

Principal Investigator Dr. N.M. Abdal
 Application No. 83-019P
 Title of Study Serum Transferrin and Iron Status in Malnourished Children

Trainee Investigator (if any) _____
 Supporting Agency (if Non-ICDDR,B) 8

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).

1. 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:

- Umbrella proposal - Initially submit a 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.

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

N.M. Abdal
 Principal Investigator

 Trainee

83-019P
17/5/83

SECTION 1 - RESEARCH PROTOCOL

1. TITLE: "SERUM TRANSFERRIN AND IRON STATUS
IN MALNOURISHED CHILDREN"
2. PRINCIPAL INVESTIGATOR: Dr. N. M. Abdal
CO-INVESTIGATORS: Dr. H. Rahman, Dr. A. N. Alam
CONSULTANTS: Dr. Sultana Khanam, Dr. M. Mujibur Ral
3. STARTING DATE: May, 1983
4. COMPLETION DATE: October, 1983
5. TOTAL INCREMENTAL COST: US \$ 3625.00
(Staff commitment: US \$ 670.00
Operational cost: US \$ 2955.00)
6. SCIENTIFIC PROGRAM: This protocol has been approved by
the NUTRITION WORKING GROUP.

G. M. Molla
PROGRAM HEAD (acting)
Date: 28-4-83.

7. ABSTRACT SUMMARY:

Protein energy malnutrition is a major health problem in developing countries. Mortality due to it is very high where infection plays a significant role. Iron is said to enhance different types of infection. Studies have revealed that transferrin, a iron binding protein, is decreased in malnourished patients. So far no adequate study has yet been done to ascertain iron status in such patients.

This study will enable us to determine the iron status and rationalise the iron therapy in malnourished patients during nutritional rehabilitation.

A total of 75 children between 2-5 years of age of which 15 each with Kwashiorkor, Marasmic -Kwashirokor and Marasmus and 30 apparently healthy control subject will be selected for the present study. After resuscitation of malnourished children 1.5 ml of venous blood will be drawn for determining serum transferrin, serum albumin, serum iron and TIBC. The same amount of blood will be drawn from control group. The above tests will be repeated again for the malnourished group of patients after 1st and 3rd week.

8. REVIEW:

- (a) Research involving human subject: _____
- (b) Research Review Committee: _____
- (c) Director: _____
- (d) BMRC: _____
- (e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objective:

To estimate level of serum transferrin, serum albumin, serum iron and total iron binding capacity (TIBC) in different types of malnourished children in relation to status of iron and their changes during nutritional rehabilitation.

2. Background:

Protein Energy Malnutrition (PEM) is a major health problem in developing countries. Nutritional survey recently carried out in rural Bangladesh have shown that 18% of children less than 5 years of age suffer from 1st degree, 53% from 2nd and 26% from 3rd degree of malnutrition⁽¹⁾. Mortality due to PEM is very high, where infection plays a key role. Transferrin (siderophilin), a glycoprotein, Beta 1 globulin is a major iron binding protein which transports ferric iron in the blood and mediates iron exchange between body tissues. Its single polypeptide chain has two iron bindings sites, so that it may exist as apoferric, monoferric or diferric transferrin. Normally 20% to 50% of transferrin are saturated with iron, rest remains unsaturated ready for binding with iron. In iron deficiency anemia serum transferrin level is increased and percentage of transferrin saturation is decreased. Malnutrition and anemia are interlinked. But in Kwashiorkor serum transferrin is found to be very low⁽²⁾. Transferrin, like albumin, is synthesized in the hepatocytes and liver is one of the vital organs first affected by malnutrition.

