Attachment 1.

Date 09.02.88

17-2

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Syed A.I. Ally

Trainee Investigator (if any) X

Application No. 88007(P)

Supporting Agency (if Non-ICDDR,B)

Title of Study Detection of Vitamin A deficiency by ocular Impression cytology.

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).

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 N/A Yes No
(c) Physical risks N/A Yes No
(d) Sensitive questions N/A Yes No
(e) Benefits to be derived Yes No N/A
(f) Right to refuse to participate or to withdraw from study (Yes) No
(g) Confidential handling of data N/A Yes No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure N/A 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).
X Protocol (Required)
X Abstract Summary (Required)
X 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
X 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)

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

Saially
Principal Investigator

Trainee

88007(P)
17/2

SECTION - I : PILOT PROTOCOL

1) Title:

DETECTION OF VITAMIN A DEFICIENCY BY OCULAR IMPRESSION CYTOLOGY

2) Principal Investigator: : Dr. Syed A.I. Ally

Co-Investigators : Dr. A. Briend (CMD)

Dr. Tahmeed Ahmed

Consultant: Dr. O. Amedeé Manesme, Service de Pédiatrie, Hopital du Kremlin Bicétre, Paris, France.

3) Starting date: April 1, 1988

4) Completion date: September 30, 1988

5) Total direct cost: 4770 US $

5. b) Source of funding : UNDP / PDF

6) This protocol has been approved by the Laboratory Sciences Divis

Signature of the Scientific Programme Head: .....

Date: Febr. 07, 1988

ABSTRACT

Ocular impression cytology (OIC) is a new technique recently proposed to detect subclinical Vitamin A deficiency. Its principle is to look for mucus secreting cells by a simple staining technique on an impression made from the bulbar conjunctiva on a strip of cellulose acetate filter paper. The usefulness of this technique to detect children with Vitamin A deficiency will be determined by a) estimation the proportion of clinically deficient children who have an OIC suggestive of deficiency b) by estimating the proportion of children known not to be deficient who are correctly diagnosed as non deficient by OIC c) by assessing whether OIC brings original information compared to biochemical indicators of Vitamin A status.

8) Reviews:

i) Ethical Review Committee: .....

ii) Research Review Committee: .....

iii) Director's signature & remarks if any: .....

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SECTION - II : RESEARCH PLAN

A. INTRODUCTION

The major cause of blindness among children in many developing countries is Vitamin A deficiency (1). Xerophthalmia, a spectrum of ocular symptoms (ranging from night blindness to corneal ulcer) is the most obvious lesion and often establishes the diagnosis. Yet Vitamin A deficiency can be regarded as a systemic disease. It has been argued that the previously reported association between Vitamin A deficiency and higher mortality (2) might be the result of epithelial lesions in the gut and in the respiratory tract (3, 4) leading to increased susceptibility to infections. The clinical meaning of extra-ocular manifestations of Vitamin A deficiency is not clear however, partly because there is no simple reliable method for the assessment of Vitamin A status.

Vitamin A reserves in the body are concentrated in the liver and their direct assessment by needle biopsy cannot be done routinely. Conventional methods indirectly assessing vitamin A reserves include (a) plasma retinol concentration, (b) ratio retinol/retinol binding protein (RBP) in the plasma (c) intravenous relative dose response test. Traumatic blood sampling, sophisticated techniques and laboratory facilities are required in all these methods. Expense is another drawback.

Ocular impression cytology (OIC) of the bulbar conjunctiva has been suggested as a simple, practical technique to detect vitamin A deficiency in animals (5) and in humans (6). It has been claimed that this method can detect subclinical vitamin A

deficiency and can distinguish between children with normal vitamin A status and those with preclinical vitamin A deficiency (7). A simplified DIC method for detecting vitamin A deficiency has been recently reported (8).

DIC should lend itself well to its development and application in developing countries, including Bangladesh, as a simple, safe and inexpensive technique to detect vitamin A deficiency. Until now however, this technique has been used mainly in developed countries for the assessment of Vitamin A status of children with various hepatic disorders. This protocol proposes to evaluate, standardise and validate the DIC procedure to detect Vitamin A deficiency at an early stage in a malnourished population.

B. SPECIFIC AIMS

1. To assess the feasibility of DIC in Bangladeshi children.
2. To perform DIC on children with clinical signs of Vitamin A deficiency (estimation of sensitivity).
3. To perform DIC on control children with no clinical signs of vitamin A deficiency who recently received a Vitamin A capsule (VAC) (estimation of specificity).
4. To compare how serum indicators of Vitamin A status and DIC discriminate Vitamin A deficient and control children.

C. MATERIAL AND METHODS.

OIC requires some co-operation from the child to be examined. It will not be carried out in children below 2 years of age.

