**Ethical Review Committee, ICDDR,B**

**Principal Investigator**
Dr. A.N. Alam

**Registration No.** 84-004 (P)

**Title of Study** Iron Deficiency and Cholera

**Trainee Investigator (if any)**

**Supporting Agency (if Non-ICDDR,B)**

**Project status:**
- [x] New Study
- ( ) Continuation with change
- ( ) No change (do not fill out rest of form)

---

### Source of Population:

<table>
<thead>
<tr>
<th>(a) Ill subjects</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) Non-ill subjects</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>(c) Minors or persons under guardianship</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

### 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 |

### 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 |
| (d) Sensitive questions | Yes | No |
| (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 |

---

5. Will a signed consent form be required:

| (a) From subjects | Yes | No |
| (b) From parent or guardian (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

---

*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 Ctte. for review.

---

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

Principal Investigator

22 Jan 1984

Trainee
SECTION I - RESEARCH PROTOCOL

1. TITLE: IRON DEFICIENCY AND CHOLERA

2. PRINCIPAL INVESTIGATOR: Dr. A.N. Alam
   CO-INVESTIGATORS: Dr. Paul A. Goff
                     Dr. M. A. Rashid (IPGM&R)
                     Dr. N. M. Abdal
                     Dr. M. M. Rahman

3. STARTING DATE: February 15, 1984

4. COMPLETION DATE: September 15, 1984

5. TOTAL DIRECT COST: US $1,129.6

6. SCIENTIFIC PROGRAMME HEAD:
   This protocol has been approved by the Nutrition Working Group.

   Signature of Scientific Programme Head: [Signature]
   Date: 8/11/1984

7. ABSTRACT SUMMARY:
   One hundred adult male subjects, fifty admitted to ICDDR,B hospital with cholera and fifty as control, will be selected for a case-control study to determine the association between iron deficiency and cholera. Finding a causal relation between the two would encourage future interventions e.g., iron supplementation and/or fortification in endemic cholera areas.

       ......./2.
A. Introduction

1. Objective: The objective of this study is to determine whether iron deficiency is more prevalent in cholera patients than in a matched control population. The findings will test the hypothesis that iron deficient subjects are more susceptible to cholera.

2. Background: A correlation between infection and iron deficiency is not clear. Studies have argued both ways, that is that iron deficiency provides resistance to infection and that iron deficiency also can impair immune resistance. The former view is supported by studies of malaria in which patients with iron deficiency were found to have fewer attacks\(^1\). Also, the finding that newborns treated with iron have an eight fold increase in the rate of bacterial infection supports the contention that iron itself is necessary for bacterial proliferation\(^2\).

   On the other hand a number of studies indicate that lack of iron impairs resistance to infection. Iron deficient rats have been shown to be more susceptible to S. typhuminiun\(^3\) and strepto pneumonococcal infection\(^4\).

   Explanation of these observations have ascribed the problem caused by iron deficiency to the faulty granulocyte function, impairment of cellular response and a mucosal defect\(^5\).

   Mucosal atrophy has been related to mucocutaneous candidiasis. In one study 27 of 31 patients were deficient in iron and responded to iron treatment\(^6\). Our hypothesis of a relation between iron deficiency and cholera also depends on this mechanism. Chronic gastritis and gastric atrophy frequency occur in patients found to be iron deficient. A causal relation between these factors has not been established, in many cases the gastric improves with iron therapy.

   Ghosh et al\(^7\) showed decreased acid secretion in iron deficient individuals. Naiman\(^8\) et al found a mucositis and enteropathy associated with iron lack that improved remarkably with only iron treatment. Shearman et al\(^9\) reporting on 17 patients with moderate to severe iron deficiency observed that there are two groups of patients with gastric and iron
deficiency: One in whom the gastritis is dependant on the iron status and the other group in which the gastric atrophy has occurred independently of the iron status.

It is our hypothesis that in a poorly nourished population iron deficiency and the development cholera are related. The mechanism of this correlation is the development of gastritis and hypochlorhydria in iron deficient patients. The decreased gastric acid production compromises the acid barrier to Vibrio cholera and results in increased incidence of cholera in those who are iron deficient.

Studies of the epidemiology and pathogenesis of cholera support this concept of a relation between iron deficiency and cholera. First, the major groups affected by cholera are children under 10 and women of childbearing age. These groups are same as those with the highest prevalence of iron deficiency. Secondly, it has been shown that cholera occurs more frequently in patients with gastric hypoacidity. Acid in the stomach apparently protects against the development of cholera. This contention is supported by the observations that the administration of bicarbonate with a dose of cholera organisms greatly increases the chance of infection. In no study to this date has the cause of the gastric hyposecretion and atrophy found in cholera patients been identified. However, iron deficiency is well known to cause gastric atrophy and hypoacidity. The effect of iron deficiency on the acid secretory capacity of the mucosa can be profound and long lasting.

Because of these intriguing observations, a comparison of the iron status of cholera victims with matched controls is indicated. The hypothesis that iron deficiency causes increased susceptibility to cholera would be confirmed by finding lower hematocrits and ferritin levels in the test group as opposed to the comparison group.

