Library A-000024

Attachment 1.

Date 20/6/95

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. M.A. Khaled
Dr. G. Fuchs

Trainee Investigator (if any) 69

Application No. 95-014

Supporting Agency (if Non-ICDDR,B)

Title of Study The effect of antioxidative nutritional intervention on clinical outcome of acute shigellosis

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
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, (hospitnl, 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
4. 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 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)
   NA 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.

(PTO)

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

M. Ab. Khaled & G. Fuchs
Principal Investigator

Trainee

A-032001

RESEARCH PROTOCOL

TITLE: The effect of antioxidative nutritional intervention on clinical outcome of acute shigellosis

Principal Investigators :

Dr. M.A. Khaled
Senior Advisor
Clinical Sciences Division
ICDDR,B

Dr. George Fuchs
Actg. Divisional Director
Clinical Sciences Division
ICDDR,B

Co-investigators :

Dr. Iqbal Kabir
Scientist
Clinical Sciences Division
ICDDR,B

Dr. Mohammed Ali
Senior Medical Officer
Clinical Sciences Division
ICDDR,B

Starting date : September 1, 1995

Completion date : 2 years after start

Total budget requested : US $ 210,476

Funding source : USAID/AUSAID

Programme Head :

Dr. George Fuchs
Actg. Divisional Director
Clinical Sciences Division

Abstract summary

Shigellosis is a major cause of death in children in developing countries. Approximately 300 million children suffer from the disease, of whom about 600,000 die. Many of those who survive an episode of shigellosis go on to develop malnutrition. Supplementation with megadose Vitamin A in these children has not been found to improve their Vitamin A status, as febrile and infective conditions are associated with a higher urinary excretion of the vitamin. More importantly, it is speculated that higher oxidative stress in these children lead to vitamin A depletion, as vitamin A is highly sensitive to oxidative stress. Studies conducted at ICDDR,B have shown that children attain a rapid catch-up growth after recovery from shigellosis if fed on a high-protein diet. The vitamin A status of these children on long-term follow-up was also normal, even without megadose supplementation with the vitamin. It is hypothesized that albumin, an effective antioxidant, present in the high-protein diet, could be responsible for the improved vitamin A status.

Beta-carotene, a vitamin A precursor, is an effective antioxidant whose absorption in the human body is facilitated by vitamin E. Vitamin E, itself another antioxidant, provides antioxidative protection to beta-carotene. As vitamin A helps in preventing infections and promoting growth in children, it is important to investigate whether antioxidative intervention with beta-carotene and vitamin E helps in improving the clinical outcome of acute shigellosis, as well as improve vitamin A nutriture and promote growth in children. This is the rationale behind the proposed study.

The study will be conducted in children aged 18-60 months presenting with shigellosis at the Dhaka Hospital of the ICDDR,B. Children will be randomly assigned to one of 4 dietary interventions and kept in the hospital for 7 days during which their clinical course and vitamin A and oxidative stress parameters will be monitored. The diets tested will be (1) normal hospital diet (2) a protein-enriched diet (3) the hospital diet supplemented with beta-carotene and vitamin E and (4) the protein-enriched diet supplemented with beta-carotene and vitamin E. The outcome variables that will be measured are: (a) clinical- duration of illness, fever, character and number of stool motions, and absence/presence of straining and tenesmus (b) body composition-weight, height, skin fold thickness at triceps, biceps, subscapular and suprailiac levels, bioelectrical impedance assays and (c) biochemical- serum estimations of vitamin A, vitamin E, beta-carotene, and markers of oxidative stress such as TBARS and GSSG.

