) test will be used for comparison of binary variables, e.g., the incidence of *H. Pylori* infection following the therapy, number of children whose anemia has been corrected and diseases morbidity. The odds ratio and confidence interval will be calculated. Multivariate analysis (logistic regression or multiple regression) will be done to adjust the confounders, e.g., age, socioeconomic status, nutritional status. Data will be analyzed with a microcomputer using data analysis package (SPSS/PC + Inc., USA).

F. Relevance of findings for policy and program formulation:

Iron deficiency anemia is the most common single nutrient deficiency in the world. Although inadequate and poor qualities of diets, together with parasitism and infections, are the most common immediate cause; the role of poor absorption of iron in causation of iron deficiency anemia has not been properly studied. The mounting evidence that low acid output

is associated with poor absorption of iron, it is important to identify the factors related to such abnormalities. Moreover it has been suggested that iron deficiency anemia in the third world countries is refractory to iron supplementation therapy. At present the cause of treatment failure of an iron supplementation program in correcting iron deficiency anaemia in those countries is not fully known. As *H. pylori* infection is associated with low acid output, it seems reasonable that iron deficiency anemia and treatment failure of iron therapy are linked to such infection. The proposed study is designed to investigate the role of *H. Pylori* in IDA and treatment failure of iron supplementation. In addition, the study will help to estimate the reinfection rate of *H. Pylori* following eradication in developing countries. If the hypothesised relation between *H. Pylori* in IDA is established, this information would be of fundamental importance for addressing the formulation of appropriate policy to eradicate the infection with appropriate antimicrobials particularly in a community where a reinfection rate is low.

G. Time schedules

-Procuring supplies and establishing methods' & recruitment and training study personnel	1 month (July 97)
-Expected time for initiation of data collection	August 1997
-End of data collection	1 yr 9 months after starting
-Analysis of data	2 months after end of data collection

H. Ethical consideration

UNDER PROCESS

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SECTION III: BUDGET

Project Title: "Is H. pylori infection ... anemia in children in Bangladesh?"

Project Duration : 2 years from starting

Name of P.I : Dr. Shafiqul A. Sarker

Amount in US$

Line Item	1st year	2nd year	Total
PERSONNEL LOCAL SALARIES			
Dr. Shafiqul A. Sarker (25%)	3,919	4,193	8,112
Dr. G. Fuchs (5%)			
Dr. N. H. Alam (10%)	1,568	1,677	3,245
Dr.T. Ahmed (10%)	1,109	1,187	2,296
Dr. P. K. Bardhan (5%)	835	894	1,729
Study Nurse x 1 (100%)	5,400		5,400
Research Physician x 1 (100%)	3,000	3,000	6,000
Health Assistant x 1 (100%)	2,868	2,520	5,388
Secretarial Service (5%)	1,096	1,173	2,269
Sub-total	19,795	14,644	34,440
INTERNATIONAL TRAVEL			
-for attending conference & seminar	0	5,000	5,000
Local (General transportation & conveyance)	300	300	600
Sub-total	300	5,300	5,600
SUPPLIES AND MATERIALS			
Hospital supplies	500	500	1,000
Office supplies	500	500	1,000
Staple Isotope 13c - Urea58 57 Fe,Fe	10,000	10,000	20,000
FE and there measurement			
Others	300	300	600
Sub-total	11,300	11,300	22,600
OTHER CONTRACTUAL SERVICES			
Rent communication and utilities	300	300	600
Printing & Publication of Forms & Annual Report	300	300	600
Patient food & diet, ICDDR,B	500	500	1,000
Guest house and lodging			
Service charge, daily wager & short term staff	500	500	1,000
Consultant	5,000	5,000	10,000
Sub-total	6,600	6,600	13,200
INTERDEPARTMENTAL SERVICES			
Transport, land & water	100	100	200
Medical illustration	50	50	100
Xerox, Memio & Library services	300	200	500
Laboratory Tests	12,500	12,500	25,000
Patient hospitalization	4,500	4,500	9,000
Sub-total	17,450	17,350	34,800
CAPITAL EXPENDITURE			
Equipment & Furniture	500	500	1,000
Sub-total	500	500	1,000
Total operating cost	55,945	55,694	111,640
Indirect cost (31%)	17,343	17,265	34,608
TOTAL PROJECT COST	73,288	72,959	146,248

