

27/7/88

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## SECTION - I: RESEARCH PROTOCOL

1. TITLE: Comparison of the effects of green leafy vegetables and vitamin A capsule in under-nourished children with and without non-corneal xerophthalmia.
2. PRINCIPAL INVESTIGATORS: Mr. M. Mujibur Rahman  
Dr. S.K. Roy
- CO INVESTIGATOR: Mr. M.A. Wahed
- CONSULTANT: Dr. I. Ciznar  
Prof. M.A. Malek
3. STARTING DATE: As soon as possible
4. COMPLETION DATE: One year after the starting date
5. TOTAL DIRECT COST: US \$ 43680.00
6. SCIENTIFIC PROGRAM HEAD: \_\_\_\_\_

This protocol has been approved by the Laboratory Sciences Division.

Signature : \_\_\_\_\_

Date : \_\_\_\_\_

## 7. ABSTRACT SUMMARY:

Non-corneal xerophthalmia due to vitamin A deficiency is reversible and 6.5 per cent rural children of 0-6 years age group suffer from non-corneal xerophthalmia in Bangladesh. Green leafy vegetables (GLV) are rich source of provitamin A and are abundant in the rural areas of Bangladesh. The green leafy vegetables may be used in the treatment of non-corneal xerophthalmia. A study

will be conducted among fifty children with non-corneal xerophthalmia and equal number of control children to compare the effects of GLV and vitamin A capsule 2,00,000 I.U. Lal sak (Amaranthus gangeticus) will be the principal green leafy vegetables and 50 g of Lal sak will provide 4023 ug beta-carotene or 670 ug (2233 I.U.) vitamin A retinol equivalent. Two groups of malnourished children having xerophthalmia and not having xerophthalmia will be supplemented with vegetables for two weeks and the third group will receive vitamin A capsule (VAC) in a randomised trial. Improvement of xerophthalmia and change in the serum vitamin A levels will be measured after supplementation and its effect on the prevention of further xerophthalmia will be studied over a follow-up period of one year.

8. Reviews:

i. Ethical Review Committee : \_\_\_\_\_

ii. Research Review Committee : \_\_\_\_\_

iii. Director, ICDDR,B : \_\_\_\_\_

## SECTION II - RESEARCH PLAN

.. Introduction:

### 1. Objectives

- i. To compare the effects of supplementation with green leafy vegetables (GLV) with those of vitamin A capsule (VAC) on the serum vitamin A levels in the undernourished children having xerophthalmia (XN,X1A,X1B).
- ii. To see the effect of vegetable (GLV) supplementation in undernourished children on the prevention of xerophthalmia.

### 2. Background

In Bangladesh, vitamin A deficiency is a public health problem (1,2,3). For many, the only dietary source of vitamin A is green leafy vegetables (GLV) and the present intake meets only about one third (763 I.U.) of the requirement (1,18). Vitamin A deficiency is one of the most common causes of preventable blindness in children. The incidence of blindness due to vitamin A deficiency in Bangladesh has fallen from the early 1970s when 17,000 young children were estimated to become blind each year (3,5,). The current figure of blind children under six year may be nearer to 9-10,000 (3). About 6.5 percent of the rural children suffer from non-corneal xerophthalmia. Among 181,51850 rural children below 6 years, 3.6 per cent were suffering from nightblindness (XN), 2 per cent from conjunctival xerosis (X1A) and 0.9 per cent from Bitot's spot (X1B) (3,5,6).

•Taking into account of serum vitamin A status 20 per cent of village children (aged 1-6 years) are vitamin A deficient; 10ug/dl(5) .

Excepting the pregnant mothers average daily intake of vitamin A for any age group did not exceed 57 per cent of their requirement. Eighty eight per cent of households have deficient intake of vitamin A. Pregnant and lactating mothers are also deficient in energy and protein intake (5,6). GLV constitute only 3 per cent by weight (20.2g/person/day) of the total diet while the dietary energy from fat is only 5 per cent (6). It may be assumed that most of the infant born to these mothers have little reserve of vitamin A in the liver. Before 15 months none of the rural children is given vegetables and only 17 per cent of the urban poor children get occasional vegetables in their diets (11). The effects of infectious diseases on the marginal vitamin A status are the precipitations of acute vitamin A deficiency syndromes (8).