1. Description of the technique

The procedure consists of simply touching the temporal bulbar conjunctiva of both eyes for only 3 seconds with pre-cut 25x5 mm strips of cellulose acetate filter paper (1 filter paper to each eye), transferring the cells so obtained from the filter paper to a glass slide by simple finger pressure, fixing it in 95% ethanol for 15 minutes, and then staining it for 20 minutes. Only one staining step is required. The staining medium, (Table 1) composed of an admixture of one volume of carbol-fuschin and two volumes of 0.2% alcian-blue in 5% acetic acid, is stable for several months at room temperature. After staining, the slide is washed with tap water and examined with a simple light microscope under low (X10) and high dry (X40) magnifications. Oil immersion lens is not required. Epithelial cells are stained pink (both cytoplasm and nucleus) and the nucleus of the goblet cells stain pink. The mucous substance of the goblet cells stain blue and are conspicuous when present. Vitamin A status is determined by the presence (normal) or absence (deficiency) of goblet cells. Although the presence of one goblet cell is enough to classify the child as non deficient, their number will be recorded for each patient along with a short description of epithelial cells.

2. Examination of OIC in children with clinical signs of Vitamin A deficiency.

If OIC is a sensitive technique able to detect subclinical cases of vitamin A deficiency, then it should be positive in all cases of clinical deficiency. This will be tested by carrying out an OIC on a maximum number of children with clinical signs of Vitamin A deficiency. During the study period, one of the investigator will ask the ward physicians every working day whether there is any child (aged above 2 years) with any sign of active xerophthalmia (with the exception of corneal ulcer, excluded to avoid unnecessary manipulation of highly fragile eyes). If this is the case, an OIC will be made immediately. All precautions will be made not to delay treatment: hospital physicians will be asked to give Vitamin A a few hours after the diagnosis of xerophthalmia even if OIC could not be done. The percentage of these children with OIC suggestive of deficiency will give an estimate of the sensitivity of the method.

3. Examination of OIC in children after VAC intake.

All children of the Nutrition Rehabilitation Unit (NRU) of Dhaka hospital are routinely given a VAC on admission. One may assume that after two weeks of treatment, once their general

condition has improved, they should have an adequate Vitamin A status. An OIC will be made on selected children from the NRU staying more than 2 weeks and aged more than 2 years acting as controls. Children who had xerophthalmia on admission will be excluded. Controls will be chosen to match by age and sex Vitamin A deficient children. The percentage of these children diagnosed as being non deficient will give an estimate of the specificity of the method.

4. Comparison of OIC and serum indicators of Vitamin A status to discriminate Vitamin A deficient and control children.

In all children tested by OIC, 200 microlitres of blood will be drawn (after obtaining informed consent from the guardian) for biochemical assessment of Vitamin A status. Retinol levels will be measured by High Precision Liquid Chromatography (HPLC) and Retinol Binding Protein (RBP) by Radial Immunodiffusion. At the end of the study, it will be determined how the different indicators of Vitamin A status differ between the two groups. In a first step, sensitivity- specificity curves will be drawn for each biochemical indicator and compared with sensitivity and specificity of OIC. Then, it will be determined (by comparing different logistic regression models) whether OIC or serum indicators are the most useful to discriminate Vitamin A deficient children from controls.

5. Sample size.

The sample size needed to estimate specificity and sensitivity for a given precision depends on the level of these indicators (9). Table I shows different values of sample sizes needed according to different hypothesis. Since there is no indication on the level of specificity or sensitivity which will be obtained with OIC in Bangladeshi children, formal estimation of sample size needed is quite difficult at this stage. The rule in this case is to start with a small scale study as the one proposed in this pilot protocol to see which hypotheses should be considered. For this reason, the number of children enrolled in the protocol will be kept below 100. One can say however, that a high precision can be obtained with relatively small sample size (below 100 for each group) for high level of sensitivity and specificity. If these indicators are low, there is little advantage in estimating them with precision.

D. SIGNIFICANCE:

Availability of a rapid, inexpensive and sensitive screening procedure for Vitamin A status should significantly enhance our ability to perform research in the spectrum of diseases associated with Vitamin A deficiencies.

TABLE I.

Carbol fuschin solution (Ziehl-Neelsen)

Phenol crystals (melted)	2.5 ml
Alcool, 100%	5.0 ml
Basic fuschin	0.5 g
Distilled water	50 ml

Filter before use.

TABLE II.

Sample size needed (in each group of children) for different levels of sensitivity and specificity.