FILE NAME :PYLORI

JK

Abdul Ahad
Budget & Cost Officer
ICDDR, B. Mohakhali
Dhaka-1212, Bangladesh

CONSENT FORM

(This form will be read and explained clearly in local language before consent is obtained)

Your child is suffering from anemia and *Helicobacter pylori* infection (a germ) which are major problems in our country. The germ (*H. Pylori*) is often responsible for low gastric acid output which in turn lead to low iron absorption and thereby cause iron deficiency anemia. It has further been noted that iron supplementation some times fails to correct anemia in some children.

International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) is planning to conduct a study to examine whether the germ *H. Pylori* is a cause or treatment failure of iron deficiency anemia in children in Bangladesh. We therefore, request you to allow your child to participate in this study.

If you agree, the following procedures will be followed.

1. A physician will examine your child with particular emphasis in diagnosing anemia, nutritional disorders, and other health problems.

2. Half (0.5) mililitre blood from a vein of the fore arm will be taken for diagnosis of iron deficiency anemia.

3. Stool will be examined microscopically.

4 A breath sample will be collected from your child before and after feeding of a powder 13 C-Urea to detect the *H. Pylori* germ.

5. Your child will be provided one of the four groups of treatment
   a) Antibiotics for treatment of the germ for 14 days
   b) Iron therapy for 30 days
   c) Antibiotic plus iron therapy (antibiotic 14 days, iron 30 days)
   d) Glucose syrup for 30 days

6. Half mililitre (0.5 ml) blood will be again taken at 1 and at 3 month of therapy to see any change in iron status. A breath test will also be repeated at the same time.

7. The study involves no risk. We will maintain the confidentiality of the medical records.

8. At any time of the study, you may withdraw your child from the study, but his routine care by us will not be hampered. If you have any question to ask, we will be happy to answer them.

9. If you agree to participate in this study, please sign below.

Signature of the Investigator	Signature of witness	Signature or thump impression of the Guardian

A-31999

K 1

GUIDE FOR EVALUATING USAID/CHR PROPOSALS

Name of proposal: Is *Helicobacter pylori* infection, a cause or treatment failure of iron deficiency anemia in children in Bangladesh?

Name of proposed investigator: Dr. S.A. Sarker
Associate Scientist, Clinical Sciences Division (CSD), ICDDR,B

1) Goals: The main goal of the project, to determine whether *H. pylori* infection is a cause of treatment failure in iron supplementation, is reasonable.

2) Design:

The definitions and most methods of the project are well-described. However, the section on the basic design of the study and sample size is unclear. The investigators state that an ongoing study suggests that "in anaemic patients...70% of the iron supplemented group do not respond (Rahman)." This statement is graphically presented in Table 1.

Table 1.

Anemic			
+ Iron			
Response + (not anemic) 30%	Response - (still anemic) 70%		

The investigators go on to say that "the prevalence of *H. Pylori* infection in the 'proposed study population' is considered to be 50%". First, it is unclear whether this statement means that the prevalence of *H. Pylori* is 50% in the total population less than five years in the community of Nandipara (as implied on p. 9) *or* if this means that the expected prevalence of *H. Pylori* is 50% in the population of anaemic children to be enrolled (as might be inferred from pp. 10 and 11). This is a key point that must be addressed by the investigators.

A related point is what exactly is the design of the study according to sample size calculations presented. My best reading of the design of the study is presented in Table 2.

Table 2.

