1. Title: Effect of zinc supplementation during pregnancy on infant birth weight, growth, morbidity and response to BCG.

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EFFECT OF ZINC SUPPLEMENTATION DURING PREGNANCY ON INFANT BIRTH-WEIGHT, GROWTH, MORBIDITY, AND RESPONSE TO BCG

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7. Abstract Summary:

Little is known about the effects of zinc deficiency during pregnancy on infant birth weight, growth, morbidity, and immunity. Although observational studies suggest a positive association between zinc status during pregnancy and pregnancy outcome, so far no controlled supplementation trials have been performed in developing countries.
We propose a study to examine the effects of zinc supplementation during the last six months of pregnancy on infant birth weight, growth, morbidity and immune response during the first six months of life. The study will be performed in the slums of Dhaka, where low birth weight is highly prevalent and where zinc deficiency is likely to be a problem.

A double-blind, community-based intervention design is proposed. Women will be identified through an already established identification system and enrolled at 4 months gestation. They will be stratified by parity and randomly assigned in two groups, receiving either 30 mg zinc/day or a placebo. Supplementation will continue until delivery. At 4 and 7 months gestation woman's plasma zinc concentration will be assessed by atomic absorption spectroscopy and hemoglobin concentration will be measured. Woman's weight and health will be measured monthly.
Infant anthropometrics and gestational age will be determined within 72 hours after birth. Infant anthropometry will be repeated monthly until the infant is 6 months old. Information on infant morbidity will be obtained two-weekly. At 1 and 6 months post partum infant plasma zinc status will be measured from a capillary blood sample and immune response for BCG will be tested by skin reaction on PPD.

8. Reviews:

i Ethical Review Committee: _____
(Approved/Not Approved)

ii Research Review Committee: _____
(Approved/Not Approved)

iii Director's signature and remark, if any:

A. INTRODUCTION

1. Goal and objectives

This study aims to determine the importance of zinc supplementation during the last six months of pregnancy on infant birth weight, health and growth during the first six months of life. The study will be performed in the slums of Dhaka.

The specific objectives are:

1. Determine the effect of zinc supplementation during the last six months of pregnancy on infant birth weight.

2. Determine the effect of zinc supplementation during pregnancy on infant growth during the first six months of life.

3. Determine the effect of zinc supplementation during pregnancy on infant cell-mediated immunity (CMI) as measured by response to BCG vaccination during the first six months of life.

4. Determine the effect of zinc supplementation during pregnancy on infant morbidity from infectious diseases during the first six months of life.

5. Determine the effect of zinc supplementation during the last six months of pregnancy on maternal and infant zinc status.

2. Background

Severe protein-energy malnutrition is seen in a relatively small proportion of the population compared to marginal nutritional deficiencies. Young children and pregnant and lactating women, in particular are most at risk for micronutrient deficiencies which may significantly affect their health, growth, development and reproductive outcomes (Neumann et al., 1994; Jameson, 1993). An inadequate intake of either macro- or micronutrients may directly result in failure to grow, but may also indirectly contribute to growth impairment through decreased immunocompetence and consequent increased burden or severity of infections. Impaired growth is one of the most consistent signs of malnutrition and, while it is not in itself hazardous to health, it may be an early marker of malnutrition, and associated with conditions of poverty and poor health, leading to high morbidity and mortality and irreversible developmental impairment.

Most research and intervention efforts are concentrated on the prominent micronutrient deficiencies of vitamin A, iron and iodine. Deficiencies of other micronutrients, however, may be equally prevalent and harmful, not in the least because of the interactions between many

micronutrients. The same dietary factors leading to iron deficiency for example, contribute to inadequate zinc nutriture (Allen, 1994; UNICEF, 1993). Murphy et al. (1992) estimated that virtually all Kenyan and most Mexican preschool children have inadequate intakes of zinc. The richest sources of bioavailable zinc are flesh foods. The bioavailability of zinc is decreased by phytate in the diet. Thus, zinc deficiency is likely where intake of animal foods is low and the prepared staple is high in phytic acid (UNICEF, 1993). This is likely to be the case in the urban slums of Bangladesh, where the intake of foods from animal origin is very low (Hassan and Ahmed, 1991) and where undernutrition is widely prevalent (Appendix I; Chen et al.,1980; Helen Keller International,1993-1994). The prevalences of malnutrition among children of the urban slums is even higher than among children of the rural poor (Hassan and Ahmed, 1991) or the non-slum urban children (Bangladesh Bureau of Statistics, 1994).

Bangladesh has among the highest incidence of low birth weight (LBW) in the world. The incidence of LBW is estimated to be 40-50% of all lifebirths in rural areas (Canosa, 1989). There is no birth weight data available for the slum population of Dhaka City, estimates from earlier studies conducted in the slums are around 40% (El Arifeen, personal communication). Zinc deficiency has been implicated in pregnancy complications, intrauterine growth retardation, low birth weight, increased perinatal mortality, reduced immunocompetence, and growth failure in infants and children (Gibson, 1994; Filteau and Tomkins, 1994).
Even in the absence of a linear growth response, for example in slow-growing age groups, zinc supplements may effect changes in body composition (Cavan et al., 1993). Moreover, as the influence of nutrition on growth already starts before birth, maternal zinc status can be an important factor affecting birth weight, breast milk quality, neonatal zinc status, morbidity and mortality. Zinc is also important for recovery of intestinal integrity during diarrhoea, and thus zinc supplements can limit the mucosal damage, growth faltering and subsequent morbidity caused by gastrointestinal infection (Roy et al.,1992).

Zinc

The importance of zinc in the nutrition of infants was recognized during the 1970s. It is necessary for growth, sexual maturity, wound healing and cellular immunity, and is a constituent of over 200 metalloenzymes (Hambidge et al., 1972). Zinc has a fundamental role in gene replication, activation and repression, is critical for transcription and translation, and affects nucleic acid metabolism. Zinc is especially needed in times of rapid growth. This is due not only to effects on gene replication and nucleic acid metabolism but also as a mediator of growth hormone action (Chesters, 1982).
The size of total body zinc store is extremely limited, so that there is a day-to-day requirement for dietary zinc (Wilkins et al., 1972). Requirements for zinc are increased during pregnancy for the growth and development of the fetus and maternal tissues. If 20% bioavailability of zinc is assumed, then the metabolic requirement for pregnant women is 13.0 mg Zn/day during the latter half of pregnancy (Gibson, 1994). Zinc is generally considered a relatively nontoxic metal. Homeostatic mechanisms regulate the body burden of zinc such that increased intake is associated with decreased absorption and increased excretion. Clear evidence of zinc toxicity in

human pregnancy has not been reported (Walsh et al., 1994).

The lack of sensitive and specific methods for assessing zinc status has been a major reason for exclusion of zinc from the list of micronutrients receiving public health attention. Plasma zinc concentration is recognized to be a relatively insensitive index of zinc nutritional status, but more sensitive indexes unfortunately have not been identified (Krebs et al., 1995). Currently the most reliable way to assess zinc status is to measure growth rate after zinc supplementation (Michaelsen et al., 1994).

Zinc and growth during pregnancy

While a few studies have investigated the effect of zinc supplementation during pregnancy on pregnancy outcome, the results are still not clear. Cross-sectional studies have suggested a relationship between maternal plasma zinc and birth weight, but only in women with low plasma zinc levels (Swanson and King, 1987). Plasma zinc has also been reported to correlate with pregnancy complications such as prolonged labor, hypertension, postpartum hemorrhage, spontaneous abortion, and/or congenital malformation (Gibson, 1994).
Eight controlled zinc supplementation trials in pregnant women are summarized in Table 1. All studies so far were performed in developed countries. Only one of the zinc intervention trials produced an effect on birth weight and head circumference (Goldenberg et al., 1995). In this study only women with low plasma zinc levels at enrollment were selected. In some studies, significant reductions in pregnancy complications have been observed in the zinc treated compared to the placebo group (Hunt et al., 1984; Cherry et al., 1989; Jameson et al., 1990; Simmer et al., 1991), perhaps in some cases associated with alterations in prostaglandin metabolism (O'Dell et al., 1977).
Several adaptive mechanisms exist during pregnancy to meet the increased demands for zinc, including an increase in zinc absorption, a reduction in endogenous losses of zinc, redistribution of tissue zinc, and an efficient maternal-fetal transfer of zinc (Swanson and King, 1987). Although such adaptive mechanism may be adequate in developed countries to prevent zinc deficiency, they may not be sufficient for pregnant women in developing countries, in whom zinc status may be especially poor because of frequent reproductive cycling, excessive losses of endogenous zinc, and diets low in readily available zinc (Gibson, 1994). Unfortunately, no zinc supplementation trials among pregnant women in developing countries have been implemented so far. However, in an observational study in Egypt, maternal zinc status during the second trimester of pregnancy and early pregnancy weight (3 months) formed the best predictor model of birth weight (Kirksey et al., 1994), whereas in Bangladesh, no association was found between maternal or cord zinc status and birth weight (Islam et al., 1991).