There are many biochemical indices of nutritional status, so far various biochemical indices like ^{serum} amino acid, ⁽³⁻⁴⁾ serum albumin ⁽⁵⁾, and serum transferrin concentration ⁽⁶⁾ has been studied for diagnostic criteria. Transferrin is said to be one of the most accurate index for assessing the outcome from Kwashiorkor ⁽⁶⁾. Another study has shown that both prealbumin and transferrin could be useful for assessing nutritional status in surgical patients ⁽⁷⁾. Transferrin was found to be a reliable indicator for assessing marginal undernutrition ⁽⁸⁾. The body fluid of vertebrates contains only 10^{-18} M Fe^{3+} as ferric iron ⁽⁹⁾. Extremely low concentration of free iron in blood, lymph and external secretions such as bronchial secretion is due to presence of iron binding proteins ⁽¹⁰⁾. Transferrin in blood and lactoferrin in external secretions are normally only partially saturated with iron ⁽⁹⁾. There is plenty of iron in the body (about 4.5g for average man) ⁽¹¹⁾ but in normal individual it is locked up in proteins such as hemoglobin, myoglobin, ferritin and hemosiderin. The lack of readily available free iron in vivo prevents enhancement of bacterial growth ⁽¹²⁾. In blood concentration of transferrin is about 30 micro M, and that of Lactoferrin is about 0.01 micro M. But in external secretion the situation is reversed. Lactoferrin has a higher affinity for Fe^{3+} than transferrin ⁽¹³⁾ and unlike the latter, it can bind iron under acidic concentration ⁽¹⁴⁾. Ability of transferrin and Lactoferrin to bind iron is usually essential for anti-bacterial properties of body fluids. Since fresh

serum is an essential ingredient for tests of bacteriocidal properties, all such assays are done on partially saturated transferrin. The addition of enough iron to saturate the transferrin shows that the unsaturated iron binding capacity of serum has a bacteriostatic effect. If iron is added bacteriostatic properties are abolished and bacteria can grow. The loss of bacteriostatic properties is not due to interference with complement or lysozyme since saturation of transferrin does not prevent complement fixation or destruction of susceptible bacteria by lysozyme (15-16). It has been shown that Patients recovering from near starvation where transferrin saturation could be as high as 50 - 100% are unusually susceptible to malaria, and iron therapy in such increase the attack rate of malaria (17). Administration of iron to milk drinking African nomads to correct their dietary iron deficiency sharply increased their susceptibility to amebiasis. Thus it seems that competition for iron is a major factor in the battle between pathogenic organism and their host (19).

MacFarlane in his study has seen many malnourished children with low serum transferrin died immediately after treatment started when iron was one of the major drugs (20). Iron is said to enhance the growth of many bacteria. Such enhancement has been recorded for *Yersina pestis* (21) *Pasteurella multoacida* (*Pasteurella septica*) (22), *Clostridium perfringens* (*Clostridium welchi*) (23), *Listeria monocytogens* (24), *Vibrio cholerae* (25), *Neisseria meningitidis* (26), *Neisseria gonorrhoea* (27) *Salmonella typhimurium* (28), *Klebsiella pneumoniae* (29), *Clostridium septicum* (29), *Escherichia Coli* (30), *Pseudomonas aeruginosa* (31) *Erysipelothrix rhusiopathiae* (29).

More and more microorganisms are being included in the list.

In normal individual serum transferrin strongly binds free iron, reducing its amount below the requirement for bacterial growth. When serum transferrin goes down and most of the transferrin become saturated then free circulating iron may go up, it may reach to the amount required for bacterial growth.

All these evidences suggests that malnourished patients when have a low transferrin level and if free iron is increased due to any reasons then they may be susceptible to overwhelming infection and death may occur. In hospital a large number of malnourished patients are dying due to overwhelming infection of unexplained reason during course of treatment.

There is also a body of informations that infers that iron lack affects adversely macrophage functions that hampers defensive function of body. Adam et al ⁽³⁷⁾ studied South African infant with PEM showed that hemoglobin concentration significantly increased in those subjects who received iron and those who did not receive iron during nutritional rehabilitation

Adequate knowledge about the status of iron in different types of malnutrition and their changes during course of nutritional rehabilitation will give us a guideline of appropriate time of iron therapy in malnourished patients.

3. RATIONALE:

Majority of preschool children of developing countries, including Bangladesh suffer from PEM and increased mortality where infection plays a significant role. Adequate knowledge about iron in malnourished children is essential as iron is found to enhance bacterial growth.

The status of iron in relation to serum transferrin level and the time of iron therapy in these patients during nutritional rehabilitation will be seen. Transferrin as a prognostic parameter in different types of malnourished children will also be seen.