Anemic children			
randomized to receive either			
Iron		Placebo	
some percentage of whom are			
H. Pylori + (A%)	*H. Pylori* - (B%)	*H. Pylori* + - (A%)	*H. Pylori* - (B%)
who after follow-up, differ in anemic status			
Anemia + (P1%)	Anemia-	Anemia+ (P2%)	Anemia-	Anemia +	Anemia +	Anemia +	Anemia +

If this is a correct interpretation of the design of the study, and the first of the two alternatives, i.e. that prevalence of *H. Pylori* is 50% in the *total* population (since the statement on p. 9 seems clear on this), the question then is: what percentage of children who are anaemic are expected to have *H. Pylori*? (i.e. what are the values for A% and B% in Table 2)

Next, the sample size calculations suggest that the investigators believe P1% in Table 2 will be 70%, apparently based on the observations by Rahman. This reviewer does not see that this would necessarily be the case. The lack of response to iron supplementation will likely be higher in children who are anemic children *and* positive for *H. Pylori* than the 70% lack of response in the general population of anemic children observed by Rahman.

Finally, it should be pointed out, even as calculated, that the denominator (P1-P2) does not equal 35.

In sum, the design of this study needs to be clarified and the sample size calculations redone.

Based on clarifying the above, there are also a number of other issues the investigators should consider. First, it is highly likely that children with and without *H. Pylori* differ in a number of ways other than just potential response to iron (e.g. socioeconomic status, hygiene, etc.). Therefore, it may be advisable to stratify the randomization scheme by *H. Pylori* status. Also, alternative designs might more efficiently answer the questions of interest. For example, including only *H. Pylori* positive children randomized to (1) treatment for the infection + iron or (2) just iron would answer the question: does treatment of H. Pylori increase the absorption and effectiveness of iron supplementation?

3) Appropriateness:

Iron deficiency anemia is an important global child health problem. The investigators should clarify, however, what can be done about decreasing the very high prevalence of *H. Pylori* if the hypothesized relations are found.

4) Timing and budget:

In regards to timing and feasibility, the investigators state that they will be drawing from a population of 500 children under 5 years. However, they do not say how many of these children are mild-moderately anemic (i.e. between 70 and 110 g/L). Even if 50% of children meet this criteria, the investigators will need to enroll nearly all children in the community in the study based on current sample size calculations (those these may change based on the above comments).

5) Ethics: Acceptable.

6) Background: Investigators have an excellent knowledge of subject area.

7) Other: None

R2

3. Is *Helicobacter pylori* infection a cause of treatment failure of iron deficiency anaemia in children in Bangladesh?

Proposed investigators: Sarker, Fuchs, Alam, Bardhan, Ahmed, Begue & Khaled

1. Goals

The proposed study has two distinct but related sets of goals: the first is to examine associations between *H. pylori* infection and iron deficiency anemia in young children, and a possible mechanism to explain this association (Objectives 1 & 3 in the study proposal), and the second is to determine whether *H. pylori* infection is associated with lack of response in iron supplementation (Objective 2).

2. Design

The study design proposed will not enable the investigators to examine whether there is any association between *H. pylori* infection and iron deficiency anemia because - apparently - no non-anemic children will be recruited into the study. Furthermore, if the investigators truly wish to establish whether *H. pylori* infection is a CAUSE of iron deficiency anemia, then they must examine changes in iron status (or hemoglobin levels) either when *H. pylori* infection is cured (if this is possible) or before and after it is first acquired.

In order to establish whether *H. pylori* infection is a CAUSE of non-response in iron supplementation, it is necessary to randomly assign the infection, not the iron. Since it

R2

is not ethical to induce infections in humans, the only options are a) to randomly assign infected children to treatment for *H. pylori* infection or no treatment and then assess response to iron supplementation, or b) to assess response to iron supplemented children in infected and uninfected children, and then treat the infected children and re-assess their response to supplementation. The placebo group in the design proposed serves no purpose whatsoever.

The investigators need to explain whether their proposed diagnostic test (urea breath testing) is both sensitive and specific for *H. pylori* infection. If it is not, then an alternative test needs to be identified. It is not clear why the investigators have carefully defined iron deficiency anemia using a combination of three different biomarkers in Section D2 and then given a very non-specific outcome measure (a "rise" of undetermined magnitude in either hemoglobin or serum ferritin). No details are give as to how the secondary outcomes - "anthropometric indices" and "disease morbidity" - are to be measured.