Table 1 Studies on effect of zinc supplementation in pregnant women.

Reference	Subjects duration	Treatment	Suppl. mg Zn/ day	Daily Zn from diet	Growth effect	Other responses
Hambidge et al. (1983) USA	46 middle income, 9-7 mo	not random. double blind placebo	15	11	None	None
Hunt et al. (1984) USA	213Hispanic low income, < 27 wk	random. double blind	20	9	Not measured	Decreased number of low serum zinc
Hunt et al. (1985) USA	138Hispanic teenagers, last 3 mo.	random. double blind	20	9.8	No effect on birth weight	None
Cherry et al. (1989) USA	556 poor adolescent last 3 mo.	random. double blind	30	?	No effect on birth weight	Reduced rates prematurity
Mahomed et al. (1989) UK	494 middle class, last 4 mo.	random. double blind	20	9	No effect on birth weight	None
Jameson et al. (1990) Sweden	1231	not random. double blind	15-90 (depending serum Zn)	9.4	Fewer preterm (= <33 wk)	Reduction perin.death +spont. abortions
Simmer et al. (1991) UK	56 risk of SGA infant	random. double blind	22.5	?	Lower incidence IUGR	Labor induced+C -section less often.
Goldenberg et al. (1995) USA	580 African-American, low plasma-Zn, >19 wk	random. double blind	25	12.8-13.1	Higher birthweight and larger head circumf.	None

Zinc and growth

The results of studies on zinc interventions to improve growth in developing countries are not consistent. Studies carried out up until now are reviewed in Table 2 and show that mild zinc deficiency may limit the growth potential in infants, children, and adolescents. In Middle Eastern countries, adolescent nutritional dwarfism characterized by delayed sexual maturity and poor growth is related in part to zinc deficiency (Prasad, 1976; Carter et al., 1969). However, a zinc

supplementation trial to adolescent dwarf boys in Egypt and Iran did not improve growth (Mahloudji et al., 1975; Ronaghy et al., 1969). Increasing zinc supplementation to 40 mg/day for two years however, produced a growth effect (Ronaghy et al., 1974). In Bangladesh, supplementation with zinc was performed in children, recovering from protein-energy malnutrition, resulting in a significantly higher increase in weight (Khanum et al., 1988; Simmer et al., 1988).

Zinc deficiency may also contribute to poor growth in otherwise healthy infants and children. Infants consuming zinc supplemented formula experienced better growth rates than the control infants fed the same formula without additional zinc (Walravens et al., 1992). Suboptimal zinc status is characterized by reduced physical growth, impaired appetite and diminished taste acuity (Hambidge et al., 1972; Buzina et al., 1980). In these studies, the children were otherwise healthy but short. Supplementation with zinc improved growth and produced an increase in height velocity (Walravens et al., 1983; Gibson et al., 1989).

In developing countries as well, certain studies have shown healthy, but growth retarded children to benefit from zinc supplementation. In China, 1-6 year old children with poor growth supplemented with 1-2 mg zinc/day for 6 months, significantly increased their weight and length (Xue-Cun et al., 1985). In Ecuador, children between 12 and 50 months of age were given 10 mg zinc/day for 15 months. Supplementation significantly increased the height and height-for-age Z-score (Dirren et al., 1994). In Chile, 68 small-for-gestational age infants were supplemented with 3 mg zinc/day from birth till 6 months of age. Zinc supplementation significantly increased weight and length gain (Castillo-Duran et al., 1995). In the Gambia (Bates et al., 1993) and Guatemala (Cavan et al., 1993) however, zinc supplementation to healthy, but growth retarded children did not improve growth but affected only body composition.

Some studies have shown that boys seem to benefit more than girls from zinc supplementation (Walravens et al., 1983; Schlesinger et al., 1992). However, in Ecuador a higher weight-for-age Z-score was observed in the supplemented girls but not in boys (Dirren et al., 1994) and the effect of zinc supplementation in Chilean infants was more pronounced in girls than in boys (Castillo-Duran et al., 1995).

Table 2. Studies on effect of zinc supplementation on growth in developing countries.

Reference	Age Status	Supplement mg Zn/day	Duration mo	Height gain	Weight gain
A. Malnourished infants					
Golden and Golden (1981) Jamaica	6-17 mo PEM	0.2-1.0 mg/kg	< 1	NS	+
Khanum et al. (1988) Bangladesh	5-60 mo PEM	10 mg/kg or 50 mg	?	NS	+
Castillo-Duran et al. (1987) Chile	8 mo marasmic	2 mg/kg	2	NS	+
Simmer et al. (1988) Bangladesh	12-84 mo marasmic	50	0.5	NS	+
Golden and Golden (1992) West Indies	6-31 mo marasmic	5	?	NS	+
Schlesinger et al. (1992) Chile	8 mo marasmic	10	3.5	+	+
Castillo-Duran et al. (1995) Chile	birth SGA	3	6	+	+
B. school boys/adolescents					
Carter et al. (1969) Egypt	11-19ygrowth /sex retarded	14	5.5	NS	NS
Mahloudji et al. (1975) Iran	6-12yrgrowth retarded	20	24	NS	NS
Ronaghy et al. (1969) Iran	12-18 yr low Wt/Ht	28	17	NS	NS
Ronaghy et al. (1974) Iran	13 yr malnouris.	40	18	+	+
C. Healthy infants and children					
Xue-Cun et al. (1985) China	12-72 mo short	1-2 mg/kg	6	+	+
Bates et al. (1993) The Gambia	7-27 mo all	20	15	NS	NS
Cavan et al. (1993) Guatemala	81.5±7.0 mo all	10	25 (wk)	NS	NS
Dirren et al. (1994) Ecuador	12-48 mo all	10	15	+	+(girls)

* This table is partly adapted from a table produced by C.E. West.

Zinc and immunity

The importance of zinc for immune function has been clearly demonstrated but its precise role is not always clear (Keen and Gershwin, 1990; Vallee and Falchuk, 1993). Severe zinc deficiency is accompanied by a markedly increased susceptibility to infection, but the effects of marginal zinc status are less well documented (Beisel, 1993).
In zinc deficiency, epithelial barrier functions are likely to be impaired, as skin lesions and mucosal defects are common findings. In animal models of zinc deficiency, athropy of lymphoid organs and lymphopenia have been noted (Keen and Gershwin, 1990). Lymphopenia is largely due to a reduced number of T-cells, but zinc deficiency also has major effects on B cell numbers and antibody responses (Fraker et al., 1993). Macrophage function in zinc deficiency is also impaired. That explains why, in zinc deficiency the delayed type hypersensitivity (DTH) skin reaction, which depends on the interaction of both macrophages and T-cells, is attenuated (Vallee and Falchuk, 1993).
Several studies have demonstrated a reduction in infant morbidity due to infections after zinc supplementation. Schlesinger et al. (1992) observed that a zinc-fortified formula improved linear growth and the immune response in 7 month old Chilean infants. In a study among preschool children in rural Mexico, supplementation with 20 mg zinc/day for 12 months reduced the number of episodes of diarrhea and respiratory diseases (Rosado et al., 1995). In rural Bangladesh, normal fullterm infants showed significantly better growth and less diarrhoeal morbidity after zinc supplementation (Talukder et al., 1991).

3. Rationale

Little is known about the effects of zinc supplementation during pregnancy on infant birth weight, growth, morbidity and immunity. Although results from observational studies suggest a positive association between zinc status during pregnancy and pregnancy outcome, so far no controlled zinc supplementation trials have been implemented in developing countries, where zinc intakes are habitually very low and where zinc deficiency is likely to be a problem.
We propose a study to examine the effects of zinc supplementation of pregnant women on pregnancy outcome in an area where LBW is highly prevalent. The results of such a supplementation study, will be useful in implementing health and nutrition programs and making existing programs more cost-effective, in a very poor urban community with a high incidence of LBW.