In the United States, the recommended daily allowances of retinol equivalents for various age groups are: infants 420 ug, children 0.5 to 4 years old 400 ug, adult females 800 ug, adult males and pregnant women 1000 ug and lactating women 1200 ug (9,18,23). The current recommendation by WHO is to give 200,000 units of vitamin A per child every 6 month (7,20).

Recent studies from India showed that carotenoids from vegetables contribute 82 per cent of dietary vitamin A in developing countries

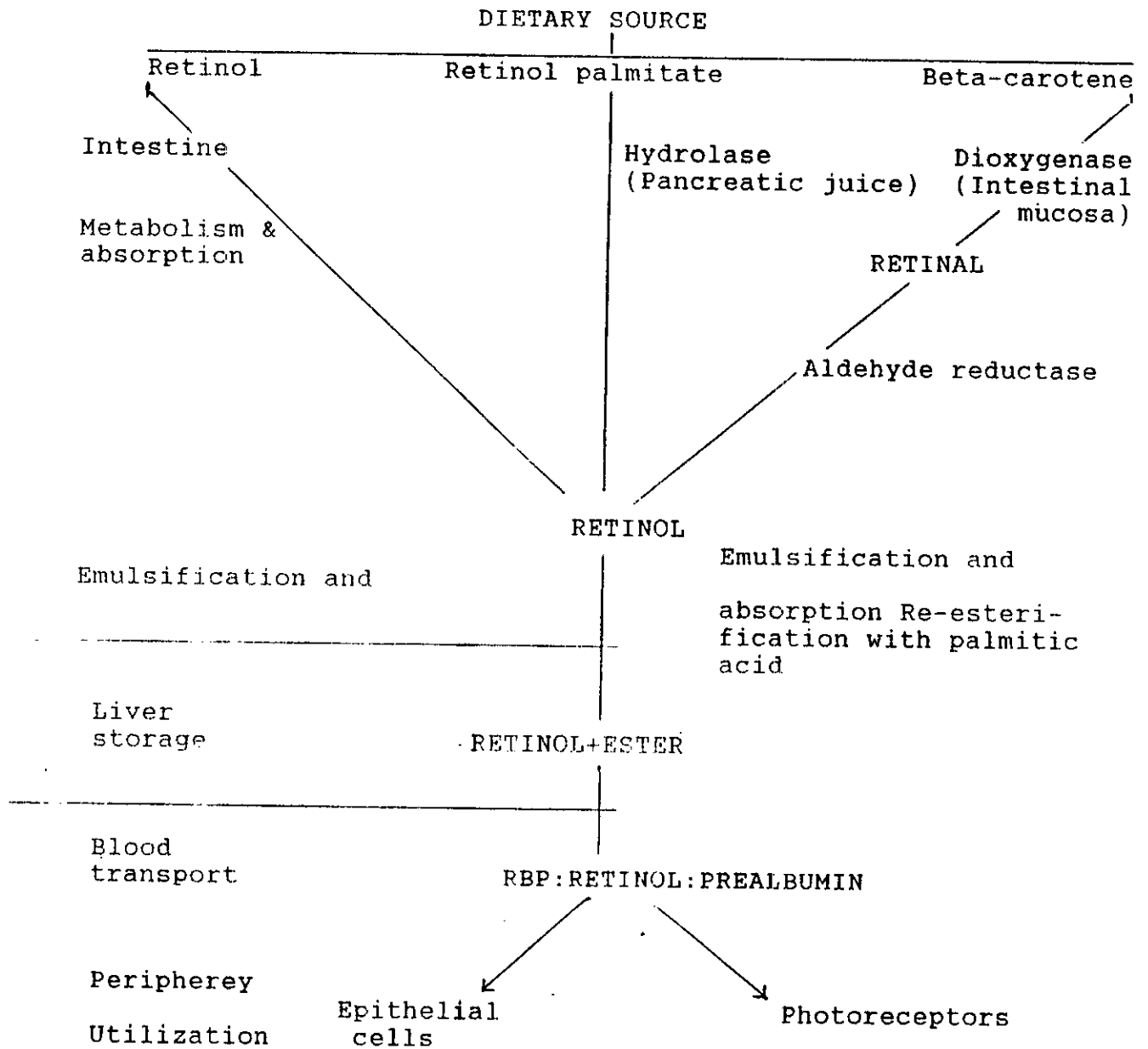
). The average consumption of other vegetables per person per day 134 g and the consumption of green leafy vegetables is only 15.2 g in Bangladesh (1,2). It is interesting to note that about 15-20 g of appropriate vegetables containing high amount of beta-carotene may be sufficient for a young child (15,16). Lala et al. found significant increase (from 15ug/dl to 28 ug/dl) in serum vitamin A level feeding 30 g of green leafy vegetables daily for two weeks compared to the controls, without feeding vegetables (17.5 ug/dl to 17.9ug/dl) (13). Jayrajan et al. found that supplementation of spinach led higher level serum vitamin A levels in preschool children from poor community after a period of four weeks (10). The same study also showed that small amount of dietary fat enhances the absorption of vitamin A. Studies from India showed that addition of 40 g of green leafy vegetables to the daily diets significantly improved the vitamin A status of preschool children where intake of protein, fat and calories were inadequate (13). They also found that severely malnourished children (below 60% wt. for age) without diarrhoea can consume 30 g of green leafy vegetables in one meal without any side effect.