Hypotheses:

Since oxidative stress is highly prevalent in the gastrointestinal tract during infections, it is hypothesized that:

1. antioxidative intervention will improve the vitamin A status

2. antioxidative intervention during acute shigellosis will improve clinical outcome (reduce duration of illness)

3. antioxidative intervention during acute shigellosis will enable improved growth (weight and linear gain)

4. antioxidative intervention during acute shigellosis will be associated with decreased oxidative stress (reduced levels of TBARS and GSSG)

Objectives:

1. To assess the effects of the antioxidative micronutrients, beta carotene and vitamin E (alpha-tocopherol), on the clinical course of acute shigellosis in children and on their vitamin A status after clinical recovery

2. To observe the above effects with and without high protein energy diet supplementation

3. To determine the effect of antioxidant supplementation on growth of children recovering from acute shigellosis.

Significance of the study

The study will answer three important questions relating to childhood nutrition and infections. One, whether antioxidant supplementation will improve clinical outcome in children with acute shigellosis. Two, whether antioxidant supplementation will result in improved growth and vitamin A nutriture and whether this effect is dependant on a high-protein diet. Three, baseline information on the levels of oxidative stress in children with acute shigellosis can be determined.

If the study demonstrates the effectiveness of antioxidants in infection and nutrition, this could spell a major development in the field of child survival. Antioxidants could then be routinely used in vitamin A and nutritional rehabilitation programmes and in the clinical management of shigellosis.

Ethical considerations

There are no ethical concerns with respect to the interventions. Even if the antioxidant supplementations proved out to be of no benefit, the dietary interventions themselves are expected to be beneficial in point of energy and/or protein intake.

As study patients are usually more closely monitored than normal inpatient children, a high degree of clinical care will be ensured. Normal hospital guidelines will be strictly adhered to with regard to management of the shigella cases. A total of 4 ml of venous blood will be collected for biochemical assays during the duration of hospital stay of each patient. Finally, informed parental consent will be obtained prior to recruitment of each subject.

Background:

Shigellosis is a major cause of diarrhoeal disease in underdeveloped countries. Although malnourished populations are more prone to this bacterium, it also induces malnutrition particularly in children unless adequate dietary management is followed. Recently, Kabir et al (1) have demonstrated rapid catch-up growth in children receiving a high-protein diet after their recovery from shigellosis. It has also been observed that the serum level of vitamin A in these children reached normal values in 3 to 4 weeks, without even administering a megadose supplementation of this vitamin. In contrast, children who received a megadose vitamin A supplementation without high protein energy intake did not show any change in their serum vitamin A level, i.e. remained vitamin A depleted (2). Protein-energy supplemented children had higher levels of serum albumin which is considered one of the most effective extracellular antioxidants (3). Oxidative stress concomitantly was found to be considerably less in these children (4) whose level of vitamin A reached the normal value. Infections and febrile conditions usually enhance oxidative stress (5). Recently it has been reported that vitamin A excretion through urine is maximized by febrile conditions (6). It is therefore speculated that higher oxidative stress in malnourished children, particularly in those with infections, might be a causal factor in vitamin A depletion since this vitamin is highly sensitive to oxidation. Vitamin A, on the other hand, is intimately involved in the cellular proliferation required for growth and normal function of mucosal epithelium in addition to other important biological functions like vision, immunity etc (7).

The provitamin A carotenoids, particularly beta-carotene, produce vitamin A in the human body and are also effective antioxidants (8). It is of interest that vitamin E facilitates the absorption of vitamin A and also provides antioxidative protection to it (9). It appears therefore that a combined dose of beta-carotene and vitamin E may be highly beneficial in enhancing/improving vitamin A nutriture in human body. Since it is well accepted that vitamin

A helps in preventing infections and promoting growth in children, it is important to investigate whether antioxidative nutritional intervention with beta carotene and vitamin E would be able to reduce duration of disease course caused by shigellosis and promote catch-up growth, possibly by enhancing vitamin A status in these children. A study design, as described in Methods, is therefore developed in order to examine this hypothesis.

Methods:

Study design

A clinical trial will be conducted on children with *shigella* dysentery who will be hospitalized for 7 days in a metabolic ward. The children will be randomly assigned to one of 4 dietary groups, as mentioned below, and their clinical, body composition and laboratory parameters observed over the next 7 days. The patients will be followed up 3 months after discharge to monitor their growth.