B. SPECIFIC AIMS

Hypothesis Testing

Hypotheses:

1. Zinc supplementation during the last six months of pregnancy will improve infant birth weight.

2. Zinc supplementation during the last six months of pregnancy will improve infant growth during the first six months of life.

3. Zinc supplementation during the last six months of pregnancy will improve infant cell mediated immunity during the first six months of life.

4. Zinc supplementation during the last six months of pregnancy will improve infant morbidity due to infectious diseases.

5. Zinc supplementation during the last six months of pregnancy will improve maternal and infant zinc status.

C. METHODS OF PROCEDURE

Study design

A prospective double-blind intervention design is proposed. Women will be enrolled when they are 4 months pregnant, will be stratified by parity and will be randomly assigned to one of two groups, zinc or placebo. The women will be prospectively followed from 4 months gestation until birth and their newborns from birth until 6 months postpartum. .

Study population and sample size

The study population will consist of all pregnant women living in five thanas of the former Urban Surveillance System (USS) area in Dhaka. They form a representative sample of households from Dhaka's slum population. The Urban Surveillance System was operated by the Urban MCH-FP Extension Project (UEP) of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The target population of the UEP consists of the slum population of five thanas (Mohammadpur, Lalbagh, Kotwali, Sutrapur and Demra) of Dhaka City. The total slum population in these five thanas is about 400,000. There are approximately 800 slums with sizes varying from 20 households each to many thousands (Paljor, 1991).

An identification system to identify pregnant women was established in the former USS areas for use in a previous study on "determinants of low birth weight, gestational age, and perinatal morbidity". A community worker has been assigned by UEP in selected clusters. In these clusters, the volunteer will be utilized as an identifier and report all pregnant women in her neighborhood to the fieldoffice. Each pregnant women should be identified before 16 weeks of gestation. The community workers will be intensively trained to ensure timely enrollment. The target population includes all pregnant women in selected clusters of the five thanas. In the previous studies on low birth weight in this population, a mean number of 150 pregnancies were identified monthly, therefore 600 pregnancies are expected to occur in this area in 4 months.

Since the last recruited woman will enter the study after 4 months and will have to be followed for 12 months (6 months prepartum and 6 months post partum), the total length of the data collection period will be 16 months.

Inclusion criteria

All pregnant women, living in the study area will be eligible for this study provided they: (a) are identified by the identification system, (b) meet the criteria of residence and length of gestation, (c) agree to participate, and (d) remain at/near their residence in Dhaka for the delivery.

Exclusion criteria

Women with an established medical risk for having reduced or excessive birth weight of infants (hypertension, renal disease, diabetes, thyroid disease, or other significant medical

complications such as heart disease) will be excluded from the study.

Data collection

Women will be recruited through the identification system before 4 months pregnancy and verbal informed consent will be obtained. At 4 months gestation, a standard questionnaire concerning age, date of last menstrual period, parity, number of live and dead children, level of schooling, religion, occupation, husband's occupation, and other socioeconomic variables will be administered to the women. A capillary bloodsample will be obtained and baseline data on anthropometry, zinc and haemoglobin will be collected. Women will be randomly assigned to the two treatment groups and supplementation will begin when women are 4 months pregnant. Supplementation will continue until delivery.

Women will be visited by health workers weekly for administration of a one-week supply of the zinc supplements (30 mg zinc /day in tablets). Compliance will be assessed by counting the remaining tablets at the next visit. Women will be visited by health workers monthly for weighing and health checks and for a qualitative dietary assessment on intake of foods rich in zinc. At 7 months of pregnancy, i.e., when supplements have been given for 3 months, their effects on pregnant women will be ascertained by repeating zinc and heamoglobin assays.

Infant weight, length, head, chest and arm circumference will be measured and gestational age will be estimated, within 72 hours of delivery. The infant will be given BCG vaccination, the vaccine will be provided by the standard EPI program. Infant morbidity during the previous two weeks from cough, diarrhea, fever, vomiting, ear, eye or skin problems, and refusal of food or breastmilk, will be determined two-weekly by standard questionnaire. Infant and maternal anthropometry will be repeated at monthly home visits until 6 months post partum. More detailed investigations will be conducted at the ICDDR,B clinic when the infant is 1 and 6 months old. Immune response for BCG will be tested by skin reaction on PPD (TBC microbacteria). A finger prick blood sample will be taken from infants for measurement of zinc status.

Figure 1 shows a time schedule for the different measurements and supplementation periods. Details of the data collected at all visits have been presented in Appendix II.

Figure 1. Time schedule

	pregnancy					deli-	postpartum		
	4mo	5mo	6mo	7mo	8mo	very	1mo	2mo.....5mo	6mo
Zn suppl mother	x----------x								
Maternal Zn status	x			x					
Maternal Hb	x			x					
Maternal anthrop.	x	x	x	x	x	x	x	x.....x	x
Mat. morbidity (weekly)	x	x	x	x	x	x			
Infant:									
Gestational age						x			
anthropometry						x	x	x.....x	x
morbidity (2 weekly)						x	x	x.....x	x
Zn status							x		x
Immune response							x		x

Anthropometrics

Anthropometric measurements of the mother will be taken monthly from baseline (4 months pregnancy) until 6 months post partum. The women will be measured to the nearest 0.1 kg on a bathroom weighing scale (Seca 770), their height will be measured to the nearest 0.1 cm on a length board. Triceps and biceps skinfold thicknesses and midupper arm circumference (to the nearest millimeter by TALC numeral insertion tapes) will be measured.

Anthropometric measurements of the infants will be taken monthly from birth until 6 months postpartum. The nutritional status of the children will be assessed by measuring nude or lightly clothed children. The children will be measured to the nearest 10 g on beam balance scales (Seca 725), which will be regularly calibrated against standard weights. Recumbent and supine lengths will be measured to the nearest 0.1 cm on a length board. The head circumference will be measured to the nearest millimeter by using TALC numeral insertion tapes. From these measurements 4 indicators will be derived: Weight for Height (WT/Ht), Weight for Age (Wt/Age), Height for Age (Ht/Age) and Head circumference. These indicators reflect acute and chronic undernutrition. The anthropometric measurements will be related to the US NCHS reference curve or another WHO reference curve (WHO working group, 1995) by standard deviation scores (Z-scores). According to outlines of the WHO (WHO working group, 1986) for children 0-5 years of age, underweight (low Wt/Age), stunting (low Ht/Age) and wasting (low Wt/Ht) are defined with the Z-score cut-off point at -2.00 and less (-2 SD of the reference population). Health workers will be extensively trained in the anthropometric measurements, and measurements will be repeated until minimum in-between variance is reached.

Randomization, blinding and zinc supplementation

When entering the study, at 4 months of pregnancy, the women will be randomly assigned into two groups. The randomization procedure will be stratified to ensure equal distribution of women according to previous pregnancies as parity is an important potential confounding factor. The study has a double-blind design, therefore registration of the women will be done by numbers, which will correspond with the letters or colours of the capsules (with either the supplement or the placebo) and all the registration cards and questionnaires. Only the company, who will provide the capsules, will know whether a certain letter/colour resembles the supplement or the placebo. The code linking the letters/colours with the supplement or placebo will only be known at the company providing the capsules, and will not be broken until the fieldwork has been finished. A register linking the numbers and letters with the names of the women will be kept locked at the UEP office.

At 4 months of gestation capsules of 30 mg of elemental zinc (zincacetate) will be supplemented daily among half of the pregnant women. Zinc acetate in general, results in less nausea and gastro-intestinal irritation then other salts (UNICEF, 1993). The amount of 30 mg zinc/day is based on the recommended daily intakes for zinc during pregnancy (Gibson, 1994) and is in accordance with most previous supplementation trials among pregnant women. The other women will receive a placebo, containing an inert substance (sucrose, maize starch, cellulose). Supplement and placebo will be indistinguishable in appearance and taste. The zinc content of the supplements will be regularly verified independently in the ICDDR,B laboratory. Health workers will deliver a one week supply of the supplement or the placebo at the houses weekly. They will instruct the women to consume the capsules between the meals and not together with other vitamin/mineral supplements (mostly iron). Compliance will be checked by counting the remaining tablets at the next visit. It will be registered if the woman had diarrhoea or had been vomiting after the consumption of the tablets. Regular compliance checks will be done by unexpected visits, in-between the weekly visits in a 10% sample. Healthworkers will count the remaining capsules during these visits.