B. Methods & Materials

The purpose of this study is to determine the relation if any, between iron deficiency and the occurrence of cholera in an individual. Two groups of adult male subjects, fifty in each group, will be studied. The first group will be patients diagnosed as having cholera and admitted to the ICDDR,B hospital
in Dhaka. The second will be apparently healthy individuals, matched for age, sex and socioeconomic status and recruited from the same household or neighbourhood of the index case preferably within a day or two of the cholera case and during the same period of the epidemic. Patients with a history of diarrhoeal disease within three months will be excluded from the control group.

The cholera patients will be accepted into the study consecutively as their diagnosis is confirmed by culture. Their socioeconomic status will be determined by asking for the family income number of members and education of the mother and that of the study subject.

The tests to be performed in all patients, the hematocrit and the ferritin, can be completed with blood obtained by fingerstick. Because infectious problems can interfere with the ferritin level, the optimal timing after the acute phase of cholera, for drawing the blood sample will need to be determined. To study this, a small sample of 5 patients with diagnosed cholera will have ferritin levels performed on admission and after 5, 10, 15 and 20 days. It is anticipated that the ferritin level will stabilize between 10 and 20 days after the resolution of the infection. The data from this preliminary study will guide the timing of the sample taking in study patients.

Testing Procedure:

Two tests will be done on all patients in the study--hematocrit and ferritin. The hematocrit will be performed using the standard micromethod. The ferritin will be determined using at ELISA method that allows accurate assays with only 50 to 70 lambda of serum. This amount of maternal can be obtained from one microhematocrit tube with a second tube being taken for duplicate determination. To allow for duplicate determinations a total of 4 microhematocrit tubes per patients will be used.

Interpretation of Tests:

Whether iron lack is a risk factor in the development of cholera is not known. From the comparison of the hematocrits and ferritin levels in cholera patients and controls it is hoped some relationship will emerge.
If there is not causal relation between iron deficiency and cholera there should be no difference in the prevalence of anaemia and average ferritin levels of cholera patients and controls. If on the other hand the hematocrit and ferritin levels in study patients are significantly lower than the controls it will point to a relation between iron deficiency and the occurrence of cholera.

D. Significance and Rationale:

The role of iron in infectious process in general, need clarification. There are conflicting reports indicating direct effect of iron on the immune system and bacterial replication 18,19,20,21,22. In cholera it is felt that neither of these mechanisms are active. Rather, the mechanism of the hypothesized association may be the decrease in the gastric acid production, that can occur with iron deficiency and which appears also to increase susceptibility to cholera infection.

Finding a causal relation between iron deficiency and cholera would be of great importance. Interventions including iron supplementation and fortification would assume a higher public health priority in endemic cholera areas. Such a relation would also encourage further research into the immunologic affects of iron lack and the role of iron in infectious processes.

E. Facilities Required:

ICDDR,B hospital facilities in Dhaka will be utilised for the study. The ICDDR,B clinical pathology laboratory will provide facilities for hematocrit and ferritin estimations. No animal source or new equipment will be required.

F. Collaborative Arrangements

Dr. M.A. Rashid, the Hematologist at IFGM & R has agreed to work on this study as a coinvestigator. Dr. James D. Cook of University of Kansas has agreed to perform the ferritin levels.
References


ABSTRACT SUMMARY FOR E.R.C.

1. Two groups of adult male subjects, 50 in each group will be studied. The first group will be patients diagnosed as having cholera and admitted to ICDDR,B hospital in Dhaka. Patients with complications e.g., fever, pneumonia, convulsion or any other associated illness will be excluded from the study. The control group will be subjects from same household of the first group matched for age, sex, socioeconomic conditions and not having cholera, excluded by rectal swab culture.

2. Except the mild pain associated with obtaining finger blood samples, there are no potential risks to the subjects.

3. All possible care will be taken and trained personnel will be used during fingerprick to cause minimal pain.

4. Only hospital identification numbers of the study patients will be used.

5. Informed written consent will be obtained from all study subjects.

6. The study will involve obtaining information about clinical history and socio-economic status by asking about family income, number of members in family and education level of the study subject. It will take less than 5 minutes the interview of the control subjects will be obtained at their respective homes.

7. The study patients will be treated in the hospital. All study subjects who show anaemia (from HCT determination) will be treated by proper therapy.

8. This protocol requires the use of hospital records and body flinds.
SECTION III - BUDGET

1. Personnel Services:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>% of effort</th>
<th>Dollar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. A.N. Alam</td>
<td>Principal Investigator</td>
<td>15%</td>
<td>$858.25</td>
</tr>
<tr>
<td>Dr. Paul Goff</td>
<td>Co-Investigator</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dr. M.A. Rashid</td>
<td>-&quot;-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dr. N.M. Abdul</td>
<td>&quot;-&quot;</td>
<td>25%</td>
<td>$181.18</td>
</tr>
<tr>
<td>Dr. M.M. Rahman</td>
<td>Consultant</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>To be named</td>
<td>Health Assistant (Male)</td>
<td>100%</td>
<td>$364.25</td>
</tr>
<tr>
<td></td>
<td>(Female)</td>
<td>100%</td>
<td>$364.25</td>
</tr>
</tbody>
</table>