The sample size calculations are very unclear and are based on the false assumption that the study requires random allocation to iron.

Since the study design is not appropriate to answer the research questions identified, the analysis plans are necessarily also inappropriate.

No interview or record abstract forms were provided for assessment.

3. Appropriateness

Without some discussion of the technical feasibility and cost of diagnosing *H. pylori* infections in young children, it is impossible to say whether it would be useful to know whether these infections are an important factor in the failure of iron supplementation to improve iron status. Supposing that it is both feasible and cheap, then it would be an important contribution since both the infection and the disease outcome (iron deficiency anemia) are highly prevalent in poor countries. Many international agencies have made the reduction of iron deficiency anemia a major priority, but little concrete progress has been made so far.

Research has concentrated on the association between *H. pylori* infection and poor growth in young children - little is currently known about its association with iron deficiency anemia. A well conducted study in this area would therefore making a significant contribution to existing knowledge.

4. Timing and Budget

No information presented in the proposal to evaluate these areas.

5. Ethics

Ethical questions would have to be reviewed once the design of the study has been reformulated.

6. Background

No background information is presented on either of the two secondary outcomes: growth and morbidity, and their associations with *H. pylori* infection. The data on the association between *H. pylori* infection and anemia in Bangladeshi children referred to in the Introduction should be presented in detail.

R3

Name of proposal: Is *Helicobacter pylori* infection, is a cause or treatment failure of iron deficiency anemia in children in Bangladesh? (3)

Name of investigator: Sarker et al.

Date of review: December 1996

Overall comments

The proposal is addressing an interesting and important question which has received little attention to date. The proposal, however does not address an equally fundamental question related to *helicobacter pylori*, which is: what could and should be done about it? If infection rates are so high in developing countries as estimates tend to indicate, attention should be directed to how to prevent it and how to cure it. What do we know about etiology, treatment, re-infection rates, etc.? These are questions that should at least be addressed in the relevance and policy implications section of the study.

Specific comments

Hypothesis: The first 3 hypotheses stated are not tested in this study. For instance, the fact that *H. pylori* causes gastritis and hypochlorhydria is not tested; nor the association between hypochlorhydria and poor iron absorption. What is tested is whether there is an association between *H. pylori* and iron deficiency.

Literature review: The literature review should also discuss the potential association between *H. pylori* and B12 deficiency, which has been documented recently.

Objectives of the study: The objectives of the study should be modified to better reflect what the study can really achieve. Objective 1, for example, should be reworded to say whether *H. pylori* is **associated** with iron deficiency anemia, as opposed to is **a cause**. The study can only test for an association between the two variables because it cannot randomly assign the *H. pylori* infection to a group and no infection to the other group and test for the occurrence of iron deficiency. Thus, causality cannot be inferred; only an association can be documented. The same applies to the second objective. The third objective should be reworded to say: To compare iron absorption between children with and without *H. pylori*. Finally, the real objective of the study is missing. It should say something like: To compare the response to iron supplementation between anemic *H. pylori*-infected and non infected children.

Design: The design of the study should be modified to better address the main question of interest. The main interest is to select a sample of anemic children and compare those who have *H. pylori* with those who do not have the infection with respect to their response to iron supplementation. In order to have a more efficient design, the authors should stratify their sample of anemic children into those who have the infection (+) and those who do not have it (-) at

baseline and randomize the supplementation intervention within each group. This would allow a more balanced design than just counting on the fact that approximately half of all anemic children will have the infection.

Methods: The impact of mild infections on the indicators of iron deficiency used (particularly ferritin) should be discussed.

Sample size calculations: Sample size calculations are done on the wrong outcome: prevalence of anemia after supplementation, comparing *H. pylori*-infected and non-infected children. This is the wrong outcome because randomization is not based on these two groups. If one follows the current proposed randomization, sample size calculations should be based on comparing the iron-supplemented and the placebo groups relative to their final iron status. Obviously, this does not answer the main question of the study, and this is why the current design is inappropriate. Therefore, the design needs to be re-formulated as suggested above, and the sample size calculations modified accordingly.

