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ETHICAL REVIEW COMMITTEE, ICDDR,B.
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Principal Investigator Dr Aysha Molk 100-10 Trainee Investigator (if any) 20
Registration No. 84-024(P) Supporting Agency (if Non-ICDDR,B) CNU

Title of Study Effect of zinc Supple-Project status:
mentation on the utilization of macro- (✓) New Study - Pilot
nutrients in children of Bangladesh () Continuation with change
() No change (do not fill out rest of form)

Give 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 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

- 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 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.

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

Aysha Molk 21 MAY 1984
Principal Investigator Trainee

SECTION I - RESEARCH PROTOCOL

1. TITLE : EFFECT OF ZINC SUPPLEMENTATION ON THE UTILIZATION OF MACRONUTRIENTS IN CHILDREN OF BANGLADESH
2. PRINCIPAL INVESTIGATOR : Dr. Ayesha Molla
- CO-INVESTIGATORS : Dr. A.M. Molla
Dr. Sultana Khanam
Dr. A.N. Alam
Mr. Akbar Ali
3. STARTING DATE : July 1984
4. COMPLETION DATE : ~~February 1985~~
December 1984
5. TOTAL DIRECT COST : US \$ 2584.00
6. SCIENTIFIC PROGRAMME HEAD : This protocol has been approved by the Nutrition Working Group.

SIGNATURE OF THE PROGRAMME HEAD :

M. M. Rahman

DATE:

17/5/1984

ABSTRACT SUMMARY

This study will be carried out in the Children Nutrition Unit (Eskaton), utilizing the same research subjects of protocol #84-012-P, recently approved by the Ethical Review Committee of ICDDR,B and CNU. Thirty children aged between 2 and 3 years, completely weaned, with severe degree of malnutrition, without acute diarrhoea and symptoms of systemic disease will be selected to study the effect of zinc supplementation on the absorption of macronutrients.

Seventy-two hour balance study will be done during zinc supplementation as per protocol #84-012-P, 3 weeks after initial recovery when body weight increases steadily. Half of the children will receive 5 to 10 mg/kg/day of zinc sulphate, made into syrup and the other half matched for nutritional status and age will receive placebo. Usual practice of feeding regimen and vitamin supplementation by CNU will be kept unchanged. Effect of zinc on absorption of nutrients will be compared between this two groups at the 3rd week of rehabilitation. This study will provide information whether zinc supplementation improves absorption of nutrients from G.I. tract. If this is found useful, it will help us in formulating a better diet for malnourished children.

SECTION II - RESEARCH PROTOCOL

A. INTRODUCTION

1. Objectives - The overall objective of this protocol is to study the impact of zinc supplementation on the utilization of nutrients in severely malnourished children during nutritional rehabilitation.

2. Background - The importance of zinc in the nutrition of animal and human diet has been well recognized (1). Deficiency of zinc causes growth retardation, anorexia, diarrhoea, dermatitis, mental lethargy and hypogonadism in males (2-3). Abnormal dark adaptation in alcoholic cirrhotics has been found to be related to deficiency of zinc (4). Zinc administration to these patients corrected the abnormal dark adaptation. It was proposed that the effect of zinc on the retina probably is mediated by an enzyme, retinal reductase, which is known to be zinc-dependent (5).

Some patients with celiac disease who failed to respond to diet, steroids or nutritional supplements, recovered completely after administration of zinc. They also gained weight and their d-xylose absorption test and steatorrhea improved following zinc therapy (6). Malabsorption syndromes other than celiac disease were also known to be improved after zinc supplementation (7). Therefore, one should be aware of the occurrence of zinc deficiency in malabsorption syndromes. The activities of zinc dependent enzymes have been found to be affected adversely in zinc-deficient tissues. Three enzymes, alkaline phosphatase, carboxypeptidase and thymidine kinase were found to be the most sensitive enzymes and their activity reduced sharply with simultaneous institution of a zinc deficient diet to experimental animals (8-9). Zinc is known to be an important constituent of several metalloenzymes (10-11), which participate in many metabolic processes including carbohydrate, lipid, protein, and nucleic acid synthesis. In other words it is natural to speculate that the level of zinc in human body controls the physiological

processes through the formation and regulation of zinc dependant enzymes.