Laboratory analyses

A capillary bloodsample from the mothers at 4 and 7 months pregnancy and a capillary bloodsample from the infant at 1 and 6 months post partum will be taken for the zinc assessments. The assays will be performed at the ICDDR,B laboratory, where the methods have been established. Blood sampling will be performed by trained medical nurses from the Clinical Science Division of ICDDR,B. Fasting morning specimens will be obtained for plasma zinc determination by finger prick using trace mineral-free lancets and put into heparinized (50 IU/ml) plastic tubes. Plasma will be immediately separated by centrifugation (600 x g for 5 min) and 200 μl of plasma will be stored at -20C until analyzed.

Zinc status will be estimated from plasma zinc concentrations analyzed on a flame atomic absorption spectrophotometer (Pye Unicam SP9; Hackley et al., 1968).

Hemoglobin (Hb) will be analysed in whole blood (Hemocue, Helsingborg, Sweden). Samples with hemoglobin concentration of <11g/dL will be defined anemic (WHO/UNICEF/UNU, 1993) and weekly iron supplementation will be provided. Women

will be instructed not to take the irontablets together with the zinc supplements, to avoid potential competition between zinc and iron for intestinal absorption (Vallee and Falchuk, 1993).

Birth weight and gestational age

Birth weight will be measured within the first 72 hours after birth. This limit was chosen as a feasible and still valid field measure of birth weight in Bangladesh in the previous studies. Standardized equipment and procedures will be used for the measurements. The infants will be weighed on a portable beam balance scale, accurate to the nearest 10 g.

Gestational age of the infants will be assessed using the method described by Capurro (1978). This is a simplified version of the Dubowitz (1970) method, using only five somatic signs of maturity and is thus easier to use in the community. The correlation found between the score obtained by Capurro and the time of amenorrhea was similar to that obtained by Dubowitz (Capurro et al., 1978). The Capurro method has been successfully used in developing countries, in both clinical and field situations (Ferraz, 1990). However, it has been observed that gestational age assessments overestimate gestational age in the preterm and underestimate in the postterm baby (Alexander,1990). In a previous ICDDR,B study on birth weight and infant mortality, the Capurro method was validated against the recorded LMP in a hospital setting. Gestational age assessments with the Capurro method were +/- 2 weeks of the recorded LMP's (El Arifeen, personal communication).

Infants showing low birth weight (< 2500 g) can be subdivided into two subgroups: preterm (i.e. < 37 weeks gestation) and small for gestational age or intrauterine growth retardated (IUGR) (Canosa, 1989). IUGR newborns will be classified in accordance to their Ponderal Index (PI = weight x 100/ length^3), where type I neonates will be those exhibiting a normal PI at birth and a normal reduction in height, weight and head circumference ("symmetric" ;stunted), while type II neonates will exhibit a low PI at birth and a reduction in weight relative to their length and head circumference ("asymmetric"; wasted) (Pérez-Escamilla and Pollitt, 1992; Adair, 1989).

Assessments of birth weight and gestational age will be done by the same doctors who have been working on previous studies on "determinants of low birth weight, gestational age, and morbidity" and "birth weight and infant mortality"at ICDDR,B. These doctors had extensive training on measuring birth weights and on the use of the Capurro method followed by periodically reliability checks.

Notification of births within 72 hours after birth might be delayed. However, since the supplementation schedule requires weekly visits, notification of births will not be delayed by more than one week.

Response to BCG

All children in the study-area will receive the standard vaccination with Bacille Calmette Guerin (BCG) at birth. BCG vaccines will be provided by the local EPI program and consist of 0.05 ml reconstituted live freeze-dried vaccine made from an attenuated strain of *Mycobacterium bovis* (Japan BCG Laboratory) and trained medical doctors will vaccinate the children intradermal with BCG at the 72 hour post partum visit.

At 1 and 6 months postpartum the response on BCG vaccination will be checked by looking at scar formation. About 2-3 weeks after intradermal injection of a potent vaccine, a papule develops at the site of the vaccination, which slowly increases in size and then breaks into a shallow ulcer. This heals spontaneously leaving a tiny scar, which is a normal reaction (Dam et al., 1976).
Cell mediated immuneresponse of the infant will be tested in vivo at 1 and 6 months post partum by delayed hypersensitivity skin test (DTH) on purified protein derivate (PPD) tuberculosis obtained from the State Serum Institute in Copenhagen, Denmark. Tuberculin testing and reading will be performed by trained health workers. They will apply tuberculin solution (0.1 ml) to the volar surface of the forearm using an Omega glass PPD syringe with platinum needles. After 72 hours the size of the duration will be read in millimeters as the transverse diameter of induration developed. A cutaneous reaction will be considered positive when an induration > 5mm is observed. Infants negative to the tuberculin test at the age of 1 months of age will be re-immunized with BCG and tuberculin test will be repeated at 6 months of age.
Healthworkers will be extensively trained and standardized in the administration and interpretation of PPD skin testing. Reproducibility of skin test reading will be tested before the study starts in 30 hospital patients with tuberculosis. Variability between reading by the same health worker and between the different health workers will be measured.

Morbidity and mortality
Questions about maternal morbidity during the past week will be asked weekly during the compliance visits and information on dietary zinc intake of the women will be assessed monthly during pregnancy. A qualitative 24 hour dietary recall will be administered and seperate questions on the intake of foods known to be rich in zinc (meat, shellfish, pulses) will be asked.
Questions about infant history in the past two weeks of fever, diarrhea, acute respiratory infections, vomiting, ear, eye, or skin problems and refusal of food or breastmilk will be asked two-weekly after delivery. Case-definitions for pneumonia, asphyxia, respiratory distress syndrome, measles, and diarrhoea according to international standards are given in Appendix III. If the child dies in the period between two visits questions about the exact date and cause of death will be asked if possible.
To collect information on morbidity and mortality, the same questionnaire will be used for this study as has been used in the previous studies on birth weight and infant mortality. This questionnaire have been pretested previously.

All information will be obtained verbally from the mother or caretaker of the child. Trained healthworkers will conduct all interviews and assessments. They will be assisted by a female helper. This study will use the same healthworkers as the previous study on "birth weight and infant mortality in the slums of Dhaka city". These interviewers have been trained at a maternity clinic and a children's hospital on anthropometric measurements and have undergone fieldtraining on the use of questionnaires, morbidity follow-up and verbal autopsy. They will be repeatedly trained for this study, and measurements will be repeated

until minimum in-between variance is reached. The reliability of these measurements will be periodically verified throughout data collection.

Logistics

For the enrollment of the women the same procedure will be followed as has been used in the other studies on "the determinants of low birth weight and perinatal mortality" and " the effect of low birth weight on infant mortality". A pregnant woman should be identified by community workers before 16 weeks of pregnancy. The community workers will be trained to ensure timely enrollment. An interviewer will visit the woman between the 16th and 18th week of pregnancy to describe the study, enlist the woman's participation, and take the baseline measurements. Each participant will receive a participants card.
In order to detect births the community worker will be utilized as a reporter. She will be encouraged to report all births in her neighborhood to the field office to ensure maximum reporting of births. The volunteers will notify the UEP office, which will be open every day. All volunteers will be encouraged to notify the office in time and will receive travel reimbursement.
Some of the births in this population may take place in hospitals or clinics. Arrangements has been made to identify those births and to visit the mothers and children in the hospitals. Anthropometric measurements at birth will take place according to the procedures of the previous studies.

The measurements at 4 and 7 months of pregnancy and at 1 and 6 months post partum include bloodsampling and skintesting. Therefore these measurements will take place in the ICDDR,B hospital. After centrifugation the bloodsamples will be stored properly at -20C for examination. The other measurements will take place during home visits.

All attempts will be made to undertake the visits on time, as required by the protocol. Visits may be made within ±1 week of the schedule. For participants who move out of the USS clusters, but are still in Dhaka City, whenever possible, their addresses will be obtained from family members or neighbors in an attempt to continue follow-up of these participants outside the USS area.

For logistic reasons, a female helper will accompany the interviewer on the monthly visits. The female helper will not only assist in the transport of the equipment but also during actual measurements. The female helper and the interviewer will use autorickshaws/rickshaws for transporting the equipment to the slums.

