 Date 25/10/93 / 20/12/93

tachment 1.
CE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B

incipal Investigator Dr. Nigar S. Shahid

Trainee Investigator (if any)

plication No. 93-032 (Revised)

Supporting Agency (if Non-ICDDR,B)

le of Study Retinol & B-carotene
ntent in breast milk and the reflection
these levels in infants' serum.

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

ele 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 NA
(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 treatments 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.

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

Nigar S. Shahid
Principal Investigator

Trainee

1.	Principal Investigator	:	Dr. Nigar Shahid
2.	Co-Investigators	:	Mr. M.A. Wahed Ms. Amy Rice
	Consultants	:	Dr. Chris Kjoldhe Dr. R. Bradley Sack
3.	Title	:	Retinol & β-carotene content in breast milk and the reflection of these levels in infant's serum.
4.	Starting Date	:	When funds are available
5.	Date of completion	:	6 months from starting date
6.	Total Budget Requested	:	US$ 9,610.00
7.	Funding Source	:	
8.	Head of Programme	:	Dr. R. Bradley Sack Associate Director Community Health Division

9. Abstract Summary

Vitamin A intake of lactating infants is expected to come from the breastmilk of mothers. Bangladesh has a very high rate of Vitamin A deficiency in its population. It is also observed that the diet of lactating and pregnant mothers in Bangladesh is also deficient.

The objective of this protocol is to examine the relationship between mothers breast milk content of retinol and β carotene and its reflection on their infants serum levels.

Serum and breast milk samples are being collected in an ongoing project(92-025) entitled "Maternal immunization with pneumococcal polysaccharide vaccine". We are having blood from mothers at delivery. Infant blood is collected at the age of 6, 14 and 20 weeks. Colostrum are collected within 48 hrs of delivery and breast milk samples at 1, 3 and 5 months of the birth of their babies. The maternal and infant pair will provide the data on the content of breastmilk retinol and β-carotene in Bangladesh population and the reflection of these levels on infants.

The data generated may have policy implications on future

Vitamin A supplimentation programs.

10. Aims of the Project

a) General Aim : 1) To investigate the relationship between mothers breastmilk retinol and - β carotene levels and its reflection on their infants' serum retinol levels.

b) Specific Aims: 1) To assess the fat content, β-carotene and retinol levels of breastmilk of mothers living in Dhaka.

II) To determine the serum retinol and β-carotene levels of breast feeding infants.

III) To correlate the infant serum retinol and β-carotene levels of the infants with that of the breastmilk and serum of their mothers.

c) Significance

The nutritional demand of suckling babies is expected to be met by the breastmilk of their mothers. Studies carried out so far have not examined the mother and child pair as one entity. This timely and important study will provide the missing data examining the relationship between the breastmilk content of retinol and β-carotene and the serum levels of their infants. This piece of information is vital in the understanding of the dynamics of vitamin A metabolism and also have an impact on future vitamin A supplementation programs in developing countries.

11. Ethical Implications

Mothers who are already enrolled in the protocol "Maternal Immunization with polysaccharide pneumococcal vaccine (92-025)" will be subjects of the study. Samples (serum and breastmilk) - from mothers and (serum only) from their infants are already being collected at set intervals. The mothers will be requested not to feed her baby on her right breast for about 2 hours before collection of the breastmilk sample for the vitamin A estimation. She will be free to suckle her infant from her other breast ad libitum. This sampling is only required on 3 occasions in 5 months i.e. at the end of 1,3 and 5 months. The withdrawal of one breast for feeding for only 2 hours will not have any deleterious effect on the infants' health.

Various strategies to combat vitamin A deficiencies are being advocated. One of them is supplementing mothers with vitamin A. Studies on providing supplementation have not shown any conclusive results. The association between vitamin A levels of mothers breastmilk and that of the child's serum is missing. This piece of information is necessary for understanding the dynamics of vitamin A metabolism and transfer.