#### Vitamin A Metabolism and effect of malnutrition :

Vitamin A is formed in the body from carotene pigments of GLV, the most important of which are beta-carotene. The bio-synthesis of vitamin A from beta-carotene takes place in the intestinal mucosa and absorption of beta-carotene depends upon the presence of dietary fat, bile and antioxidants mainly in the intestinal mucosa and after absorption stored in the liver. From liver, vitamin A is mobilised and transported in the plasma, in association with a complex

nsisting of one molecule of retinol binding protein (RBP) and one molecule of prealbumin, to the target tissue where the metabolic requirement exists (21). The bioavailability of stored vitamin A also depend upon the child's nutritional status. In severely malnourished children synthesis of RBP takes place at a much reduced rate (14,19,21). Serum retinol level may therefore remain subnormal, inspite of the storage of vitamin A in the liver is high. So, a diseased liver cannot store as much vitamin A or make as much RBP, as a normal one (19,21). Zinc is also essential for mobilization of vitamin A from liver mainly by the synthesis of RBP in order to maintain the normal concentrations in the circulating blood (14). If the intake of vitamin A is less than the amount required for a prolonged period, the liver store become depleted, serum retinol level drops, epithelial function is impaired, and leads to the development of xerophthalmia. The duration of reduced vitamin A for development of xerophthalmia depends upon (a) the amount of vitamin A or precursor ingested, (b) the extent of pre-existing liver stores, and (c) the rate at which vitamin A is being utilized by the body (19).

SCHEMA OF VITAMIN A METABOLISM



WHO 78061 (19)

Classification of xerophthalmia(19,20)

|     |   |
|-----|---|
| XN  | Night blindness   |
| X1A | Conjunctival xerosis                                      |
| X1B | Bitot's spot  |
| X2  | Corneal xerosis   |
| X3A | Corneal ulceration/Keratomalacia<br>< 1/3 corneal surface |
| X3B | Corneal ulceration/keratomalacia<br>≥ 1/3 corneal surface |
| XS  | Corneal scar  |
| XF  | Xerophthalmia fundus                                      |

Common and easily available green leafy vegetables and their values are given below (17) :

| Edible green leaves with a high content of beta-carotene | Available season | Beta-carotene value ug/100 g |
|--|------------------|------------------------------|
| 1. Lal sak<br>( <i>Amaranthus gangeticus</i> )           | Winter/Summer    | 8046                         |
| 2. Mula sak<br>( <i>Raphanus sativus</i> )               | Winter           | 6263                         |
| 3. Palank sak<br>( <i>Spinacea oleracea</i> )            | "                | 6415                         |
| 4. Helencha sak<br>( <i>Enthydra fluctuan</i> )          | Summer           | 8219                         |
| 5. Kachu sak<br>( <i>Colocasia antiquorum</i> )          | "                | 8415                         |
| 6. Kalmi sak<br>( <i>Ipomoea reptanes</i> )              | "                | 8261                         |
| 7. Pat sak<br>( <i>Corchorus capsularis</i> )            | "                | 10263                        |



Vitamin A activity in food is expressed in international units (I.U.), 1 I.U. is equivalent to 0.3 ug of retinol, 0.344 ug retinol acetate, 0.55 ug retinol palmitate, 0.6 ug of beta-carotene and 1.2 ug of provitamin A carotenoids other than beta-carotene (4,18).