Definition of variables

An overview of the main outcome variables for mother and infant is given in Table 3. Operational definitions of these variables are given in Appendix IV. This study will also use some additional baseline information. These variables are listed in Appendix V and will be used as covariates in the multivariate analyses.

Table 3. Main outcome variables for mother and infant.

Variable	Definition
A. Mothers	
Nutritional status	Maternal weight in kg, height in cm, and MUAC in mm
δ Nutritional status	Maternal weight (kg) and MUAC (mm) between 5 and 7 months pregnancy
δ Maternal zinc status	Maternal plasma zinc concentration between 5 and 7 months of pregnancy
B. Infants	
Birth weight	Birth weight in 0.01kg, 72 hours after birth
Gestational age	Gestational age according to the Capurro method
Infant growth	Infant weight (10 g), length (0.1 cm), headcircumference, chest circumference and MUAC (mm), length/age, weight/age, and weight/length at 1-6 mo of age
Infant immune response	Scar formation after BCG vaccination and skinreaction on PPD at 1 and 6 mo of age.
Infant morbidity	Number and duration of episodes from fever, pneumonia, respiratory infections, measles and/or diarrhoea.
Infant zinc status	Infant plasma zinc concentration at 1 and 6 mo of age.

Power and sample size of the study

Sample sizes were estimated using available data in order to detect differences between treatment groups of at least 80% power and 95% significance. The following assumptions have been used for important differences in primary outcome variables: a 10% increase in mean birth weight, 15% increase in mean weightgain during the first six months of life, a 15% decrease in mean number of epsiodes from diarrhoea, 40% reduction in the decline of maternal plasma zinc concentration during the last six months of pregnancy, and a 20% increase in mean plasma zinc concentration among the infants in the first 6 months postpartum. Assumptions were made, based on available data, to calculate sample sizes (Appendix VI).

The following formula for sample size calculation was used, considering type I and type II errors of 0.05 and 0.20, respectively:

$$n_p = \frac{2(Z_\alpha + Z_\beta)^2 \sigma^2}{(\mu_p - \mu_z)^2}$$ (Modified from: Meinert, 1986)

$n_z = n_p$

$N = (r+1)n_p$

where μ_p = true mean of the outcome measure for placebo-treated women,

μ_z = true mean of the outcome measure for zinc-treated women,

σ^2 = variance of the outcome measure for a single individual (assumed to be the same for all women in both treatment groups).

Estimated sample sizes required (per group) will be 133 for the number of episodes from diarrhoea, 128 for change in weight gain, 98 for the infant plasma zinc concentration, 63 for birth weight and 26 for the reduction in maternal plasma zinc concentration (Appendix VI). Given these estimates, 150 women per treatment group will be required. Little data is available on which to base sample size calculations for the expected number of negative and positive responders to the PPD test. However, available information from other studies (Sazawal et al., 1995), suggests a sample size of 150 per treatment group should be sufficient to detect differences of about 50% in mean skin duration (in mm) between groups.
Assumptions were made on an expected 5% refusal, 20% loss to follow up during pregnancy, 5% miscarriages/still births, 10% dropout postpartum and 8% infant mortality. Therefore 150/(0.95x0.80x0.95x0.90x0.92)=250 subjects per group will be recruited. The expected number of life births for 500 recruited women will be (0.95 x 0.80 x 0.95)x 500 = 380.

Data analysis

All questionnaires and data forms will be reviewed by the investigators for completeness and accuracy. When possible, an interviewer will return to the field to clarify answers, or collect missing information. After editing, the data will be entered into a database. Epi-Info 6.0 will be used for data entry, this program incorporates range and consistency checks. Data will be periodically checked by running frequencies to identify outliers.

Descriptive statistics (mean, median, variance, frequency tables) will be used to assess the distribution characteristics of the independent (supplementation) and the outcome variables. Some of these variables will then be recoded and grouped into categories for bivariate analyses as appropriate. Estimates of rates and proportions will be expressed within 95% confidence intervals.

Bivariate associations will be identified using the chi-square test and other appropriate tests. Some adjusted bivariate associations or interactions will be estimated by stratifying the data on potential confounding characteristics. A test on normality (Kolmogorov-Smirnov) will be

performed on the outcome variables.

The main hypotheses of this study will be tested by measuring the differences between the supplemented and non supplemented groups in means of the outcome variables (birth weight, immune response, growth). Student t-tests (non-paired) or the non-parametric Kruskal-Wallis test will be used for this purpose. To assess differences between initial and final values, Student's paired t-test or Wilcoxon signed-rank test will be performed. Multivariate variance analysis models (MANOVA) will be used to control for the simultaneous effect of several multiple confounding factors and identification of interactions. Several different models will be created by including potentially confounding and interacting variables, e.g. age, socioeconomic status, morbidity, height of the mother etc.

Analyses will be done using the statistical programs SPSS/PC+ and Epi-Info 6.0.

Ethical Implications, Informed Consent and Confidentiality.

Verbal informed consent will be obtained from each participating woman after thorough explanation of the purpose of the study, requirements of participation, risks and benefits to the participant. Because the vast majority of the study population is illiterate, verbal consent is the only option.

Women will be supplemented with 30 mg zinc/day. This amount of zinc is based on twice the RDA and there is no evidence of any teratogenic effects for either the mother or fetus, due to this amount of zinc. However, in the rare event of any signs of possible zinc-related toxicity (headache, nausea, stomach cramps, and diarrhea), supplementation will immediately be stopped.

All women and infants requiring medical treatment will be referred to the nearest health care facility with whom collaborative arrangements for such referral have been made during the previous studies. Arrangements will be made for treatment at the nearest hospital whenever necessary. Women defined anemic (Hemoglobin < 11 g/dL) will be supplied with weekly irontablets.

Some of the information collected might be considered sensitive in nature. Therefore, to protect each participant's privacy, interviewers will conduct all interviews in private, exceptions only being made if the respondent insists on the presence of others. Each questionnaire and data form will be given an identification code. The linkage between the code and the study participant's name will be kept in a locked register at UEP office. All staff and interviewers will be counseled about the sensitive nature of some of the information collected, and on the need for strict confidentiality of the records.

D. SIGNIFICANCE

Very little is known about the effect of zinc supplementation during pregnancy on infant birth weight, growth, morbidity and immunity. Although supplementation trials have been performed in industrialized countries, to date, virtually nothing is known about effect of zinc supplementation during pregnancy in developing countries where the prevalence of zinc deficiency, low birth weight and growth retardation are very high. As far as we are aware, the proposed study would be one of the first zinc supplementation trials among pregnant women in a very poor urban community with a high prevalence of low birth weight.

Information derived from this study will be essential for planning and implementating health intervention programs aimed at reducing the incidence of low birth weight, improving maternal health, and improving infant growth and immunity. The information derived from this study can also be used to make existing programs more cost-efficient.

We expect that the information obtained from the proposed study will increase the understanding of the importance of zinc supplementation during pregnancy on infant birth weight, growth, immune response, and morbidity in urban populations of developing countries.

E. FACILITIES REQUIRED

Existing facilities within the Urban MCH-FP Extension Project and Clinical Science Division will be utilized for this study. Office space and administrative support already available in the project will be adequate for this study. Laboratory equipment of the Clinical Science Division will be used for this study. We do not anticipate excessive demands on project resources. However, the necessary chemicals and laboratory supplies will have to be purchased for this study. Finally, additional personnel would be needed.

F. COLLABORATIVE ARRANGEMENTS

Collaborative arrangements will be worked out with Azimpur Maternity Clinic and with Dhaka Shishu (children's) Hospital for referral and treatments of study participants and their mothers.
Collaboration between the principal investigators of the previous studies on "determinants of low birth weight, gestational age and perinatal morbidity" and "birth weight and infant mortality" has been ongoing.

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APPENDIX I

AVAILABLE INFORMATION ON INFANT NUTRITIONAL STATUS IN BANGLADESH.