Exclusion criteria: Hb < 7 as an exclusion criteria is very low. For ethical reasons, children should be treated for anemia at Hb < 9 or 8.

Screening Tests: The sensitivity and specificity of the test for *H. pylori* and for iron absorption should be discussed. These two tests are not widely used and a discussion of the percentage of misclassification that they entail should be presented.

The proposed supplementation period of 30 days seems short to obtain a significant reduction in the percentage of anemia. It may be wise to increase the supplementation period by 1 month and also to compare mean Hb and ferritin levels as the outcomes, rather than the prevalence of anemic children.

Outcomes: There is an inconsistency between sample size calculations and the outcome variables mentioned. The sample size calculations are based on differences between groups in the prevalence of anemia at the end of the study and the outcome variables are 'rise in Hb or ferritin at the end of the study'.

Minor outcomes: Morbidity: there is no information on how the morbidity information will be collected and which variables will be studied.

Policy implications: As mentioned in the overall comments, the proposal should discuss what should be done about *H. pylori*, especially if it is shown to be associated with iron deficiency anemia and to impair the response to iron supplementation. What would be the implications in terms of prevention and treatment of the infection?

Response to the Reviewers' comments on project entitled "Is Helicobacter pylori ... anemia in children in Bangladesh?"

Response to reviewer no. 1

1. Comment on the **goal** of the study is appreciated.

2. Design

We agree with comments made by the reviewer and changed the design accordingly. We expect to able to obtain urea breath test results within 3 weeks of collection. Therefore, according to the suggestion made by the reviewer only iron deficiency anemic children with positive urea breath tests will be enrolled and assigned to one of four groups i) **anti Hp** alone **Group** (will be treated with Clarthromycin plus omeprazole ii) **Fe** $^{+}$ alone group (will be treated with iron only) iii) **anti-Hp plus Fe** $^{+}$ group (will be treated with combination of antimicrobial as above and iron) and iv) **placebo.** The new design has been incorporated in page 10, section D.1.3, para 2 in the revised version.

The sample size calculation has been redone and added to the section D.1.4, page11-12. We will also stratify nutritional status, socioeconomic status to the analysis according to the suggestions of the reviewer.

3. Appropriateness

If the hypothesized relation is found between *H. Pylori* infection and iron deficiency anemia (IDA), this information would be of fundamental importance to determine the need for addressing the formulation of appropriate policy to eradicate the infection with appropriate antimicrobial. The comment has been incorporated in section F, page 14-15.

4. Timing and budget

At present, the under five populations in the study area are 750. The statement in section D.1.1 has been changed. We expect that around 75% children are moderately anemic and are equally infected with *H. Pylori*. Therefore, we expect to get the stipulated sample easily from the population described in the proposal. In the unlikely situation that the stipulated sample could be not collected from the proposed study area, attempts will be made to enroll children from the adjacent area.

Response to reviewer no. 2

Design

We agree with the comment and have revised the design of the study. The comment on whether *H. Pylori* infection is a CAUSE of iron deficiency anemia has been addressed (see response to reviewer no. 1).

The urea breath test in detecting *H. Pylori* infection is highly sensitive (94%) and specific (100%) and is considered as a safe and gold standard test for diagnosing *H. Pylori* infection in children. The diagnostic value of the test has been referred in section D.1.1 (screening of *H. Pylori* infection part, p 9, para 1).

We interprete the reviewer's comment to indicate uncertainty about assessment and confirmation of etiology of anemia. It is important to keep in mind that not all anemia is due to iron deficiency. Different limitations of the markers, we have selected for the assessment is the basis for their inclusion and group interpretation. Please refer to the enclosed manuscript which briefly describes this in further detail. While different combinations of markers (serum iron, TIBC, transferrin etc.) can be used to assess iron status, Hb and SF have been the most widely used reference standards for the determination of iron status. We have included sTFr because of the more recently defined limitation of SF.
In the revised version we will compare all the biomarkers in different treatment groups with ANOVA and mutivariate analysis (section E, page 14).