12. Background and bibliography

Vitamin A deficiency is a major public health problem in developing countries. In Bangladesh, it is estimated that 30,000 children become xerophthalmic every year and half of them die within 5 years of age[1]. It was noted that in urban and rural Bangladesh 20% of children aged between 1-6 months had serum vitamin A levels of less than 10 ug/dl[2,3]. Recent investigations on breastfed children carried out at ICDDR,B have revealed that 75% of children <1 year of age have serum vitamin A levels of <20 ug/dl. (M.A. Wahed - personal communication).

All three national dietary studies conducted in Bangladesh have shown that protein-energy malnutrition and specific nutrient deficiencies exist and that the intake of vitamin A in particular is very low. Moreover, caloric intake has been shown to decrease in Bangladesh through the years[4,5,6]. The 1981-82 survey has shown that the average intake of vitamin A was 38% of the Recommended Daily allowance (RDA). Lactating and pregnant mothers were receiving 28% and 19% of RDA respectively. Subsequent studies have shown that 70% of vitamin A in the diets of Bangladeshi children upto 20 months of age is derived from breastmilk[7,8]. It is not traditional to provide β-carotene rich food to babies.

Supplementation of prescribed aged children with vitamin A has brought about a reduction in mortality rates. Moreover, a reduction of measles case fatality rates and incidence of measles-associated pneumonia and diarrhoea has been observed after supplementation with vitamin A. However, no reduction in incidence has been shown for pneumonia or diarrhoea. Various means of improving the vitamin A status to reduce xerophthalmia and mortality rates have been implemented. One means now being considered is to supplement lactating mothers with Vitamin A capsule to increase the content of vitamin A in the breastmilk, and thus improve the vitamin A status of their infants. However, the relationship between breastmilk vitamin A levels and the vitamin A status of breastfeeding infants is poorly understood, especially among populations in the developing world.

In a study conducted in 1989 Roy et al[9] supplemented mothers with 200,000 IU of vitamin A, 24 hours after delivery and followed them for 7 months. This study did not observe statistically significant differences in the serum or breast milk vitamin A levels of the supplemented or non-supplemented mothers. However, both serum and breastmilk samples were analysed for their retinol content according to the method of Neeld and Pearsons[10] which has now appeared to be less sensitive than current HPLC methods of analysis[14]. Moreover this study did not estimate serum vitamin A of the suckling babies.

However, a more recent retinol supplementation study conducted by Stoltzfus et al[11] in Indonesia did show a statistically significant difference in the breastmilk retinol levels upto 6 months after the women were supplemented with 300,000 IU of retinol palmitate. The proportion of infants with low serum retinol levels was less in the supplemented group as compared to the non-supplemented group.

We are proposing a study to investigate the relationship between the breastmilk content of retinol and β-carotene in Bangladeshi mothers and the serum levels of their infants.

13. Methodology of Protocol 92-025

A double blind control trial was planned on 60 middle class urban mothers of Dhaka who were vaccinated around 32 weeks of pregnancy either with the polysaccharide S pnuemoniae (Spn) or the Meningococcal meningitidis (Nm) vaccine. Gestational age, neuromuscular and physical maturity of the infants are assessed. Mothers are requested provide sera before and after immunization. Colostrum is collected within 48 hours of delivery and breast milk is requested at 1,3 and 5 months of delivery . Cord bloods are collected at delivery. Infants are providing 2 ml blood at the age of 6, 14, and 20 weeks. The family is being visited every fortnight for collection of data on morbity, mothers dietary history and the infants naso-pharyngeal swabs which are cultured for identification and serotyping of S pneumoniae.
The maternal-cord serum pairs will provide data on the transplacental transfer of antibodies against the vaccines. The infant sera will be used to determine the passively acquired antibodies. Nasopharyngeal cultures will be correlated with the presence and level of specific breast milk antibody and with infant serum antibody level.

Methodology of Present Study

This project will share data and blood samples collected in the above protocol. Colostrum is collected within 48 hours of birth together with spot breastmilk samples at 1, 3 & 5 months of birth.

For the purpose of this protocol we will get a full expression of the milk of one breast (20-40ml) which has not been sucked for the previous 2 hours. The time of collection will be mornings for all mothers so as to minimize the variability of breastmilk fat content[12].