Prevalences of wasting (weight-for-height/WFH), stunting (height-for-age/HFA) and underweight (weight-for-age/WFA) of urban and rural children aged 6-11 months in Bangladesh

	Yes	No
WFH < -2 Z score	6.9%	93.1%
WFA < -2 Z score	47.5%	52.5%
HFA < -2 Z score	34.8%	65.2%

(Source: Bangladesh Bureau of Statistics, 1994)

Z-scores for WFH, WFA, HFA and MUAC found in children aged 6-59 months in urban Bangladesh

	Score ± SD*	Score ± SD**
WFH (Z-score)	-1.13 ± 0.89	-1.2 ± 0.8
WFA (Z-score)	-2.29 ± 1.01	-2.4 ± 0.9
HFA (Z-score)	-2.37 ± 1.39	-2.6 ± 1.2
MUAC (Z-score)	-	-2.4 ± 0.9
MUAC (mm)	138.2 ± 12.3	141.2 ± 12.4

(Sources: * Bangladesh Bureau of Statistics; 1994
** Helen Keller International; 1993-1994)

APPENDIX II

DETAILS OF DATA COLLECTED AT EACH VISIT

Each mother-child unit will be visited at home at enrollment, 5, 6 and 8 months pregnancy, at birth, and two-weekly after birth till 6 months postpartum. At 4 and 7 months pregnancy and at 1 and 6 months postpartum, women will be asked to come to ICDDR,B hospital for more detailed examinations. Information collected at these visits is as follows:

Enrollment:

-Women will be checked for signs of abnormal pregnancy, or serious chronic illness; they will not be enrolled if they show any of these signs.
-Information will be obtained by a standardized questionnaire on: date of Last Menstrual Period (LMP); parity, number of live and dead children, education (woman and husband), employment (woman and husband), income, religion, dwelling structure, assets, number living in the house, and water and sanitation facilities.

Visits at 4 and 7 months pregnancy (hospital):

-Women's weight, height, and midupper arm circumference will be measured using standardized equipment.
-Women's plasma zinc concentration will be measured from a capillary bloodsample (0.5-1 ml) by atomic absorption spectroscopy.
-Women's Hb concentration will be measured from a capillary bloodsample.
-Information will be collected on illness symptoms, injuries and food intake.

Weekly visits:

-After the first visit to the hospital, supplementation will start.
-A one week supply of tablets will be delivered at the women's houses, each week the remaining tablets from the previous week will be counted.

Visits at 5, 6, and 8 months pregnancy (home):

-Women's weight and MUAC will be measured using standardized equipment.
-Information will be collected on illness symptoms, injuries, and food intake.

Visit at 72 hours after birth:

-Information will be collected on perinatal outome, i.e., whether live birth or still birth, whether still alive or dead, and if dead: cause of death and gender.
-Infant will be physically examined to detect congenital anomalies.
-Gestational age will be assessed by using the non-invasive Capurro method.
-Infant's head, arm, and chest circumference, weight and length will be measured using standardized equipment.
-A simple clinical examination will be performed on the infant including respiratory rate counts, signs of respiratory distress and dehydration, and rectal temperature.
-Information will be collected on the dietary intake of the child since birth by a simple food frequency questionnaire. Breast feeding patterns will be classified as follows: *exclusive
*predominant (addition of water or non-nutrious drinks)
*partial (breastmilk and other food)
*lack (complete lack or token breast feeding)

APPENDIX II (Cont.)

Visit at 72 hours after birth (cont.):

-Information on the delivery (location, difficult or not, cord prolapse or twisting, expulsion of placenta, bleeding etc), and on symptoms of toxaemia and/or eclampsia during the pregnancy will be obtained by standardized questionnaire.
-The infant will be vaccinated with 0.05 ml BCG in the left upper arm.

Two-weekly visits:

-Information on infant morbidity in the past two weeks will be obtained using a structured questionnaire. In addition to interviewing the mother about morbidity, a simple clinical examination will be performed on the infant, including respiratory counts, signs of respiratory distress and dehydration, and rectal temperature.
-Any changes in breast feeding and intake of other food since the last visit will be noted along with time of changes.
-In case of death since the last visit, the cause of death will be determined.
-The mother will be asked about her current pregnancy status and if pregnant again, her Last Menstruation Period date will be recorded.

Visits at 1 and 6 months post partum (hospital):

-Infant weight, length, head, arm, and chest circumference will be measured using standardized equipment.
-Mother's weight and MUAC will be measured using standardized equipment.
-Infant's plasma zinc concentration will be measured from a capillary bloodsample by atomic absorption spectroscopy.
-Infant's immune response will be assessed by checking scarformation at the place of BCG vaccination and by Delayed Hypersensitivity Skin Test (vaccinating the infants intradermal with PPD, examine skin response three days later).
-Information on infant morbidity in the past two weeks will be obtained using a structured questionnaire. In addition to interviewing the mother about morbidity, a simple clinical examination will be performed on the infant, including respiratory counts, signs of respiratory distress and dehydration, and rectal temperature.
-Any changes in breast feeding and intake of other food since the last visit will be noted along with time of changes.

Visits at 2,3,4 and 5 months post partum (home):

-Infant weight, length, head, arm, and chest circumference will be measured using standardized equipment.
-Mother's weight and MUAC will be measured using standardized equipment.
-Information on infant morbidity in the past two weeks will be obtained using a structured questionnaire. In addition to interviewing the mother about morbidity, a simple clinical examination will be performed on the infant, including respiratory counts, signs of respiratory distress and dehydration, and rectal temperature.
-Any changes in breast feeding and intake of other food since the last visit will be noted along with time of changes.

APPENDIX III

CASE DEFINITIONS FOR MORBIDITY

Pneumonia

Symptoms and signs used in the diagnosis of pneumonia can be classified into three categories:

I Cough and difficult breathing
II Chest indrawing and rapid breathing (if <2 months old then ≥ 60/minute, if > 2 months old then ≥ 50/minute).
III Convulsions, noise during breathing (stridor, wheezing), nasal flaring, fever (≥38C), central cyanosis (blue lips/skin), whether feeling well, able to drink, and abnormally sleepy or difficult to wake.

Infants will be considered to have **pneumonia** if the following minimum number/signs are present, i.e., if there is a history or presence of either:

- any one symptom/sign from each of the categories, or
- any one symptoms/sign from category I and both symptoms/signs from category II.
- both syptoms/signs from category I and any one symptom/sign from category II.

An episode of pneumonia shall be considered a new incident case of at least 7 days free from any symptoms or signs seperate it from the previous episode.

(Source: WHO (1991) Acute respiratory infections in children: Case management in small hospitals in developing countries. A manual for doctors and other senior health workers. Program for the Control of Acute Respiratory Infections. World Health Organization.)

Measles

An infant will be diagnosed as having measles if there is history or presence of fever, generalized rash lasting 3 or more days and either cough, coryza or conjunctivitis.

(Source: Centers for Disease Control (1990) Measles. In: Case definitions for Public Health Surveillance MMWR. 39(RR-13):23.;
Cutts, F. (1990) Measles Control in the 1990s: Principles for the next decade. Expanded Program on Immunization. World Health Organization. WHO/EPI/GEN/90.2.)

APPENDIX III (cont.)

Diarrhoea

Diarrhoea is conventionally defined as occurrence of loose or liquid stool 3 or more times per day or bloody stools. However, this definition is very unlikely to be appropriate for the very young, breastfed infant. Therefore, the mother's perception of the episode will be used to define diarrhoea. If, according to the mother, the child's stool was unusually frequent and unusually loose then this will be defined as diarrhoea. Presence of blood will be classified as diarrhoea if there was at least one loose stool. At least 3 days free of symptoms and signs shall separate episodes.

(Source: Baqui, A.H., Black, R.E., et al. (1991) Methodological Issues in Diarrhoeal Diseases Epidemiology: Definition of Diarrhoeal Episodes. Int J Epidemiol. 20, 1057-1063.)

Respiratory Distress Syndrome (RDS)

The following history will be used to reach a diagnosis of RDS (modified from Bhakoo et al., 1975).

- Respiratory distress within 4 hours of birth, and gets steadily worse. Respiratory stress is characterized by difficult breathing, rapid breathing ($\geq$60 per minute) or chest retractions.
- Improvement of respiratory distress after 48-72 hours.

(Source: Bhakoo, O.N., Narang et al. (1975) Neonatal moribidity and mortality in hospital born babies. Ind Pediatr. 12, 443-450.)

Perinatal Asphyxia

A history of the following will be used to reach a diagnosis of perinatal asphyxia (modified from Cochran, 1990).

- Baby showed some signs of life after birth
- Baby did not cry or cried weakly after birth
- Baby's breathing was weak and irregular (not distressed)
- The baby did not suckle normally
- Baby was lethargic, floppy
- Baby's skin was bluish

(Source: Cochran, W.D. (1990) Management of the normal newborn. In: Oski, F.A. (ed). Principles and Practice of Pediatrics. JB Lippincott Co. Philadelphia, p276-281.)