Level of precision	Sensitivity/Specificity (in %)	Sample size
+ or - 5%	80	245
	90	138
	95	72
+ or - 10%	70	80
	80	61
	90	34

References :

1. Sommer A, Tarwotijo I, Hussaini G, et al.: Incidence, prevalence and scale of blinding malnutrition. Lancet 1981;1:1407-1408.
2. Sommer A : Nutritional Blindness : Xerophthalmia and Keratomalacia. New York, Oxford University Press, 1982.
3. Sommer A, Tarwotjo I, Hussaini G, et al: Increased mortality in children with mild vitamin A deficiency. Lancet 1983;2:585-588.
4. Sommer A, Katz J, Tarwotjo I: Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. Am J Clin Nutr 1984;40:1090-1095.
5. Hatchell D, Sommer A : Detection of ocular surface abnormalities in experimental vitamin A deficiency. Arch Ophthalmol 1984;102:1389-1393.
6. Wittpenn J, Iseng S, Sommer A : Detection of early xerophthalmia by impression cytology. Arch ophthalmol 1986; 104:237-239.
7. Amedee-Manesme O, Zuzeaw R, Witpenn J, et al.: Impression cytology detects subclinical vitamin A deficiency. Am J Clin Nutr (in press).
8. Amedee-Manesme O, Luzeau R, Carlier C, Ellrodt A : Simple impression cytology method for detecting vitamin A deficiency. Lancet 1987; 1: 1263.
9. Armitage P. Statistical methods in medical research. Oxford: Blackwell Scientific Publication, 1971.

BUDGET (in US $)

A. INTERDEPARTMENTAL SERVICES

BIOCHEMISTRY

Tests	Number	Per test	Total
Retinol	200	4.6	920
RBP	200	13	2600

XEROX AND MIMEO GRAPH	150
COMPUTER (DATA ENTRY)	100

B. SUPPLIES AND MATERIAL

STATIONERY	150
LABORATORY SUPPLIES	250
CHEMICAL AND MEDIAS	200
GLASSWARE	100

C. OTHER COSTS

REPAIRS AND MAINTENANCE	200
POSTAGE, TELEPHONE, TELEX	100

TOTAL DIRECT COST (US $)	4770

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

This protocol aims at evaluating a cytologic technique recently proposed to detect Vitamin A deficiency. This technique is performed by touching the conjunctiva with a cellulose acetate filter paper for three seconds and then transferring the cells which adhered to the paper to a glass slide for staining and microscopical examination.

In all tested children, Vitamin A status will be tested by measuring serum levels of Retinol and Retinol Binding Protein (RBP) to see how this correlates with cytologic findings. The blood (200 microlitres) will be taken by finger prick after informing the guardian (see consent form).

1. The proposed study will be done on children since they comprise the highest-risk group of Vitamin A deficiency and it's associated morbidity and mortality.

2. No potential risks are involved in this study. The procedure will be performed personally by the investigators, all three of whom are physicians. In addition, no adverse reactions or risks have been reported in the previous studies done elsewhere using this technique involving over 200 children (refs. 6,7,8). The procedure has also been tried on the investigators themselves and other local adult volunteers without any risks or adverse effects whatsoever.

3. To avoid unnecessary manipulation of fragile eyes, children with

corneal ulcers will not be included in the protocol. For other children, precautions will be taken to perform this test rapidly in order not to delay treatment (by high potency Vitamin A capsule) for more than 6 hours.

4. Subjects enrolled in the study will be assigned a unique number thus safeguarding confidentiality.

5. No potential risks are involved. However, since the study involves minors, a signed consent form will be obtained from the guardians prior to enrolment in the study.

6. Interview : Not applicable.

7. Ocular impression cytology has been suggested as a simple, safe, practical technique to detect subclinical and clinical Vitamin A deficiency versus the other techniques which require sophisticated laboratory facilities and are expensive.

8. Only hospital records will be used.

CONSENT FORM

To assess the value of a new method to detect Vitamin A deficiency, we would like to touch the white part of your child's eyes with a small piece of special soft paper for a few seconds. We will also take a small quantity of blood from his finger (equivalent to 4 drops) for analysis. This test is done for research purposes and is not needed for the treatment of your child. You are free to refuse this examination but your co-operation will be appreciated.

Date: ___/___/_____

Guardian's signature: _____

(or left thumb impression)

Name of the guardian: _____

Relationship with patient: _____

সম্মতি পত্র

মানুষের দেহে "ভাইটামিন ই" -এর অভাব আছে কিনা সেটা দেখার জন্য আমরা আপনার কিশোর চোখের সাদা অংশে একটি বিশেষ ধরনের নরম কাগজের ছোট্ট টুকরো কয়েক মুহূর্তের জন্য জোয়াবো। আমরা শুধু হাতের আঙুল থেকে কয়েক ফোঁটা রক্ত নেবো। এই পরীক্ষাটি গবেষনার কাজে ব্যবহৃত হবে এবং আপাতত আপনার কিশোর চিকিৎসার জন্য দরকার নাই। আপনি ইচ্ছা করলে এই পরীক্ষা নাও করাতে পারেন। আমরা আপনার সহযোগিতা কামনা করছি।

তারিখ :

অভিভাবকের স্বাক্ষর / টিপসহি :

অভিভাবকের নাম :

রোগীর সাথে সম্পর্ক :