Sub-Total: $1767.45 = Tk.44,175.00

2. Laboratory Test:

i) HCT                     Taka
ii) Ferritin                900
iii) Rectal swab culture    1575

3. Equipment:

Microhematocrit tube       1250

4. Hospital patient         7500

5. Out patient care         -

6. ICDDR,B Transport        14800

7. Printing and Reproduction 5000

8. Travel & Transport of person -

9. Transportation of materials (HCT tube for ferrition estimation) 5000

10. Medicines               1000

Sub-Total: Tk.29,465.00

Grand total = Tk.44,175 + 29,465 = Tk.73,640 = $2946 (2Tk.25/- = $1)

Incremental Cost = Tk.29,465 + 10% overhead cost = Tk.2947

Total: Tk.32,412 = $1296 (2Tk.25 = $1)
Consent Form

International Centre for Diarrhoeal Disease Research, Bangladesh is carrying on research on cholera. We are working on the relationship between cholera and anaemia.

We will examine your stool and a small amount of blood taken from your finger tip (this blood test will be repeated every 5 days for 4 times). These tests will not cause any harm to you. You will be treated if any abnormalities are found. We will also have to know about your family status.

Your co-operation will help us to know more about cholera. You will still be treated if you do not participate or if you withdraw from the study. If you agree, please put your signature/thumb impression below.

Signature: __________________________

Name: ________________________________

Date: ________________________________

____________________________________
Investigator
Consent Forms (for CONTROLS)

Recently one of the members of your family has suffered from cholera and was treated in our hospital. We are studying the relationship between cholera and anaemia. We would like to know whether as a member of this family, you have anaemia or not. For this, we will take a small amount of blood from your finger tip. This will not cause any harm. We will provide proper treatment if anaemia is found.

Your co-operation will help us to know more about cholera. You may or may not like to participate in this study. If you agree to participate, please put your Signature/Thumb impression below.

Signature: ____________________________

Name: __________________________________

Date: ________________________________

___________________________________
Investigator
সম্পৃক্ত পত্র

আনুষ্ঠানিক উদ্দেশ্যে গবেষণা কেন্দ্র করেরা রোগ সম্পর্কে গবেষণা চালিয়ে যাচ্ছে। আমরা করেরা রোগের সাথে রোগ শুধুমাত্র সম্পর্কের উপর গবেষণা করছি।

এই গবেষণার চেয়ে আশনির পায়েরনা ও অঙ্গগুলি থেকে শায়ান পরিমার রন্ধন নিয়ে শরীর করা হবে। (এই রন্ধন শরীর ৫ দিন পরে পর এরা ৪ বার করা হবে) এই সব পরিক্ষায় আশনির রোগ শরীর হতে হবে না। এছাড়া আশনির পরিবারিক সমস্ত ঘটনা দরকার। এই সব পরিক্ষায় কয়েক রোগ ধরা পড়লে তার চিকিৎসা দেওয়া হবে।

আশনির পরিমার্গী আমাদের করেরা রোগ সম্পর্কে আরো জানতে শায়ান করবে। এই পরিক্ষায় অংশগ্রহণ না করলেও অথবা পরে প্রত্যাহার করলেও আশনির চিকিৎসার কোন অবস্থা হবে না। এই গবেষণায় অংশগ্রহণ করতে রাত্রি থাকলে পৃষ্ঠ/টিপসই দিয়ে।

---------------
গবেষণার সূচনার পত্র

---------------
পৃষ্ঠ/টিপসই
নাম-------------------------------
তারিখ-------------------------
সম্বুচ্ছন্দ পত্র

শরীর আশ্চর্য পরিবারের একজন সদস্য কলরায় আগ্রহু হয়েছিলেন এবং আমাদের হাসপাতালে চিকিৎসা নাট করেছেন। আমারা রচনায় শুনা সাধারণ পারে কলরার রোগের কোন সম্পর্ক আছে কি না তা নিয়ে গবেষণা করছি।
এই পরিবারের সদস্য হিসেবে আপনার রচনা শুনা সত্যি আছে কি না তা দেখার জন্য আপনার আংশিক থেকে শায়ীনায় পরিমাণ রক্ষা স্বরূপ চন্দ্র নেওয়া হবে।
এই শরীর আশ্চর্য কোন কর্তিত হবে না। পরিমাণ রক্ষা স্বরূপ ধরা পড়লে আপাতক্ষেত্র চার জন্য যথাযথ ধর্ম দেওয়া হবে।
এই কারণে অপরূপ না করলেও আপনার কোন অসুবিধা হবে না।

আপনার সহযোগীতা আমাদের কলরার রোগ শুনকে আরো জানতে শায়ীনায় করবে। আপনি সম্বুচ্ছন্দ থাকলে শ্রদ্ধা/টিপসই দিন।

-----------------------------

gবেষকের শ্রদ্ধা

-----------------------------

শ্রদ্ধা/টিপসই

নাম_____________________

tারিখ____________________