Other conditions

Current medical guidelines will be used to define morbidity episodes due to other causes.

APPENDIX IV

OPERATIONAL DEFINTIONS OF OUTCOME VARIABLES

Table 1a Basic data collected in present study

Outcome variables	Type	Definition
Height mother	Continuous	Height of the mother in cm measured at 4 months gestation.
Weight mother	Continous	Weight of the mother in kg measured at 8-4 months gestation.
MUAC mother	Continous	Mid Upper Arm Circumference (MUAC) of the mother in mm measured at 8-4 months gestation.
Zn mother	Continous	Plasma zinc concentration (μmol /ml) of the mother assessed at 7 and 4 months gestation.
Morbidity mother	Binary (presence/absence) Categorial (type of illness/complication)	Maternal self report of illness symptoms or complications experienced during pregancy, assessed at 8-4 monhs gestation.
Birth Weight	Binary (<2500g/ ≥ 2500g) Continous	Birth weight of the child (in g) measured within 72 hours after delivery.
Gestational age	Nominal (preterm /IUGR/NBW) Continous	Gestational age of the child (in weeks, weight for gestational age) assessed within 72 hours after delivery.
length $infant_{1-6}$	Continuous	Length of the infant in cm measured at 1-6 months postpartum
weight $infant_{1-6}$	Continuous	Weight of the infant in kg measured at 1-6 months postpartum
headcircumference $infant_{1-6}$	Continuous	Head circumference of the infant in mm measured at 1-6 months postpartum.
chestcircumference $infant_{1-6}$	Continuous	Chest circumference of the infant in mm measured at 1-6 months postpartum.

APPENDIX IV (Cont.)

OPERATIONAL DEFINTIONS OF OUTCOME VARIABLES

Table 1b Basic data collected in present study

Outcome variable	Type	Definition
MUAC $infant_{1-6}$	Continous	Mid Upper Arm Circumference of the infant in mm measured at 1-6 months postpartum.
Morbidity $infant_{1-6}$	Continuous	Total days and total number of episodes of specific morbidity summed across the recall periods of each of the two-weekly visits.
Immune response $infant_{1,6}$	Binary (induration <2 mm/ ≥2 mm) Continuous	Induration of skin (in mm) measured 72 hours after vaccination with PPD at 1 and 6 months postpartum.
Zn $infant_{1,6}$	Continuous	Plasma zinc concentration (in μmol/ ml) of the infant assessed at 1 and 6 months postpartum.

APPENDIX IV (Cont.)

OPERATIONAL DEFINITIONS OF GROWTH VARIABLES DERIVED FROM OUTCOME VARIABLES.

Table 2a Derived data

Outcome variable	Type	Definition
δ Weight mother	Continuous	Weight gain of mother in kg measured between 8 and 4 months gestation.
δ MUAC mother	Continuous	MUAC-gain of mother in mm measured between 8 and 4 months gestation.
δ Zn mother	Continuous	Change in plasma zinc concentration (μmol /ml) of mother assessed between 7 and 4 months gestation.
PI	Continuous	Ponderal Index of infant (weight x 100/ length^3) at birth.
IUGR-type	Binary (type I/ type II)	Classification of IUGR infants at birth according to their PI (normal PI=type I; low PI = type II)
Z-score Wt/Age_{1-6}	Continuous	Standard Deviation Score of the reference distribution (NCHS population) for Weight for Age at 1- 6 months postpartum.
Z-score Lt/Age_{1-6}	Continuous	Standard Deviation Score for Length for Age at 1-6 months postpartum.
Z-score Wt/Lt_{1-6}	Continuous	Standard Deviation Score for Weight for Length at 1-6 months postpartum.
δ $Length_{infant\ 6-1}$	Continuous	Difference in cm of length of the infant between 6-1 months postpartum.
δ $Weight_{infant\ 6-1}$	Continuous	Difference in kg of weight of the infant between 6-1 months postpartum.
δ Z-score Wt/Age_{6-1}	Continuous	Difference between 6-1 months postpartum in Z-score Weight for Age.

APPENDIX IV (Cont.)

OPERATIONAL DEFINITIONS OF GROWTH VARIABLES DERIVED FROM OUTCOME VARIABLES.

Table 2b. Derived data

Outcome variable	**Type**	**Definition**
δ Z-score Lt/Age_{6-1}	Continuous	Difference between 6-1 months postpartum in Z-score Length for Age.
δ Z-score Wt/Lt_{6-1}	Continuous	Difference between 6-1 months postpartum in Z-score Weight for Length.
δ $Headcircumference_{6-1}$	Continuous	Difference in mm of infant's headcircumference between 6-1 months postpartum.
δ $Chestcircumference_{6-1}$	Continuous	Difference in mm of infant's chestcircumference between 6-1 months postpartum.
δ $MUAC_{6-1}$	Continuous	Difference in mm of infant's mid upper arm circumference between 6-1 months postpartum.

APPENDIX V

OPERATIONAL DEFINITIONS OF CO-VARIABLES AVAILABLE FROM ADDITIONAL QUESTIONNAIRE AND BLOOD ANALYSIS

Variable	Type	Definition
Maternal Hb	Binary (anaemic, i.e. <11 g/dL/ not anaemic) Continuous	Hemoglobin concentration of the mother in g/dL at 4 and 7 months gestation.
Maternal age	Continuous	Maternal age in years (based on maternal self-report)
Parity	Binary (primipara/multipara) Continuous	Number of prior live births, based on maternal self-report.
Gravidity	Binary (primipara/multipara) Continuous	Number of prior pregnancies based on maternal self-report.
Prior poor pregnancy outcome	Binary (yes /no) Continuous	Number of prior stillbirth or miscarriage, preterm delivery, LBW, or perinatal death, based on maternal self-report.
Interpregnancy interval	Continuous	Number of months between prior pregnancy and this pregnancy, based on maternal self-report.
Maternal/Paternal education	Continuous	Actual measures of years of schooling.
Maternal/Paternal employment	Ordinal	Categorization of employment mother/father based on distribution of data.
Household Income	Continuous	Actual measures of households income (per week in taka).
Dwelling structure	Ordinal	Categorization of dwelling structure (straw/puca etc) based on distribution of data.
Family assets	Ordinal	Categorization of family possessings based on distribution of data.
Family Religion	Nominal(Muslim/Hindu etc)	Actual Religion
Household Size	Continuous	Actual number of household members.
Maternal dietary intake	Binary (yes/no) Continuous/ Categorial	Number of times woman ate zinc-rich foods in last 14 days/ Categorization of data based on self-report of food intake during past 24 hours.

APPENDIX VI

ASSUMPTIONS FOR SAMPLE SIZE CALCULATIONS

Outcome Variable	Expected outcome[1]	Mean expected control group	SD	Expected difference	Required N/ group
Birth Weight	↑ 10% mean	2.5 kg[2]	0.5	0.25 kg	63
Growth	↑ 15% weight gain	3.5 kg[3]	1.5	0.525 kg	128
Morbidity	↓ 15% mean # episodes diarrhoea	2.5 episodes/ child/ year[4]	1.25	0.38	133
Maternal Zn status	↓ 40% reduction in decline Zn status	5 μg/dL decline from 6 mo-9 mo preg[5]	2.5	2 μg/dL	26
Infant Zn status	↑ 20% plasma Zinc	50μg/dL[6]	25	10 μg/dL	98

Sources:

1) From various literature sources
2) El Arifeen, personal communication
3) Khan, M., Curlin, G.T. et al. (1979) Growth and development studies: Rural Meheran. Comilla. Bang Med J. 7, 74-90.
4) Baqui, A.H., Black, R.E., Sack, R.B., Chowdhury, H.R., Yunus, M., Siddique, A.K. (1993) Malnutrition, cell-mediated immune deficiency and diarrhea: a community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol. 137, 355-365.
5) Mahomed, K., James, D.K., Golding, J. et al. (1989) Zinc supplementation during pregnancy: a double blind randomized controlled trial. Br Med J. 299, 826-830.
6) Sazawal, S., Black, R.E., Bhan, M.K. et al. (1995) Effect of zinc supplementation on the incidence and prevalence of diarrhoea- A community-based, double-blind, controlled trial. (submitted).