### Hypothesis

In view of the above background, it is hypothesized that the reduction of vitamin A status with xerophthalmia in undernourished children can be reversed to normal level with common and easily available green leafy vegetables as efficiently as VAC.

### SPECIFIC AIMS:

1. To compare the improvement of xerophthalmia after consumption of
2. To study the effect of prevention of xerophthalmia (clinical) by vegetables supplementation.
3. To study the effect of prevention of xerophthalmia by vegetables supplementation.

### C. Materials and Method:

The present study will be conducted at Nandipara, 10 kilometre north-east of Dhaka city, a periurban village settled by people of low socio-economic status (SES) (27,28). There are about 500 under five children who are under surveillance of a weekly clinic, run by ICDDR,B.

### Subjects:

Primary screening will be done by one of the investigators for

xerophthalmia and blood level of vitamin A by biochemical method from the 550 children of the study area after taking informed consent. Children between 1-3 years of age below 76 per cent of weight for age (32) belonging to poor socio-economic group will be investigated for xerophthalmia (XN,X1A,X1B) and serum vitamin A level below 20 ug/dl and from them 50 cases and 50 clinically non-deficient children of similar nutritional status (but with vitamin A level above 20 ug/dl) will be selected for this study. SES will be determined according to the year of schooling, income, assets, structure of the house and occupations of the head of the family (28).

Fifty children suffering from vitamin A, will be randomised for 2 groups for green leafy vegetables (Group A), and one dose of vitamin A capsule (2,00,000 I.U.) with usual home diet. Green leafy vegetables with home diet will be received by the 50 control (Group C) non xerophthalmia PEM children. Group C will serve to identify the ability of prevention of xerophthalmia by leafy vegetables. Lal sak (*Amaranthus gangeticus*) will be the study leafy vegetables. Fifty gram Lal sak will provide 4023 ug beta-carotene or 670 retinol equivalent will be given once daily with their main meal. Vegetable (Lal sak) 1250 g will be measured on a beam balance and then cooked with soybean oil, onion (*Allium cepa*), and salt for 8-10 minutes (17). Cooked vegetables will be weighed again and divided for 25 children equally (each will be equivalent to 50 g raw vegetables) and will be served daily for 2 weeks. In group A, each subject will receive 596 ug vitamin A retinol equivalent (from Lal sak) as 11 percent vitamin A loss due to cooking (17). Twenty four hours dietary intake will be

measured by recall method (30) daily during the study period, and vitamin A content of the diet will be estimated.

In group A 25 children with xerophthalmia will receive one VAC (200,000 I.U.) and instruction on not taking further GLV. Their diet will be daily recorded in precoded forms. For group C 25 children having no signs and symptom of xerophthalmia will receive only GLV as group and instruction not to take VAC .

Left over GLV will be measured on a beam balance for record (see appendix). For baseline level of nutrients 0.5 ml blood will be collected from finger tips before and 14 days after feeding of vegetables . Weekly follow-up up to 3 months, 2 weekly up to 6 months and 1 year. Non responders : The non responders of xerophthalmia to GLV will be given VAC 2,00,000 I.U. after 2 weeks. Cooked GLV will be supplemented once daily with their meal. Health assistant will provide the measuring assistance to the mothers for food intake and vitamin A intake.

#### Anthropometric measurement:

A thorough clinical examination will be done by the Co. PI on 1st day and 14th day of admission and on the same day, height for age, weight for age and height for weight (31) will also be calculated.

#### D. Methods of Analysis:

1. Beta-carotene: A rapid and simple methods for determining carotene in plant material (26).
2. Serum vitamin A : Vitamin A estimation by HPLC (24).

