ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 19.1.84

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ncipal Investigator Dr. A.N. Alam

lication No. 84-004(P)

le of Study IRON DEFICIENCY AND CHOLERA

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

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

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

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects (Yes) No
(c) Minors or persons under guardianship Yes No

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 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:
   N-A 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
   NA Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   NA 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.

ree to obtain approval of the Ethical Review Committee for any changes ving 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 $.1296.-

6. SCIENTIFIC PROGRAMME HEAD:

This protocol has been approved by the Nutrition Working Group.

Signature of Scientific Programme Head: M Lutfar Rahman

Date: 8/2/1984

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.

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SECTION II - RESEARCH PLAN

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 susceptibile 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. typhuminium[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 secretation 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[10]. These groups are same as those with the highest prevalence of iron deficiency[11,12]. Secondly, it has been shown that cholera occurs more frequently in patients with gastric hypoacidity[13]. 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[14]. 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[9,15,16,17].

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 occurrance 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 occurrance 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 the 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 IPGM & R has agreed to work on this study as a coinvestigator. Dr. James D. Cook of University of Kanasas has agreed to perform the ferritin levels.

References

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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, convalsion 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:

Name	Position		% of effort	Dollar
Dr. A.N. Alam	Principal Investigator	:	15%	$.858.-
Dr. Paul Goff	Co-Investigator	:	-	-
Dr. M.A. Rashid	-"-	:	-	-
Dr. N.M. Abdal	-"-	:	25%	$.181.-
Dr. M.M. Rahman	Consultant	:	-	-
To be named	Health Assistant (Male)	:	100%	$.364.-
-"-	-"- (Female)	:	100%	$.364.-

Sub-Total: $.1767.-
= Tk.44,175.-

2. Laboratory Test:	Taka
i) HCT	900
ii) Ferritin	-
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 Reporduction	5000
8. Travel & Transport of person	-
9. Transportation of materials (HCT tube for ferrition estimation)	5000
10. Medicines	1000

Sub-Total: Tk.29,465.-

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

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

Total: Tk.32,412
= $.1296 (@Tk.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

সম্মতি পত্র

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র কলেরা রোগ সম্বন্ধে গবেষণা চালিয়ে যাচ্ছে। আমরা কলেরা রোগের সাথে রক্ত শূন্যতার সম্পর্কের উপর গবেষণা করছি।

এই গবেষণার জন্য আপনার পায়খানা ও আংগুল থেকে সামান্য পরিমান রক্ত নিয়ে পরীক্ষা করা হবে। (এই রক্ত পরীক্ষা ৫ দিন পর পর আরো ৪ বার করা হবে) এই সব পরীক্ষায় আপনার কোন প্রকার ক্ষতি হবে না। এছাড়া আপনার পারিবারিক অবস্থা জানা দরকার। এই সব পরীক্ষায় কোন রোগ ধরা পড়লে তার চিকিৎসা দেওয়া হবে।

আপনার সহযোগীতা আমাদের কলেরা রোগ সম্বন্ধে আরো জানতে সাহায্য করবে। এই পরীক্ষায় অংশগ্রহণ না করলেও অথবা পরে প্রত্যাহার করলেও আপনার চিকিৎসার কোন অসুবিধা হবে না। এই গবেষণায় অংশগ্রহণ করতে রাজী থাকলে স্বাক্ষর/টিপসই দিন।

গবেষকের স্বাক্ষর

স্বাক্ষর/টিপসই

নাম----------

তারিখ----------

সম্মতি পত্র

সম্প্রতি আপনার পরিবারের একজন সদস্য কলেরায় আক্রান্ত হয়েছিলেন এবং আমাদের হাসপাতালে চিকিৎসা লাভ করেছেন। আমরা রক্ত শূন্যতার সাথে কলেরা রোগের কোন সম্পর্ক আছে কি না তা নিয়ে গবেষণা করছি। এই পরিবারের সদস্য হিসাবে আপনার রক্ত শূন্যতা আছে কি না তা দেখার জন্য আপনার আংগুল থেকে সামান্য পরিমান রক্ত পরীক্ষার জন্য নেওয়া হবে। এই পরীক্ষায় আপনার কোন ক্ষতি হবে না। পরীক্ষায় রক্ত শূন্যতা ধরা পড়লে আপনাকে তার জন্য যথাযথ ঔষধ দেওয়া হবে। এই কাজে অংশগ্রহণ না করলেও আপনার কোনরূপ অসুবিধা হবে না।

আপনার সহযোগীতা আমাদের কলেরা রোগ সম্বন্ধে আরো জানতে সাহায্য করবে। আপনি সম্মত থাকলে স্বাক্ষর/টিপসই দিন।

গবেষকের স্বাক্ষর

স্বাক্ষর/টিপসই

নাম----------

তারিখ----------