VERBAL CONSENT FORM FOR PARTICIPATION IN THE STUDY:

Effect of zinc supplementation during pregnancy on infant birth weight, growth, morbidity and response to BCG.

Urban MCH-FP Extension Project at the ICDDR,B (Cholera Hospital) is conducting a special study on women who have babies delivered in Dhaka. The purpose of this study is to find out more about women in Dhaka, who are having babies, and to find out whether a daily vitamin/mineral supplement will help to improve the health of the mother and the baby. We want to do this by asking you to take a vitamin/mineral supplement every day until your delivery. We will ask you some questions before and after delivery.

If you agree to participate in the study, I will ask you to come to the Cholera Hospital two times before delivery. During these visits we will measure your weight, height and your arm size and the doctor will take a small amount of blood from your finger. We will ask some questions about your health. We will visit you at home every week to give you a weekly supply of vitamin/mineral tablets and we will ask you to take one tablet every day. Once a month we will measure your weight, height and arm size at these home visits.
We will visit you as soon as possible after delivery to see how you and your baby are doing. Then we will ask you to come to the Cholera Hospital together with your baby, two times after delivery. The doctor will take a very small amount of blood from your baby's finger and will inject him/her with a vaccine. We will measure the weight, height, and arm size of your baby and ask you some questions about the health of your baby and the feeding practices. We will visit you at home monthly till your baby is 6 months old to measure your baby.

There is no possibility of harm coming to you or your newborn as a result of participating in this study, rather the measurements we perform will help us to assess how healthy you and your baby are. The vitamin/mineral tablet will not harm your health or the health of your unborn baby. The doctor will take a very small amount of blood from you and your baby, which will not cause any harm. If your blood is not okay, the doctor will give you some extra mineral tablets. We will give you some money for your travel to the hospital. If you or your baby are ill we will help to seek treatment at a nearest hospital.

We know that some questions we will ask you about your health, pregnancy, and family will be personal. Therefore, we will keep all of your answers secret. That is why this questionnaire does not have your name on it.
If there are any questions which you do not want to answer, that is okay. If you don't want the doctor to take your blood or the blood of your baby, that is okay. Even if you decide not to answer the questions or not to participate in this study, you will always be able to use the services at the Cholera Hospital as usual. If you have any questions about the study, you can contact the principal investigator Saskia Osendarp, or the Urban MCH-FP Extension Project, at the telephone number 600003.

Do you agree to participate in this research?

_____ _____

Signature of Interviewer Date of Consent

_____

Signature of Principal Investigator

Responsibilities of Investigators and Project Staff

International fellow/Principle Investigator:

Has ultimate responsibility for all aspects of study design, methodology and accountability to ICDDR,B review, Urban MCH-FP Extension Project director, Clinical Science Division director and research director. Develops computerized tracking system to manage data; writes reports and articles on progress of the study and findings.

Research Investigator:

Assists in hiring and training of health assistants; assists in hiring and training of nurses; supervises nurses and Field Research Officer; helps coordinating and monitoring the clinical visits at 4 and 7 months antepartum and 1 and 6 months postpartum; maintains contacts with laboratory for proper coordination between the two divisions; secures necessary equipment and supply of supplements; communicates with collaborating and non-collaborating clinics and hospitals; codes questionnaires for data-entry; translates questionnaires and consent forms into Bengali.

Medical Officer:

Conducts the anthropometric and gestational age assessments at the 72 hour postpartum visit; vaccinates the newborn with BCG after birth.

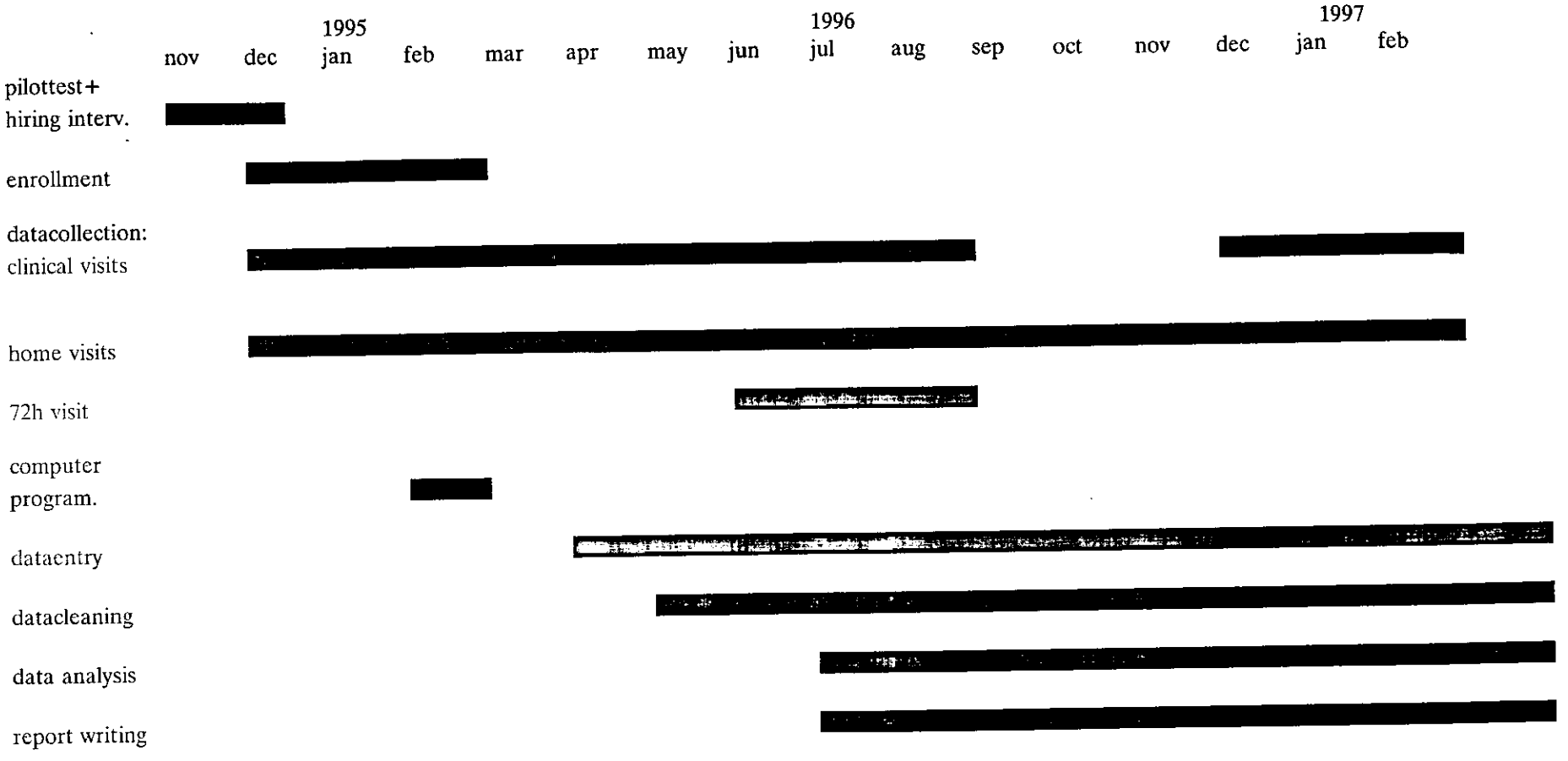
Nurses:

Participates in all training and reliability sessions; conducts anthropometric assessments during the clinical visits; conducts interviews during clinical visits; conducts bloodsampling during clinical visits; is responsible for proper storing and transportation of the bloodsamples to the laboratory for the analyses; performs the intra-dermal vaccination with PPD on the infant and checks the skin repsonse during homevisits three days after the clinical visits.

Field Research Officer:

Manages field logistics of project which includes the following activities:
Assists and supervises health assistants; copies questionnaires and other forms and distributes to field offices; distributes supplements and placebo's to the field offices; controls the supplementation schedules; reviews and edits all completed questionnaires; accompanies health assistants to the field on selected interviews to ensure standard quality of interviews and anthropometric assessments and to ensure proper supplementation use; assists in coding questionnaires.

Health Assistant:

Participates in all training and reliability sessions; conducts weekly homevisits to supply supplements and check proper consumption; conducts interviews and anthropometric assessments during the 12 homevisits; conducts the morbidity interviews during the two-weekly visits after delivery; reviews and edits interviews after completion; returns to the house to make corrections if necessary; reports to Field Research Officer on progress of the study and on supplementation.

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