3. Albumin : Dye Binding method (22)
4. RBP : SRID Techniques (25)
5. Stool M/E : Parasite, Giardia

Sample collection, preparation, and preservation:

Finger tip blood, 0.5 ml will be collected into netleson tubes and will be stored in ice box for carrying to the laboratory within 2-3 hours of collection. Serum will be separated by centrifuge machine at 3000 RPM and then, these serum will be stored at  $-20^{\circ}$  C in deep freeze till biochemical analysis is done.

Cooked GLV samples, 10 gm in triplicate will be collected and transported to the laboratory for beta-carotene, vitamin A estimation.

Sample size calculation: Ref. Statistical method by Cochran (29)

We have 2 types of outcome variables for comparison of the effects of vitamin A capsule with those of leafy vegetables

- a) change in serum vitamin A levels.
- b) clinical signs and symptoms of xerophthalmia

Ref.11 from India on serum vitamin A level change by leafy vegetables.

2

$$\frac{2 \times SD^2 \times f(\alpha - \beta)}{d^2} \quad \begin{array}{l} \text{Type I Error} = 5\% \\ \text{Type 2 Error} = 10\% \end{array}$$

gives 15 for 2 groups 30; for rejection (25+25)=50

Ref. 21 from Bangladesh on prevalence of xerophthalmia

$$n = \frac{P_1(100-P_1) + P_2(100-P_2) \times f(\alpha, \beta)}{(P_1 - P_2)^2}$$

$$\alpha = 0.05$$

$$\beta = 0.1$$

$P_1$  = Percentage of improvement on VAC treatment

$P_2$  = Percentage of improvement on GLV treatment

For 2 groups  $25 \times 2 = 50$ , and control 50. total=100

We take a sample of 50 for xerophthalmia and <20 ug/dl vitamin A and equal 50 for non xerophthalmic children . Total 100 children.

Effect of feeding green leafy vegetables on serum vitamin A

| Group Status | Serum vitamin A level ug/100 |               | Difference between before & after feeding | Difference between vegetables & capsules |
|--------------|------------------------------|---------------|---|--|
|              | Before feeding               | After feeding |   |  |
| Group A      |                              |               |   |  |
| Group B      |                              |               |   |  |
| Group C      |                              |               |   |  |

SIGNIFICANCE:

There is no information on the effect of green leafy vegetables on serum vitamin A level of children suffering from xerophthalmia in Bangladesh. From studies in other countries, it is evident that under-nourished children are able to get benefits of vitamin A from the green leafy vegetables efficiently (4,10,13). Since xerophthalmia is a problem of public health significance in Bangladesh (1,2,3,20) it can be combated by easily available green vegetables which are inexpensive and can totally meet vitamin A requirements of children. However, no data are available on the clinical benefit of green leafy vegetables in vitamin A deficient children any where in the world. The study will provide data and facts that can be utilized to solve the problem of vitamin A deficiency which causes unnecessary blindness in the developing countries .

Facilities required

ICDDR,B Biochemistry Laboratory will provide the laboratory facilities for beta-carotene, vitamin A, albumin, and RBP estimation. Nandipara clinic area will be used.

Data analysis:

Differences of serum vitamin A between before and after feeding green leafy vegetables (cooked) and vitamin A capsule group will be statistically tested by using 'student t' test.

Data will be entered into micro-computer and necessary test will be done. A micro-computer will be needed. Statistical service will be available.

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Appendix

SURVEY OF VEGETABLE INTAKE

1. Date of admission : \_\_\_\_\_
2. Name of the patient : \_\_\_\_\_
3. Age : \_\_\_\_\_ 4. Height : \_\_\_\_\_ 5. Weight \_\_\_\_\_
6. Eye symptom : \_\_\_\_\_ 7. Diagnosis : \_\_\_\_\_
8. Nourished/undernourished : \_\_\_\_\_
9. Name of vegetable : \_\_\_\_\_
10. Amount of vegetable to be taken : \_\_\_\_\_ gms.
11. Left over total amount of food : \_\_\_\_\_ gms.