Subjects

Children aged 18-60 months with a history of bloody diarrhoea for 3 or less than 3 days will be recruited into the study after informed parental consent has been obtained. The following exclusion criteria will be applied:

a) Kwashiorkor or a weight-for-age of less than 60% of the NCHS median

b) The presence of any serious systemic conditions viz: pneumonia, suspected septicaemia, meningitis, tuberculosis

c) The presence of any complications of shigellosis viz: lethargy due to presumed hyponatraemia or hypoglycaemia, abdominal distension, history of oliguria/anuria, pallor, history of convulsion.

Randomization

After recruitment, each patient will be randomly assigned to any one of the following 4 dietary intervention groups:

Group 1: Controls receiving normal hospital-based diet (150 kcal/kg/d, protein 2.5 g/kg/d)

Group 2: A high-protein diet that delivers 150 kcal/kg/d and 5 g of protein/kg/d, such that 15% of the total dietary energy is obtained from proteins. The protein used will be vegetable-based, as animal proteins by dint of their antioxidant content may confound the study results.

Group 3: Group 1 plus beta carotene (1 mg/kg/d) and vitamin E (5 mg/kg/d)

Group 4: Group 2 plus beta carotene (1 mg/kg/d) and vitamin E (5 mg/kg/d)

Sample size calculations:

The assumption is made that 90% of patients supplemented with antioxidants will have earlier recovery as compared to 50% receiving the control diet. At a significance level of 0.05% and a 90% power to detect a difference of this magnitude amongst the 4 diet groups, 22 patients in each group or 88 for the whole study will be required. Assuming a drop-out rate of around 10-12%, a sample size of 100 patients will be sufficient for the study.

Measurement variables

Clinical:

On admission, a detailed clinical history and physical examination will be carried out by one of the investigators. Clinical progress and outcome will be measured daily during the morning rounds in terms of the duration of illness, abdominal pain, rectal temperature, straining and tenesmus, stool character, and frequency of stool motions.

Body composition (Growth monitoring):

The following measurements will be carried out on admission, on discharge 7 days later and on follow-up 3 months after discharge:

a) Body weight
b) Height
c) Mid upper arm circumference
d) Skin fold thickness at the triceps, biceps, subscapular and suprailiac regions

Bioelectrical impedance analysis (BIA) will also be carried out on the same days using BIA analyzer (RJL system, MI, USA) to determine the body composition. The BIA method is well established in our metabolic laboratories (10).

Laboratory:

On admission, a stool microscopy and a rectal swab (R/S) culture for cholera, shigella and salmonella will be carried out on each patient. Blood (2 ml) and urine (5 ml) samples will also be collected for biochemical assays on admission and at discharge 7 days later.

Biochemical assays:

Both blood and urine samples will be utilized for the biochemical assays. About 2 ml of venous blood will be collected in heparinized tubes. Erythrocytes will be collected from 200 microlitres of the heparinized blood. The erythrocytes will be washed with 2 volumes of 154 mmol/L NaCl solution at pH 7.4 and afterwards will be haemolysed with a two-fold volume of distilled water. Total glutathione (GSH + GSSG), GSH and GSSG separately will be determined by the glutathione reductase [(GRX)-(5,5'-dithiobis-(2-nitrobenzoic acid)] (DTNB) assay of Di Simplicio and Mannervik (11). The ratio of GSSG to GSH is expected to give the differential measure of oxidative stress before and after the supplementation.

Vitamin A, vitamin E and beta-carotene will be estimated from the serum by using HPLC method. Both serum and urine samples will be assayed for nitric oxide which would provide additional information on the extent of oxidative stress. Vitamin A excretion in urine will also be assessed by the HPLC method. As reported earlier (6), vitamin A excretion in urine due to infection and/or oxidative stress could be evaluated by measuring the level of vitamin A in urine before and after the supplementations.

