The Effect of Zinc Supplementation During Pregnancy on Immune Response to Hib and BCG Vaccines in Bangladesh

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Summary
An essential role for zinc in development of the fetal immune system has been documented. However, the effect of antenatal zinc supplementation on infants' postnatal immune response to vaccinations is unknown. The objective of this study was to evaluate the effect of zinc supplementation during pregnancy on immune response to the Bacillus Calmette-Guerin (BCG) vaccine and the Haemophilus influenzae type b (Hib) component of the combined diphtheria, tetanus toxoid and pertussis (DTP)- Haemophilus influenzae type-b (Hib)- conjugate vaccine in poor Bangladeshi infants. We immunized 405 infants whose mothers were supplemented daily with 30 mg elemental zinc or placebo beginning at 12–16 weeks gestation with the standard BCG vaccine at birth. A subcohort of 203 infants were in addition immunized at 1-month intervals with three doses of DTP-Hib vaccine starting at 9 weeks of age. The delayed hypersensitivity (PPD) skin test was performed in 345 infants at 24 weeks of age. Hib polysaccharide (PRP) antibodies were assessed for 91 infants at 4 and 24 weeks of age. In infants born with low birth weight (LBW) a lower proportion of negative responses to PPD skin test were observed in the zinc (66.2%) compared to placebo (78.5%) group ($p = 0.07$). No differences were observed in normal birth weight infants. There were no differences in proportion of infants above the protective thresholds for anti-PRP antibodies between zinc (81%) and placebo (89%) group. Geometric mean PRP antibody titres at 4 and 24 weeks of age were not different between groups. Zinc supplementation during pregnancy did not enhance immune response to Hib-conjugate vaccine but there was a suggestion of improved delayed hypersensitivity immune responses to BCG-vaccine in Bangladeshi LBW infants.

Introduction
The response to childhood vaccinations varies in different populations. For example, the immune response to the pure polysaccharide Haemophilus influenzae type b (Hib) vaccine of Native American populations in the USA is known to be lower than in the general US population [1, 2]. Similarly, the immunogenicity of three doses of live attenuated, trivalent oral polio vaccine (TOPV) is lower in developing countries than in industrialized countries [3–6] and the effect of prior bacillus Calmette-Guerin (BCG) vaccination on the tuberculin skin test is also known to vary in different populations [7].

Malnutrition and the occurrence of several micronutrient deficiencies may be an explanation for the depressed immune responses to vaccines in infants of developing countries. Undernourished infants have impaired immunity [8] and several micronutrients, of which zinc is among the most notable, are known to affect the immune system in characteristic ways [9].

Acknowledgements
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The essential role for zinc in the non-immune and immune host defences has been demonstrated [10, 11]. In zinc deficiency, impaired epithelial barrier functions, atrophy of lymphoid organs, a reduced number of T- and B-lymphocytes, and decreased macrophage functions have been observed [12] as well as depressed antibody responses [13]. A depressed delayed hypersensitivity reaction resulting in anergy [6] and decreased CD4+ lymphocytes have been found in zinc deficient children which was reversed after zinc supplementation [13].

Maternal zinc deficiency during gestation may contribute to an impaired immune function in the offspring. In animal models perinatal zinc deficiency resulted in impaired development of the fetal immune system, including a reduced antibody response to certain bacterial antigens. These abnormalities might even persist into adulthood [9]. Maternal zinc status may also affect in utero acquisition of antibodies due to the role of zinc in placental transport [14].

Very little information exists on the effect of maternal zinc status or antenatal zinc supplementation in humans on infants’ immune response. While there is a report of zinc supplementation during pregnancy in Peru, resulting in a 35% increase of IgG3 levels in cord blood [15], we are not aware of any reports on the effect of maternal zinc supplementation on infants’ postnatal immune response to childhood vaccines.