Zinc content of a normal person varies from 1.5 to 2.0 gm (12). Plasma zinc is mostly present as bound to albumin, α_2 -macroglobulin, transferrin, ceruloplasmin, haptoglobin and γ -globulin (13-14). Absorption of zinc from dietary source varies from 20 to 30 percent. Exact mechanism of absorption from the intestine is not clearly known yet. Absorption of zinc is known to be variable and depends on variety of factors. Zinc is better absorbed from animal protein and quantity of absorption depends on the body size, level of zinc in the diet and presence of phytate, fiber and other chelating agents in the diet. Also, intestinal zinc absorption appears to vary from individual to individual and depends on the nutritional status of the individual. Absorption was found to be high in persons with poor nutritional status. The risk of zinc deficiency in growing children is particularly high when they are fed with inadequate formula diet, diet deficient in animal protein and during diseased condition like diarrhoea. In one of the studies, carried out in ICDDR,B serum zinc levels ($\mu\text{g}\%$) were estimated in children (0 to 10 years) with diarrhoea due to all aetiology. Significantly lower concentration ($p < .001$) of serum zinc (55.3 ± 28) was obtained in the diarrhoeal children compared to the zinc level (93.6 ± 27) of normal age-matched control children (15). In India also, it was (16) observed, that plasma zinc levels varies from $41.3 \pm 1.5 \mu\text{g}\%$ to $56.1 \pm 5.7 \mu\text{g}\%$ in children suffering from kwashiorkor and marasmus respectively. These values were significantly lower compared to the level of $102 \pm 4.7 \mu\text{g}\%$ in normal children.

Golden and Golden studied the effect of zinc supplementation in 16 children recovering from severe malnutrition. They demonstrated that the children with low levels of plasma zinc achieved a definite

increase in their rates of weight gain when they were supplemented with zinc. It was concluded that children undergoing a period of "catch up" weight gain or growth should have supplemental zinc (17). However, in this study milk based diet and soya based diet were used for nutritional rehabilitation of the children. In the present study and the previously approved study (84-012-P) we will be using a known familiar diet as practiced in the CNU which might contain a minimal quantity of zinc. However, while feeding this diet Dr. Saleha Hossain (18) obtained significantly low levels of serum zinc and copper in all three types of PEM (marasmus, kwashiorkor, marasmic kwashiorkor) compared to 50 age matched healthy controls. Even after nutritional rehabilitation the serum levels of zinc and copper did not reach the normal level. In an analysis of 2136 hospital PEM cases Dr. Sultana (19) also did not find any correlation between higher energy intake (200 kcal/day) and rate of weight gain during recovery stage. Presence of zinc and copper deficiency in these children were thought to be the limiting factors. The present study intends to estimate quantitatively the absorption of nutrients while supplementation with zinc during later stage of rehabilitation. Protocol #84-012-P will provide informations with regard to intake of food and rate of weight gain during zinc supplementation and the present study will provide an additional information with regard to absorption of nutrients.

3. Rationale - Increased energy consumption is a prerequisite to nutritional rehabilitation. It is known that zinc deficiency causes anorexia and possibly compromises the absorptive function of intestine. Bangladeshi children with protein calorie malnutrition and probably with marginal zinc level, it would be highly beneficial to supplement food with zinc. The proposed study intends to estimate the effect of zinc supplementation on absorption of nutrients in children recovering from malnutrition.

B. SPECIFIC AIMS

Estimate the absorption of nutrients (fat, nitrogen, calories and carbohydrates) after zinc supplementation and compare the absorption pattern with the placebo group, during the 3rd week of rehabilitation from PEM.

C. METHODS AND PROCEDURES

1. Thirty children will be studied, 15 supplemented with zinc (5-10 mg/kg/day) as sulphate and 15 without zinc but with other supplements and regular CNU diet (age and nutritional status matched controls).
2. Malnourished children aged between 2 to 3 years and completely weaned from the breast milk will be selected.
3. Nutritional status will be determined by weight for height percentage of Harvard standard and presence or absence of oedema (ref. Protocol #84-012-P). Those with weight for height less than 70% or in case age is accurate weight for age less than 60% will be selected.
4. Children not having surgical injury, renal failure, liver diseases, tuberculosis or patients needing blood transfusion will be excluded from the study.
5. On admission, only one blood sample (3 ml) will be withdrawn according to the requirement of protocol #84-012-P. 3 ml blood sample will be needed for blood culture, TC, DC, Hct, haemoglobin and total plasma protein, results will be used for management of the patients.

6. Besides zinc, all the other vitamin supplements and dietary regime from CNU will be kept unaltered in this part of the protocol.
7. As written in protocol #84-012-P, zinc supplementation will begin from the 3rd week after initial resuscitation, a period when weight gain increases steadily. A 72 hour metabolic balance study will be done in the CNU unit from the 3rd day of zinc supplementation. The balance study procedure will be the same as was done before, in ICDDR,B Metabolic Ward (20). Similar study procedure will be followed for the control groups also.
8. All the stools and urines will be collected between the two charcoal markers from both groups of study patients and fat, nitrogen, carbohydrate and calories will be estimated.
9. Food samples will be collected also for estimation of all the macronutrients and zinc.
10. Quantitative intake and output of nutrients will be calculated and co-efficient of absorption will be determined.
11. The results between the two groups will be compared statistically using paired 't' test.
12. Correlation between weight gain and absorption of nutrients will be done.