Both the serum and urine samples will be used to determine TBARS as reported previously (12). Briefly, TBA reagent consisting of 0.375% thiobarbituric acid (TBA) and 15% trichloroacetic acid (TCA) will be made in 0.25N HCL. Half ml of serum will be added with 10 microlitre of 2% pyrogallol, a highly water soluble antioxidant, in order to prevent any peroxidation during treatment with 1 ml of the TBA reagent for 15 minutes. The sample will be brought to room temperature and centrifuged at 4000 rpm for 7 minutes. The supernatant will be used to measure the UV absorption at 532 nm. The appropriate extinction coefficient value will be used to calculate the amount of TBARS in micromol/litre.

Clinical management:

All study patients will be given the standard hospital management for shigellosis. This includes antibiotic therapy with nalidixic acid for five days, beginning from the day of admission. If the antibiogram from the R/S suggests otherwise, or if the illness is clinically severe, pivmecellinum will be used instead of nalidixic acid. If the clinical condition of the patient suggests any risk of haemolytic anaemia, acute renal failure, hyponatraemia or toxic megacolon, appropriate management measures in accordance with established hospital guidelines will be undertaken.

Data analysis:

The SAS computer software package will be used for data analysis. The significance of differences between means of the 4 groups (viz: rectal temperature, frequency of stool motions, duration of illness) will be tested with ANOVA and Student's t test. Where appropriate, the chi-square and Fisher's exact tests will be used to test the significance of differences between categorical variables. The Kaplan-Meier method and the log-rank test will be used to test the significance of differences between the 4 intervention groups.

Flow chart of research activities

Organization of the study

1. Staff recruitment: 1 month [September 1-30, 1995]

2. Training of staff: 1 month [October 1-30, 1995]

3. Pretesting: 1 month [November 1-30, 1995]

4. Actual study:

   a) Dietary intervention- 100 patients. Average 2 patients per week- 1 year [December 1, 1995- November 30, 1996].

   b) Follow-up of last patient- 3 months after last recruitment-February 30, 1997

5. Data entry and analysis: 3 months [March 1, 1997-May 30, 1997]

6. Report writing: 3 months [June 1, 1997-August 30, 1997]

References:

1. Kabir I, Malek MA, Majumder RN et al. Rapid catch-up growth in children fed a high protein diet during convalescence from shigellosis. Am J Clin Nutr 1993;57:441-445

2. Khaled MA, Kabir I, Wahed MA et al. Oxidative stress and antioxidant: implications on vitamin A status in malnourished children. Vitamin A workshop, Oct 31, 1994, International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

3. Halliwell B. Commentary: Albumin-an important extracellular antioxidant? Biochem Pharmacol 1988;37:569-571.

4. Khaled MA. Editorial Review: Oxidative stress in childhood malnutrition and diarrhoeal diseases. J Diarrhoeal Dis Res 1994;12:165-172.

5. Sies H. Oxidative stress: Oxidants and antioxidants. Acad. Press, 1991

6. Stephensen CB, Alvarez JO et al. Vitamin A is exreted in the urine during acute infection. Am J Clin Nutr 1994;60:388-392

7. West KP, Howard GR, Sommer A. Vitamin A and infection: Public Health implications. Annu Rev Nutr 1989;9:63-86.

8. Drapper HH. Nutritional modulation of oxygen radical pathology. Adv Nutr Res 1990;8:119-125.

9. Olson JA. Vitamin A, retinoids and carotenoids. In: Modern Nutrition in Health and Disease, 8th edition, (Shils ME et al eds) Lea and Febiger, 1994;pp 287-307/

10. Kabir I, Malek MA et al. Changes in body composition of malnourished chidren after a dietary supplementation as measured by bioelectrical impedance. Am J Clin Nutr 1994;59:5-9

11. Di Simplicio P, Mannervik B. Enzymes involved in glutathione metabolism in rat liver and blood after carbon tetrachloride intoxication. Toxicol Lett 1983;18:285-289

12. Khaled MA, Kabir I, Mahalanabis D. Effects of protein-energy supplementation on oxidative stress in malnourished chidren. Nutr Res 1995 (in press).