We therefore evaluated the effect of maternal zinc supplementation in Bangladeshi urban poor women during the last two trimesters of pregnancy on humoral sero-response of their infants, using Haemophilus influenzae type b conjugate vaccine as a marker and on the tuberculin skin reaction to BCG as a marker for cell mediated immune response.

Methods

Study design and study population
This study was a nested study within a community-based, randomized controlled trial, to evaluate the effect of zinc supplementation during pregnancy on birth weight and infant morbidity from infectious diseases [16, 17]. Details on the study population, intervention and data collection procedures have been reported elsewhere [16, 17]. The study was approved by the Ethical Review Committee of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) and the Committee on Human Research of the Johns Hopkins School of Hygiene and Public Health.

A total of 559 women from selected areas of Dhaka city slums were enrolled between 12 and 16 weeks gestation after obtaining written informed consent. Enrolled mothers were stratified by parity and randomly assigned to two treatment groups, to receive either 30 mg elemental zinc per day (twice the RDA, as zinc acetate) or a cellulose placebo. Individual randomization was achieved by a computer-generated random letter assignment and the codes remained unknown to both investigators and participants until the study was completed. The supplements were prepared in bubble packs of ten tablets each (ACME Ltd, Dhaka) and zinc content of both zinc and placebo tablets were independently confirmed by two different laboratories. Compliance with tablet consumption was 86%, as assessed by counting the remaining tablets in the bubble packs during unannounced home visits. Supplementation continued until delivery.

A total of 420 singleton infants born to these women were included in the postnatal surveillance and followed to 24 weeks of age. Information on morbidity was collected weekly by mother’s recall, infant anthropometrics were measured monthly and serum zinc was assessed at 4 and 24 weeks of age [17]. Prior to the onset of the nested study, we had calculated required sample sizes to detect differences between treatment groups with 80% power and a type 1 error 5%. At the time when the study was conducted, there was no prior data available on the immune response to H. influenzae type b vaccines in developing countries. We however assumed a lower immune response in the Bangladeshi infants and estimated that approximately 25% of Bangladeshi infants (25% less than the general US population) would have an antibody level of ≥0.15 μg/ml to PRP if they were given three doses of the vaccine. We also assumed that with zinc supplementation, their response would be the same as the US infants. Anticipating that 15% would not complete the follow-up a required sample size of 75 was calculated to be enrolled in each group.

Immunization
A total of 405 infants (194 in zinc, 211 in placebo groups respectively) were vaccinated with 0.05ml reconstituted live freeze-dried vaccine of Bacille Calmette Guerin (BCG) by study physicians within 72h after birth.

A subcohort of 203 infants (96 in zinc, 107 in placebo groups respectively) who had not yet reached the age of 12 weeks at the time of the onset of the nested study, were in addition vaccinated with the combined diphtheria, tetanus toxoid and pertussis (DTP) - Haemophilus influenzae type b (Hib) vaccine (Diphtheria CRM, 97 protein conjugate), TETRAMUNE® Wyeth-Lederle, NY at 9, 13 and 17 weeks of age (±3 weeks). The second and third round of vaccinations were given at 4-week intervals (±2 days). Infants were given the trivalent oral polio vaccine (TOPV) simultaneously.
Post-vaccination safety surveillance was carried out from day 1 through day 5 post-vaccination during home visits by trained study nurses. Data was collected and recorded daily on rectal temperature, local swelling and redness, generalized rash, seizures, general health of the infant, feeding history and any other unexpected reactions.