B. SPECIFIC AIM:

- (1) To see the status of iron in different type of malnourished children by ascertaining percent of unsaturated transferrin present in serum.
- (2) To see transferrin as a prognostic parameter in different types of malnourished children in nutritional rehabilitation.

C. METHODS OF PROCEDURE

The study will be carried out in 75 children, between 2-5 years, of which 45 from malnourished and 30 from normal healthy control. In malnourished group will comprise of equal no. of Marasmus, Marasmic Kwashiorkor and Kwashiorkor. The malnourished children having less than 60% (wt for age) and/or 70% (wt. for ht.) according to Harvard Standard will be selected. Then classification into Kwashiorkor, Marasmic kwashiorkor and Marasmus will be done by McLaren scoring⁽³²⁾ patient having any other organic disease will be excluded. Control subjects will be taken from RIHD and malnourished children from the Children Nutritional Unit in Dhaka.

1.5 mls of venous blood will be drawn for estimating serum transferrin, serum albumin, serum iron and total iron binding capacity (TIBC). The same test will be repeated for malnourished group after 1 and 3 week. The test for serum albumin⁽³⁶⁾, serum transferrin⁽³³⁻³⁴⁾, serum iron⁽³⁵⁾, and total iron binding capacity⁽³⁵⁾ will be done in ICDDR,B biochemistry laboratory. Percentage of of transferrin saturation will be calculated as follows:

$$\text{Percent Transferrin saturation: } \frac{\text{Serum iron (mg/dl)}}{\text{TIBC (mg/dl)}} \times 100$$

D. SIGNIFICANCE:

The result of this study will show the status of ^{serum}iron and TIBC different types of malnutrition and their acceptability of iron during disease process. This study will determine the proper time of ^{iron}acceptability during nutritional rehabilitation of malnourished patient.

E. Facilities required

- (i) No new office space is required.
- (ii) ICDDR,B. Biochemistry will be utilized for doing biochemical tests.
- (iii) Hospital resources: Malnourished patients will be selected from Children Nutrition Unit and control will be selected from RIHD Dhaka.

F. Collaborative arrangement

This will be a collaborative study between ICDDR,B and Children Nutritional Unit (CNU), Dhaka.

SECTION III - BUDGETA. Detailed Budget:

1. Personnel services:

<u>Name</u>	<u>Position</u>	<u>% of effort</u>	<u>Project requirements</u>	
			<u>Taka</u>	<u>Dollar</u>
Dr. N. M. Abdal	Principal Investigator	50%	8,624	360
Dr. A. N. Alam	Co-Investigator	10%	7,440	310
Dr. H. Rahman	M.O. (C.N.U)	-	-	-
Dr. M. Mujibur Rahaman	Consultant	-	-	-
Dr. Sultana Khanam	Consultant	-	-	-
Total Tk. 16064			US \$ 670	

2. Supplies and Materials:

	<u>Number</u>	<u>Unit cost</u>	<u>Total cost</u>
1. Disposable syringes (2.5 ml)	200	9.90	19.80
2. Hypodermic needles	200	3.90	7.80
3. <u>Laboratory test:</u>			
a) Total Iron binding capacity	170	2.3	390.00
b) Serum transferrin	170	7.4	1258.00
c) Serum Iron	170	2.3	390.00
d) Serum albumin	170	1.5	255.00

Sub Total US \$ 2320.60

Taka Dollar

3. Equipment

Nil

4. Hospital cost:

Nil

5. Out patient care:

Nil

6. ICDDR,B Transport: (6 miles X 50 days X 4.50)
Tk. 1800

1800.00

75.00

	<u>Taka</u>	<u>Dollar</u>
7. Rent, Communication and Utilities:	Nil	
8. Printing and Reproduction:	-	300.00
9. Travel and Transport of persons:	Nil	
10. Transportation of Things:	Nil	
11. Other contractual services:	Nil	
12. Construction, Renovation, Alterations:	Nil	

Total Taka US \$ 2695.60

B. Budget summary:

	<u>Taka</u>	<u>Dollar</u>
1. Personnel		670.00
2. Supplies		2320.00
3. Equipment		Nil
4. Hospitalization		Nil
5. Outpatient		Nil
6. Transport		75.00
7. Travel		Nil
8. Transport of Things		Nil
9. Rent		Nil
10. Printing		300.00
11. Contractual service		Nil
12. Construction		Nil