Amount of vegetables taken for 15 days

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| Days   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| Amount | g | g | g | g | g | g | g | g | g | g  | g  | g  | g  | g  | g  |

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ug beta-carotene

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Field Assistant : \_\_\_\_\_

Investigator : \_\_\_\_\_

SECTION III-BUDGET

A. DETAILED BUDGET

3100 Local salaries

|                       |                        | % of Effort | US\$  |
|-----------------------|------------------------|-------------|-------|
| Mr. Mujibur Rahman    | Principal Investigator | 40          | 3,500 |
| Dr. S.K . Roy         | "                      | 15          | 2,000 |
| Mr. M. A. Wahed       | Co-Investigator        | 10          | 1,000 |
| Dr.I. Ciznar          | Consultant             | 05          | -     |
| Prof. M. A. Malek     | "                      | 05          | -     |
| Field Assistant (Two) |                        | 100         | 9,000 |

3300 Consultant 500

3500 Local Travel 500

3700 Supplies and Materials

|                        |     |
|------------------------|-----|
| (a) Vials, Syringe et. | 500 |
| (b) Medicine           | 600 |
| (c) Stationary         | 300 |

4000 Other Contractual Services 500

4800 Interdept. Services

|                              |       |
|------------------------------|-------|
| 4802 ICDDR, B Transport      | 500   |
| 4806 Xerox                   | 100   |
| 4809 Biochemistry            | 7,800 |
| 4807 Pathology               | 200   |
| 4815 Medical Illustration    | 100   |
| 4813 Patient Hospitalisation | 2,400 |
| 4818 Out Patient Care        | 200   |

|                            |       |
|----------------------------|-------|
| 0300 Equipment (a) Balance | 700   |
| (b) Micro Computer         | 3,200 |

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|               |        |
|---------------|--------|
| TOTAL US \$   | 33,600 |
| Over head 30% | 10,080 |

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GRAND TOTAL US\$ 43,680

*Reviewed by  
Budget office*

*Hand  
27/7/88*

SUMMARY BUDGET

|    |                         |       |          |
|----|-------------------------|-------|----------|
| A. | Personnel               | US \$ | 16000.00 |
| B. | Supplies & materials    | ,,    | 15700.00 |
| C. | Transport               | ,,    | 700.00   |
| D. | Printing & reproduction | ,,    | 600.00   |
| E. | Medicine                | ,,    | 600.00   |

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TOTAL ..... 33600.00

Overhead 30% ..... 10080.00

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GRAND TOTAL US \$ 43680.00

Incremental cost US \$ 43680.00 - (6500.00 + 10080.00)  
US \$ = 27100.00

### CONSENT FORM

Research on the role of green leafy vegetables to prevent  
vitamin A deficiency diseases.

International Centre for Diarrhoeal Disease Research, Bangladesh has been conducting researches on problems related to nutrition and diarrhoea. Green leafy vegetables grown in our country contain significant quantity of pro-vitamin A which can adequately meet vitamin A requirement. Even then nightblindness or poor eye sight due to vitamin A deficiency is a major health problem in our country. This issue needs to be addressed. We would like to study the utilization of green leafy vegetables for vitamin A in your child. If you agree, we would carry out the following :

1. We shall give cooked leafy vegetables to your vitamin A deficient child for 2 weeks in addition to normal food.
2. We shall collect 0.5 ml blood from finger prick to measure the vitamin A level day 1 and after 2 weeks. For this, your child is not expected to have any discomfort.
3. In need, we may also give high potency vitamin A capsule to your child.
4. You can withdraw your child from the study at any time. Now, if you agree please sign or put your thumb impression below :

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Investigator

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Signature/LTI of  
the parent

আসনের মাসায়ে জিটামিন এ'র অসুস্থি জনিত কোম প্রতিকার  
ও প্রতিবেদি গবেষণা কর্মসূচী

সম্মতি পত্র

আন্তর্জাতিক উদ্বাসন গবেষণা কেন্দ্র জাইরিয়া ডিক্লোরাম নামে সুস্থি সংশোধন  
মসম্মিয়া নিবে গবেষণা কবে আসছে। আমাদেব দেশেব শাক-সজ্জিত কমেছে  
প্রচুর জিটামিন, মা আমাদেব জিটামিন এ'র অজাব মেটোলে সক্ষম। তুও  
জিটামিন এ'র অজাব জনিত সুস্থি শক্তি হীনতা এক রাতকানা একটি বড় বর্বনেব  
মসম্মিয়া। এ সসম্মিবেব সম্মাৰিত অজিব প্রয়োজন।