Samples are collected and stored under proper conditions i.e. 70° C. It has been shown that the retinol levels in plasma are stable at -20°C upto 24 months[13]. The carotenoids however start degrading earlier (i.e. at 5 months). However, nothing is reported in the literature regarding the stability of retinol and carotenoid in breast milk.

Samples of 53 mother infant pairs will be compared for this study.

Retinol and β-carotene in breastmilk and serum will be determined by HPLC[14,15]. Total fat content in breast milk will be estimated by creamatocrit method[16].

Sample Size Calculation :

The following formula for a confidence interval for a correlation was used to calculate the sample size :

$$n = \left(\frac{z_1 - \frac{\alpha}{2}}{\frac{1}{2} \ln \frac{1+r}{1-r} - \ln \frac{1+L_1}{1-L_1}} \right)^2 + 3$$

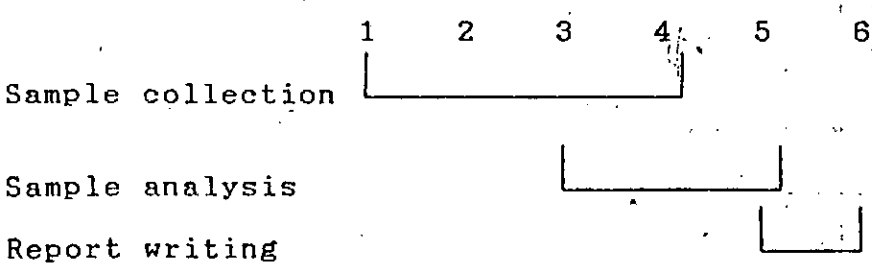
Where, r = expected correlation coefficient
L_1 = acceptable lower limit
Several assumptions were made including that a desired 'r' for biochemical outcomes would be 0.8 and that the minimum (lower C.I.) would be 0.7, $Z_1 - \frac{\alpha}{2} = 1.64$. Solving for 'n', the resulting sample size would be 53 total.

There are 60 mothers in the parent protocol.

Data Analysis

The serum and breastmilk levels will be compared for each mother-infant pair at each of several intervals. Correlation coefficients for these relations at each interval will be calculated and compared.

14. Time Frame - 6 months



15. Specific task of Investigator

Dr. Nigar Shahid, P.I. - Coordinating all the field and Laboratory activities and data analysis.

Ms. Amy Rice - Help in analysis of data and interpretation of results.

Mr. M.A. Wahed - Coordinate all the Laboratory activities i.e. vitamin A estimation of sera and breastmilk and help in interpretation of results.

16. Additional Budget

BUDGET

6 MONTHS

A.	PERSONNEL	%EFFORT	TOTAL (US$)
	Dr. Nigar S. Shahid	10%	858.00
	Mr. M.A. Wahed	10%	756.00
		Sub-Total:	1512.00
B.	SUPPLIES (STATIONERIES)		200.00
C.	MISCELLANEOUS		200.00
D.	INTERDEPARTMENTAL SUPPORT		
	Biochemistry and Nutrition -		
	53 mother - baby pairs		
	Serum:-Retinol - 159		804.00
	B-carotene - 159		1908.00
	Breastmilk: Retinol - 159		804.00
	B-carotene - 159		1908.00
	Total Operating Cost:		7336.00
	Indirect Cost:		2274.00
	Total Direct Cost: US$		9610.00

O.K. Shamima Hoi
27/10/93

References

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Abstract Summary (ERC)

Vitamin A deficiency is a major public health problem in developing countries and specially so in Bangladesh. Various strategies to combat vitamin A deficiencies are being advocated, one of them being the supplementation of mothers during pregnancy and lactation. The nutritional demand of suckling babies is expected to be met by the breastmilk of mothers alone as the young are not provided with vitamin A rich food. We wish to examine the relationship between breastmilk retinol and β-carotene levels and the infants serum vitamin levels.