Total	US \$ 3365.60
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Total Incremental cost excluding personnel salary	US \$ 2695.60
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10% overhead cost	US \$ 260.00
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Grand Total =	US \$ 2955.60
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(1 US \$ = Tk. 24)

Total	US \$ 3365.60
10% overhead cost	US \$ 260.00
Total direct cost	US \$ 3625.60

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Abstract Summary for Ethical Review Committee

1. The objective of this study is to understand the status of iron in children of 2-5 years old of different degree of malnutrition and find out appropriate time of iron therapy during nutritional rehabilitation. A total of 75 children of which 30 will be taken as healthy control and rest from 3 different types of malnourished children. Children between 2-5 years will be taken as below 5 year children are most vulnerable to malnutrition and infection.
2. There is no potential risk involved in the study.
3. Not applicable.
4. All record will be kept strictly confidential, which will remain with the principal investigator. If data is put on computer tapes, study patients will be referred by code number.
5. Inform consent will be obtained from all the parents or authorized guardian of the children. There is nothing in the study which may unmask the privacy of the subject.
6. Interview will be taken only of dietary and family history related to the nutritional status of children.

7. The study children will be benefited in management by special care and biochemical tests. Adequate knowledge of iron status in malnourished patients will give great benefit for better management of malnourished patients.
8. The study will require blood test only. 1-5 ml of venous blood will be drawn in three occasions of 10 days interval from study children.

SCORING SYSTEM FOR PEM (AFTER MCLAREN)

	<u>Signs Present</u>	<u>Points</u>
WT/HT	- 80% with Oedema	5
WT/HT	_ 70 - 80% with Oedema	4
WT/HT	_ Below 70% with Oedema +	3
WT/HT	_ 70 -80% without Oedema	2
WT/HT	- Below 70% without Oedema	1
Oedema	+ Dermatosis (WT/HT not counted)	6
Hepatomegaly		1
Hair changes		1
Dermatosis		2
<u>TOTAL PROTEIN</u>		
Loss than	3.25g%	7
	3.25 - 3.99	6
	4.00 - 4.74	5
	4.75 - 5.49	4
	5.50 - 6.24	3
	6.25 - 6.99	2
	7.00 - 7.74	1

SCORE = Sum of points

0 - 3 = Marasmus

4 - 8 = Marasmic Kwashiorkor

9 - 15 = Kwashiorkor

CONSENT FORM

The ICDDR,B and CNU are jointly trying to find out better ways of treatment of malnutrition in children. There are many problems involved in treatment of malnutrition. One of which is correct way of nutritional rehabilitation according to requirement of body. Adequate knowledge about iron status in malnourished patient is essential for better management. We would like your child to participate in our study.

1. Your child will get proper treatment.
2. Only 1.5 ml of blood will be taken in three occasions for biochemical tests.
3. All nutritious food and drugs will be supplied on need.
4. Other investigations will be done on need.
5. Your child will be discharged when recovered completely. If necessary, medicine will be given at the time of discharge.
6. You can withdraw from the study at any time. Even if you do not participate the usual treatment of your child will not be hampered.

If you are willing to let your child participate in this study, then please put your signature of left thumb impression below.

SIGNATURE OF INVESTIGATOR: _____

SIGNATURE OF LEFT THUMB
IMPRESSION

DATE: _____

RELATION WITH PATIENT _____

অনুশীলন

আন্তর্জাতিক উদ্যোগ গবেষণাকেন্দ্র এবং ডি. এ. ইউ-
 স্ট্রীটের উক্ত চিকিৎসা উদ্যোগের জন্য যৌথভাবে কাজ
 করা হচ্ছে। সুস্থিহীনতার চিকিৎসার বিভিন্ন প্রকার
 আছে। তার মধ্যে একটি হচ্ছে অগ্নির প্রয়োগ অথবা
 সুস্থিহীনতার সঠিক উপায় উদ্ভাবন। লোকের সঠিক পরিচালনা
 করে জ্ঞান এবং জ্ঞান অতিথাকম্যকীয়। আমরা আমরা কবি
 মতের বৃদ্ধির প্রক্রিয়ায় আমরা আমাদের গবেষণায় অগ্র
 সূচক করব।