The sample size calculation has been recalculated on the perspective of study objectives and on the basis of prevalence of iron non-responder to iron therapy and prevalence of *H. Pylori* infection (Section **D.1.4,** page 11-12)

The anthropometric indices will be used as minor outcome variable and will be monitored as described in the revised version (section **D.1.3.2,** page 11, para 3).

The analysis plan has also been modified in the revised version (section E, page 14).

Appropriateness: We believe that, irrespective of cost and technical feasibility of *H. Pylori* testing, it is useful to know whether this infection is an important factor in failure of iron therapy. If so, an important subsequent priority would be to determine how to apply this information into a programmatic response. We appreciate the reviewer's comment.

Timing and budget: A lay out of time frames to conduct the study has been provided in **section G,** page 15

Background: The impact of *H. Pylori* infection on growth has been provided in the background Section C, para 1, last line). The association between *H. Pylori* infection and anemia as diagnosed by measurement of packed cell volume in Bangladeshi children was based on preliminary observation on 30 children aged 4 m-24 m. The particular of the patients has been provided (UBT; 14 positive, 16 negative) in section C , page 5, para 3 *(prevalence of iron deficiency anemia)*.

Response to reviewer no. 3

Overall comments

We agree with the overall comments. The proposed study is designed to investigate the role and relative importance of *H. Pylori* in IDA and treatment failure of iron supplementation. In addition, the study will help to estimate the reinfection rate of *H. Pylori* following eradication in developing countries. If the role of *H. Pylori* in IDA is established, it will help to formulate policy to eradicate *H. Pylori* infection, particularly in a community where a reinfection rate is low. The implication of the study has been added to section F, page 14-15 of the revised version.

Hypothesis

Hypothesis 3 has been dropped in the revised version.

Literatures review

The association between *H. Pylori* infection and vit B12 deficiency is poorly defined because of paucities of study results. Recent publication demonstrated that *H. Pylori* infection is not associated with Vit B 12 deficiency (Pilott et al 1996).

Objective of the study

We agree with the comments on *H. Pylori* infection is associated with IDA as opposed to cause. Therefore, we have changed the design in the revised version (page 10, section D.1.3) in order to better address the main question of interest, i.e., infection. We hope the present design will help to establish a causal link of *H. Pylori* infection in IDA.

Design

Has been changed as mentioned earlier.

Methods

The impact of mild infection on ferritin has been discussed (page 8, L 10-15)

Sample size calculation

As mentioned earlier, the design has been reformulated and the sample size calculation has been modified accordingly. (Section D.1.4)

Exclusion criteria

We agree with the comments. In the proposed design, all of the study children will be provided iron supplementation. The children belonging to **Anti- Hp** plus Fe^+ group and Fe^+ group will receive the supplementation from the beginning of the study. The other two groups belonging Anti-Hp and placebo groups will be supplemented at the end of the study. Moreover, we will provide iron supplementation to those children who are still anemic as diagnosed by repeat Hb, ferritin and transferrin receptor. The children with low Hb level at the time of screening will also be supplemented with iron (mentioned in section D.1.3.1 page 10-11).

Screening tests

The sensitivity and specificity of UBT have been incorporated in section **D.1.1** in *screening of H. Pylori infection* part (Page 9, para 1 of the revised version). The diagnostic value for the iron absorption test has also been incorporated in section D.2 (page 14).

We agree with the comments on the period of iron supplementation. The comments are incorporated in section **D.1.3.1** (page 10-11). The supplementation will be provided for three months. Mean Hb, ferritin, and transferrin receptor has been considered as outcome measures in the revised version.

Outcomes

The comments are incorporated in **D.1.8** (page 13)

Minor outcomes

Disease morbidity as a minor outcome variable has been dropped in the revised version.

Policy implication

The comments are incorporated in the Section E (page 14-15)