1. The study will examine data and serum samples collected from an ongoing study - (92-025). For the purpose of vitamin A estimation in breastmilk, breast feeding mothers will be requested to provide a full extract of milk from one breast, as the fat content vis-a-vis the vitamin A content differ between the different parts of breastmilk.

2. NA

3. NA

4. All data will be coded and kept confidential and locked.

5. No potential risk to the subject.

6. No interview. Samples collection could take 5-10 mins.

7. The study will provide the missing data examining the relationship between the breastmilk content of vitamin A and serum levels in vitamin A in the mother and child pair.

8. Breast milk from lactating mothers.

International Centre for Diarrhoeal Disease Research, Bangladesh
GPO Box 128, Mohakhali, Dhaka-1212, Bangladesh

Consent Form for Vitamin A Protocol

Vitamin A deficiency is a major public health problem in developing countries. In breastfeeding infants the vitamin A requirement for the baby is obtained from the mothers breastmilk.

We wish to see whether there is a correlation between the infants' serum vitamin A levels with that of the breastmilk and serum of their mothers.

A full milk extract of one breast will be collected at the set intervals i.e. when your child is 1,3 and 5 month old. You are requested to withhold suckling of your baby from one breast for a period of 2 hours prior to collection. You will be free to feed your baby on the other breast during these two hours. A 2.0 ml blood is being drawn for pneumococcal vaccine study and we shall share 200 µl serum from that for doing retinol and β-carotene.

We expect you to participate in the study. Please put your signature/thumb impression in the following place. You may withdraw anytime from the study and will receive the same quality of care at ICDDR,B.

You are also at liberty to ask any question regarding the study.

Signature of subject _____

Signature of husband _____

Signature of Investigator _____

Date enrollment _____

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Bangladesh

'সম্মতি পত্র'
ভিটামিন "এ" প্রকল্প।

উন্নয়নশীল দেশে বিশেষ করে বাংলাদেশে ভিটামিন "এ"র অভাব একটি মারাত্মক সমস্যা, মায়ের দুধ-পানকারী শিশু মা'র বুকের দুধ থেকে প্রয়োজনীয় ভিটামিন "এ" পেয়ে থাকে, আমরা দেখতে চাই শিশুর রক্তের ভিটামিন "এ" মায়ের সংগে মা'র বুকের দুধ ও মা'র রক্তের ভিটামিন "এ" পরস্পর কতটুকু সহসম্পর্কযুক্ত।

আপনার বাচ্চার ৯মাস, ৩মাস ও ৫ মাস বয়সের সময় আপনার ১টি স্তন থেকে দুধ সংগ্রহ করা হবে। দুধ সংগ্রহ করার নিয়ম অনুযায়ী আপনি নির্দিষ্ট দিনে যে কোন একটি স্তন ২ঘন্টা শিশুকে পান করানো থেকে বিরত থাকবেন। ২ঘন্টা পর নির্দিষ্ট স্তন থেকে আমরা সবটুকু দুধ সংগ্রহ করবো। তবে অপর স্তনের দুধ সমান নিয়মে আপনি বাচ্চাকে অবশ্যই খেতে দিবেন। এবং বাচ্চার ৯.৫ মাস, ৩.৫ মাস ও ৫ মাস বয়সের সময় ২মি: লি: (2 ml) রক্ত নিউমেনিয়া-প্রতিরোধ ক্ষমতা পরীক্ষা করে দেখার জন্য সংগ্রহ করা হচ্ছে এবং সেই রক্ত থেকেই হেমোগ্লোবিন এবং বিটা ক্যারটিন-র মাত্রা দেখা হবে।

যদি আপনি আমাদের এই প্রকল্পে অংশগ্রহন করতে সিদ্ধান্ত নিন তবে নীচে আপনার দস্তখত/টিপসই দিন। যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করতে পারেন। সেই সময়ও আই, সি, ডি, ডি আর, বি আপনাকে একই রকম চিকিৎসা সুবিধাদি ও সেবা প্রদান করবে।

গবেষকের স্বাক্ষর: অংশগ্রহনকারীর স্বাক্ষর

নাম অন্তর্ভুক্ত করার তারিখ সাক্ষীর স্বাক্ষর