আপনি যদি বাকী থাকেন, তাহলে নিম্নলিখিত একক
 হবে।

১) আমাদের শিক্ষকে মর্মেত্তর চিকিৎসা দেয়া হবে।
 ২) পরীক্ষার বৃত্তি তিনবার করে ১৫ মিনিট বৃত্তি দেয়া
 হবে

৩) প্রযোজন অনুযায়ী সুস্থিকর খাদ্য এবং অমুখ দেয়া
 হবে

৪) অ্যান্ড প্রযোজনীয় পরীক্ষা করা হবে

৫) প্রযুক্তিগতভাবে কাজের পর আমাদের শিক্ষকে
 দেয়া হবে। প্রযোজন হলে অমুখও দিয়ে দেয়া হবে।

৬) গবেষণাচলনাকালীন যে কোন সময়ে আপনি
 আমাদের শিক্ষকে প্রত্যাহার করতে পারেন। এক্ষতকি আপনি
 দি গবেষণায় অগ্রসূহন করতে বাকী নাও হন তাহলেও
 সূচনার কোন সূচি হবে না।

আপনি যদি অগ্রসূহন এই গবেষণায় অগ্রসূহনী
 হতে বাকী থাকেন তাহলে নিচে আমাদের স্বাক্ষর
 এবং বৃদ্ধির প্রক্রিয়ায় ছাপ দিন।

আঞ্চলিক উদ্যোগ গবেষণাকেন্দ্র এবং সি এন ইউ- পুষ্টি
 উন্নত চিকিৎসা উদ্যোগের জন্য যৌথভাবে কাজ করে যাচ্ছে। পুষ্টি
 চিকিৎসার বিভিন্ন-সমস্যা আছে। তার মধ্যে একটি হচ্ছে প্রথম
 অনুযায়ী- পুষ্টিগত ক্রমিক পুনর্বাসনের উদ্যোগ উদ্যোগ। এ
 নই গবেষণায় পুষ্টিগত ক্রমিক ক্রমিক লেজের পরিমাপ- সম্মতি
 জানতে চান। এছাড়াও সুস্বাদুস্বাভাবিক স্বাস্থ্যের বাড়া
 মিক্সর বক্স লেজের পরিমাপ- সম্মতি জানা প্রয়োজন।
 আমরা আপনাদের মিক্সকে তুলনায় অন্য সুস্বাদুস্বাভাবিক স্বাস্থ্য
 মিক্স হিসাবে গবেষণায় অন্তর্ভুক্ত করতে চাই।

আমনি যদি বাকী- থাকেন তাহলে আপনার মিক্স
 ক্রমিক থেকে প্রায়শই পরিমাপে- (৩৫ মিলিলিটার) বক্স পরীক্ষার
 জন্য লেজা হবে। এই বক্স লেজার সময় যথাসম্ভব
 অবশেষে করা হবে এবং এতে আপনার মিক্সের ক্রমিক
 কোন ক্ষতি হবে না।

আমনি যদি আপনার মিক্সকে এই গবেষণায়
 পুনর্- করতে দিতে সম্মত হন তাহলে নিচে আপনার
 স্বাক্ষর কিংবা নাম হাতে বুড়া আঙ্গুলের ছাপ দিন।
 ধন্যবাদ।

গবেষণার স্বাক্ষর ৩
 তারিখ

বোম্বাই অডিটরদের স্বাক্ষর/ডি

CONSENT FORM

The ICDDR,B and GNU are jointly trying to find out better ways of treatment of malnutrition in children. There are many problems involved in treatment of malnutrition. One of which is correct way of nutritional rehabilitation according to requirement of body. In this study we want to know about iron status in malnourished children. For this we need to know about the status of iron in normal healthy Bangladeshi children under 5 yrs of age. We would like your child to be involved in this study as normal healthy control for comparison.

If you decide to participate in the study, we will take 1.5 ml of blood from vein of your child only on one occasion. This will be done with utmost precaution and will not be injurious to the health of your child.

If you are willing to let your child participate in this study, then please give your signature or left thumb impression below.

Thanks.

Investigator's Signature
with date

Guardian's Signature
or left thumb impression