BUDGET PROPOSAL

PROJECT TITLE : Antioxidative nutritional intervention during acute Shigellosis

NAME OF DONOR : USAID/AUSAID

STARTING DATE : Sept. 1, 1995

CLOSING DATE : 2 yrs after start

PROJECT DURATION : 2 years from starting

NAME OF P.Is. : Dr. M.A. Khaled
Dr G. Fuchs

RRC APPROVAL DATE :
ERC APPROVAL DATE :

Line item	1st year	2nd year	TOTAL
PERSONNEL LOCAL: SALARIES			
Dr. M.A. Khaled, PI, 25%	22,050	24,255	46,305
Dr. G. Fuchs, Co-PI, 10%	10,000	11,000	21,000
Dr I. Kabir, Co-Invest., 25%	6,000	6,600	12,600
Dr Mohammed Ali, Co-Invest, 50%	7,000	7,700	14,700
Health Assistant (1), 100%	4,400	4,840	9,240
Lab. Assistant (1), 100%	4,400	4,840	9,240
Sub-total:	53,850	59,235	113,085
SUPPLIES & MATERIALS			
-Drugs/medical supplies	1,000	1,100	2,100
-Office Supplies	1,000	1,200	2,200
-Others	1,500	1,000	2,500
Sub-Total	3,500	3,300	6,800
OTHER CONTRACTUALS			
-Postage, fax, phone, DHL etc.	500	500	1,000
-Printing & publications	100	200	300
Sub-total:	600	700	1,300
INTER DEPARETMENTAL SERVICES			
-Medical Illustration	100	100	200
-Xerox, Libarary Service	200	200	400
-Lab. and Pathological test	10,000	9,384	19,384
-Patient hospitalization (@$20 x 100 pts x 7 d)	8,000	6,000	14,000
Sub-total:	18,300	15,684	33,984
CAPITAL EXPENDITURE: Computer & accessories	5,500	0	5,500
TOTAL DIRECT COST:	81,750	78,919	160,669
Overhead (31%)	25,343	24,465	49,807
TOTAL PROJECT COST	107,093	103,384	210,476

11.6.95

CONSENT FORM

Short title: Antioxidants in shigella study

Your child is suffering from blood dysentery. As you might be knowing, this is often a serious illness in children and sometimes leads to malnutrition and failure to thrive. Previous studies have shown that certain nutrients called antioxidants may help a person to fight infections better and also prevent malnutrition after such an episode of infection. We want to see whether giving antioxidants to children with bloody dysentery helps them to recover from their illness earlier and prevent malnutrition. For this purpose, we are testing certain diets, some of which contain these antioxidants, on children with bloody dysentery.

If you agree to allow your child to participate in this study, your child will be admitted into the study ward. Your child will stay in the study ward for 7 days, during which he will be treated with the standard hospital drug for bloody dysentery and examined daily by a physician and nurses. In addition, he/she will receive any one of 4 diets, two of which contain the antioxidants, for the duration of hospital stay. To determine whether the antioxidants are working effectively, we will need to draw a total of 4 ml of venous blood from your child on admission and on discharge, which is equivalent to a little less than a teaspoonful of blood. We will also collect stool and urine samples for testing. We will also measure your child's body weight, height and composition during his/her stay in the hospital to see whether the diet helps in promoting growth or preventing malnutrition. You will also need to follow-up at our hospital 3 months after discharge, so that we can measure your child's weight and height. We shall reimburse you for the transport costs of that follow-up visit.

If you agree to participate and comply with follow-up, please place your signature or thumbprint on this form. If you do not agree to participate, your child will still receive the standard care provided by the hospital.