আমরা সবুজ শাক থেকে রুগ্ন শিমুকা কচটুকু জিটামিন এ গ্রহন এক  
তার প্রয়োজন মেটোলে সক্ষম মে সম্মাকে গবেষণা কবেছে চাই। আসনি যদি  
বাজি মাকেত তা হলে :-

- ১। আসনার জিটামিন এ'র অজাব গ্রন্থ শিমুকে ২ সসম্মিবে জিটামিন এ  
সম্মুদ্র শাক মূল খাবাবেব মাসে খেতে দেখা হবে।
- ২। উর্জিব দিন আসনার শিমুকে জিটামিন এ খাটেতিব গবিমান নিজাবন এক  
জন্তু মাত্র ২ মিলি রক্ত আস্থুল মেকে নেমা হবে। ২৪ দিন পর পুনবাস  
অনুকম পরীক্ষা করা হবে। এতে আসনার শিমুর কোম অতি হবে না।  
প্রয়োজন হলে জিটামিন এ সম্মুদ্র ব্যাসমুল দেখা হবে।
- ৩। আসনি মে কোম সসম্মিবে আসনার শিমুকে এই গবেষণা থেকে কসম্মাবে  
কবেতে পাববেন কেমনা এই গবেষণায় অল্প গ্রহনে আসনার জন্তু  
কোম ব্যব্যাপুলক নম। আসনি যদি বাজি মাকেত চবে নীচে মাসম্মিবে  
করুন কিংবা মাস হাতের বৃক্ষাশুলেব দাস গ্রহন করুন।

গবেষকের স্বাক্ষর  
তারিখ

আবিভাবকের স্বাক্ষর/  
আস্থুলেব দিন  
তারিখ



Abstract summary for ethical review committee

A study will be conducted among fifty children (1-3) years with non-corneal xerophthalmia and equal number of control children to compare the effects of green leafy vegetables (GLV) and vitamin A capsule (VAC) 200,000 I.U. Lal sak (*Amaranthus gangeticus*) will be the principal GLV and 50 g of Lal sak will provide 4023 ug beta-carotene or 670 ug (2233 I.U.) vitamin A retinol equivalent. Two groups of undernourished children having xerophthalmia and not having xerophthalmia will be supplemented with vegetables for two weeks and the third group will receive vitamin A capsule (VAC) in a randomised trial. Improvement of xerophthalmia and change in the serum vitamin A levels will be measured after supplementation and its effect on the prevention of further xerophthalmia will be studied over a follow-up period of one year.

1. This study will include children between 1-5 years of age and below 76 per cent of weight for age belonging to poor socio-economic group. Children of this group are more vulnerable for vitamin A deficiency diseases.
2. There will be no potential or any type of risks involved in this study .
3. This study will not include children with severe type of vitamin A deficiency disease (corneal xerophthalmia) rather will include mild type of vitamin A deficiency disease (non-corneal xerophthalmia) will be selected for GLV supplementation . On urgent clinical indication, high potency VAC 200,000 I.U. will be given.

4. Information on the subjects will be recorded on a prescribed form and will be kept with the Principal Investigator. Data will be identified by id . Confidentiality will be maintained.
5. Signed consent will be obtained from the gurdian of the children.
6. Interview will be taken at the subject's home ( as per appendix) for general particular and illness. Interview and filling in the forms may take 10 minutes.
7. To compare the improvement of xerophthalmia after consumption of GLV with that of VAC 200.000 I.U. and the effect of pevention of xerophthalmia by GLV supplementaion. The study will provide data and facts that can be utilized to solve the problem of vitamin A deficiency which causes blindness among the children of Bangladesh. The study is expected to directly benefit himself to prevent and cure vitamin A deficiency due to supplementation of pro-vitamin A from GLV and high potency vitamin A capsule in preventing or correcting vitamin A deficiency .This study may benifit the developing countries by using the locally available resources.
8. Only 0.2 ml blood will be collected for biochemical analysis: before and after supplementation at an interval of 2 weeks.

XEROPH. PRO