D. SIGNIFICANCE

So far no such study was done to estimate quantitatively the effect of zinc supplementation on absorption of nutrients. In view of the fact that zinc is essential for maintenance of body metabolism, control of infection and have better immunity, it will be highly significant to be able to know the role of zinc in utilization of nutrients during rehabilitation from malnutrition. The information will also be helpful in fulfilling the long term objective for the Nutrition Working Group, i.e. control of malnutrition in Bangladeshi children.

E. FACILITIES REQUIRED

1. Office space is adequate.
2. Since this is a collaborative project between CNU and ICDDR,B, available facilities for routine microbiology, biochemistry and pathology will be utilized in both the places according to our mutual understanding.
3. For statistical analysis, the help of ICDDR,B Statistics Branch will be utilized.
4. Study patients will be the same as selected for protocol #84-012-P and study will also be carried out in the CNU hospital. In case of necessity, our metabolic study trained nursing staff will spend time in the CNU for collection of food, stool and urine during the study period.

SECTION III BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

	Position	Percent of Effort	Project Required	
			Tk.	US\$
Dr. Ayesha Molla	Asso. Scientist	20% 6 mo.	49,410.00	-
Dr. A.M. Molla	Scientist	5% 2 "	-	511.00
Dr. Sultana Khanam	CNU Med. Director	-	-	-
Dr. A.N. Alam	Asso. Scientist	5% 1 "	-	150.00
Mr. Md. Akbar Ali	Head, Biochemistry	10% 6 "	-	300.00
A Physician from CNU	-	-	-	-
Ms. Makhduma Khatun	Research Officer	10% 6 "	3,861.00	-
Mrs. Naseha Majid	Res. Dietician	5% 3 "	8,076.00	-
			<u>61,347.00</u>	<u>981.00</u>
			=====	=====

2. SUPPLY & MATERIALS

Blood culture and
Routine investigations
will be done in the
CNU according to the
requirement of Proto-
col No. 84-012-P

Stationery goods	500.00	-
Xeroxing and mimeographing	500.00	-
Medical Illustrations	500.00	-
PVC bags	-	200.00
Miscellaneous	500.00	-
Transportation of things and patients	500.00	-
Rent, Communications, Utilities	-	-
Printing & Publication	1,000.00	-
	<u>3,500.00</u>	<u>200.00</u>
	=====	=====
	\$ 140.00	

3. BIOCHEMICAL ANALYSIS

	<u>No. x Cost/test (\$)</u>		
Kjeldahl N ₂	40 x 7.6	-	304.00
Fecal FAT ₂	40 x 41	-	1640.00
Calorific Value	40 x 5	-	<u>300.00</u>
			2244.00

Zinc

No new test will be needed as
these will be done for
protocol # 84-012-P.

nil.

Pre-albumin

"

Retinol binding protein

"

Ceruloplasmin

"

Total costs for supplies and assays only

US \$ 2244 + 340 = 2584

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CONSENT FORM

Children Nutrition Unit and ICDDR,B are working jointly to improve the treatment of malnourished children. Previously it was found that during rehabilitation severely malnourished children do not gain weight as rapidly as they should even though their daily intake exceeds the requirements. It was anticipated that presence of zinc deficiency in these children could be the reason for this slow process of weight gain. Thus we would like your child to participate in the following research.

1. After admission during the first 2 weeks your child will receive proper treatment for malnutrition and associated infection.
2. The present study and the previously written study (84-012-P) will be done simultaneously in the same patients. Therefore, new blood samples will not be needed.
3. Usual feeding schedule of CNU and medicines needed will be given regularly.
4. From the third week zinc (5-10 mg/kg/day) syrup will be fed till discharge from the hospital.
5. For 72 hours, all urine, stool and food will be preserved for laboratory analysis.
6. When your child has recovered completely, will be allowed to go home.
7. At any time you can withdraw your child from this study. For this reason usual treatment will not be disturbed.

If you wish to allow your child to participate in this study, please give your signature below.

Signature of the Investigator

Signature or thumb impression
of the guardian

Date

Date

ଅନୁକ୍ରମିକ ଉପଲବ୍ଧ - ମାଧ୍ୟମ - ଡକ୍ଟର ଏଡ଼ି, ସି.ଏମ. ୧୫ ବିଷୟ-
ଧର - ସୂଚିତ ହେଉଅଛି - ବିଭିନ୍ନମାନ ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ - ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ-
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ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ କ୍ଷେତ୍ର କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ -

ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ କ୍ଷେତ୍ର କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ -
ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ କ୍ଷେତ୍ର କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ -
ଡକ୍ଟରଙ୍କ କାର୍ଯ୍ୟ କ୍ଷେତ୍ର କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ କାର୍ଯ୍ୟ -