----------	----------
Signature of the investigator	Signature/thumb impression of the guardian

Composition of high-protein and standard hospital diet per 100 gm

	Veg HP		Standard	
	Protein (~~kcal~~) g	Energy (kJ)	Protein (~~kcal~~) g	Energy (kJ)
Breakfast				
Bread	8.75	1105	8.75	1105
Sugar	-	-	-	1674
Milk-Suzi	-	-	1.34	345
Lunch & Super				
Cooked Rice	6	350	1.98	521
Dal (lentil)	25	350	1.63	510
Oil	-	180	-	180
Snack 4 P.M.				
Banana	1.42	418	1.42	418
Milk-Suzi 10,16,22	-	-	1.34	345

Title: Antioxidative Nutritional Intervention during Acute Shigellosis

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology		✓	
Feasibility within time period	✓		
~~Appropriateness of budget~~	-	-	-
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: Mark J.S. Miller, Ph.D Signature: [signature]

Date: 11/25/95

Position: Professor

Institution: Louisiana State University Medical Center

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title: Antioxidative Nutritional Intervention during Acute Shigellosis

PI: Dr. M.A. Khaled, Dr. George Fuchs

Reviewer: Dr. Mark J.S. Miller

General Comments:

The concept that antioxidant supplementation may assist in the host defense response to Shigellosis is very plausible and certainly worthy of investigation. Mediators released to ward off the infection causing injury to the host and contribute to the symptoms (secretion, cramping, etc). Many of these mediators are oxidants or oxidizing agents. Additional antioxidants, while not compromising the clearance of the infection may well limit host inury, symptoms and facilitate recovery.

In this study, the use of β-carotene and vitamine E are excellent choices. The inclusion of the water soluble antioxidant, ascorbic acid, could have been justified but is not critical.

The date of 3 months, while facilitating a reasonable return of data may be on the edge of detectability for anthropometric analyses. An infection free control group was not is included and it is likely that improved growth may reflect events distinct from diarrhoeal symptoms and improved host defense. A background assessment of nutritional status would help this problem.

The measurement of TBARS and GSSG in blood are routine measures of oxidative stress. However, they can suffer from false positive signals, in the case of TBARS from eicosanoids and cyclic fatty acids, and GSSG should be measured in the context of total glutathione (oxidized and reduced).

Finally, as nitric oxide is a free medical implicated in gut injury, host defense peristalsis, epithelial secretion and hyperemia, it may be worthwhile to extend the study into the plasma and urinary levels of this oxidant. It can be easily measured as nitrate and would suffice as another index of pro-oxidant, free radical mechanisms.

Title: Antioxidative Nutritional Intervention during Acute Shigellosis

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
~~Appropriateness of budget~~	-	-	-
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ✓

b) with qualification

- on technical grounds ✓
- on level of financial support ✓

I do not support the application ✓

Name of Referee: DR. M. A. MALEK, Ph. D.

Signature: Malek

Date: 21-5-95

Position: Professor,

Institution: Institute of Nutrition & Food Science, Dhaka University, Bangladesh.

DR. M. A. MALEK, Ph. D.
Professor,
Institute of Nutrition & Food Science
Dhaka University, Bangladesh.

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel thay are justified.

(Use additional pages if necessary)

Title: Antioxidative Nutritional Intervention during Acute shigellosis.
PI: Dr. M. A. Khaled
Dr. George Fuchs
Reviewer: Professor Md. Abdul Malek

The research protocol, ''Antioxidative Nutritional Intervention during Acute Shigellosis'' is well designed. The principal investigators may add something regarding the ratio of GSSG and GSH. It may be useful for this research work.

It is an excellent piece of research work to be done. I hope it will add new knowledge in this field.

Malek 21-5-95

DR. M. A. MALEK, Ph.D.
Professor,
Institute of Nutrition & Food Science
Dhaka University, Bangladesh

RESPONSE TO REVIEWERS' COMMENTS

Reviewer # 1

The reviewer suggested the inclusion of nitric oxide (NO) measurement as another important marker of nitric oxide synthetase (NOS) activity associated with oxidative stress. This has been included in the protocol.

Reviewers # 1 and 2

Both the reviewers suggested the analysis of GSH and GSSG data in terms of their ratio. This has also been added to the protocol.