**Assessment of cell mediated immune response**

Cell mediated immune response was tested at 24 weeks of age by delayed hypersensitivity skin test (DTH) using the purified protein derivate (PPD) test (Staten Serum Institute, Copenhagen, Denmark). Tuberculin solution (0.1 ml) was applied by trained nurses to the volar surface of the forearm intradermally using an Omega glass PPD syringe with platinum needles. The nurses who administered the vaccines to the infants were blinded as to the Zinc supplementation status of the mothers. After 72 h the size of the induration in millimeters developed was read as the transverse diameter. A cutaneous reaction was considered positive when an induration of >5 mm was observed [18]. Six nurses were extensively trained and standardized in the administration and interpretation of PPD skin testing. Reproducibility of skin test reading was tested prior and during the study in hospitalized patients with tuberculosis attending the Institute of Chest Diseases, Chest Hospital, Dhaka. Variability between reading by the same nurse and between the different nurses was measured. Overall, Pearson correlation coefficients were high, ranging between 0.98 (p < 0.000) and 0.73 (p = 0.03) and consistent with inter-rater agreements observed in similar studies [19].

**Assessment of humoral immune response and serology**

Antibody response to *H. influenzae* b polysaccharide (polyribosylribitol phosphate (PRP)) was assessed pre-vaccination at 4 weeks of age and post-vaccination at 24 weeks of age. Non-fasting blood specimens (5 ml) were obtained by antecubital venipuncture in the morning hours. Serum was separated a maximum of 6 h after collection and stored at –20°C until analysis. Antibodies to PRP were measured by enzyme linked immunosorbent assay (ELISA). All assays were performed at the laboratory of Wyeth Lederle Vaccines (NY, USA) using standard procedures [20]. Geometric mean antibody titres were calculated as the antilog of the mean of the logarithms of titres. Mean paired differences between pre- and post immunization titres and percentage of infants with PRP antibody levels ≥0.15 μg/ml, associated with immediate protection [21], and ≥1.0 μg/ml, associated with long-term protection [22] were calculated.

Differences between groups in proportion of anergic infants from the Tuberculin skin test and proportion of infants with antibody titres above the protective thresholds before and after immunization were assessed with the Chi-square test and Mac Nemar’s test respectively. Differences between treatment groups in mean size of induration, geometric mean antibody titres and paired differences between pre and post immunization titres were assessed with the Mann-Whitney U-test.

Analysis of Covariance (ANCOVA) and Logistic Regression Analysis were performed to control for potential confounders (SPSS7.5 FOR WINDOWS; SPSS Inc, Chicago). Separate models were made based on subgroups for birth weight and gender. P-values of <0.05 were considered statistically significant.

**Results**

**Description of the study population**

Of the 405 singleton infants (194 in zinc and 211 in placebo group) who were born to mothers from the trial of zinc supplementation in pregnancy and immunized with the BCG vaccine at birth, 39 (21 or 10.8% in zinc-supplemented and 18 or 8.5% in placebo-supplemented group) were lost-to-follow-up before the end of the study due to the following reasons: infant deaths (n = 5 zinc, n = 5 placebo), migration from the study area (n = 4 zinc, n = 7 placebo) or refusal to further participate (n = 12 zinc, n = 6 placebo). Reasons for loss-to-follow-up were not different for infants from zinc and placebo groups. An additional 7 infants could not receive the PPD skin-test at 24 weeks of age because they were sick or absent at the time of the test. Therefore, 359 infants (167 zinc, 192 placebo) were administered with the PPD skin test at 24 weeks of age. For 14 infants, skin test readings could not be performed within 72 hours, thus, the final sample size for the effects on the tuberculin skin test consisted of 345 infants (163 in zinc, 182 in placebo group). A comparison of baseline characteristics (Table 1) revealed that infants included in the tuberculin skin test had significantly greater body weights and larger gestational age at birth compared to infants who were lost-to-follow-up. In the multivariate analysis we therefore controlled for infant’s birth weight and gestational age.

A sub-cohort of 203 infants (96 in zinc and 107 in placebo supplemented group) were eligible for inclusion in the Hib-immunization trial since they had not yet reached the age of 12 weeks. Twenty-seven infants (16 or 16.7% in zinc supplemented and 11 or 10.3% in placebo group) were lost-to-follow-up before the end of the study for the following reasons: infants deaths (n = 3 zinc, n = 2 placebo), migration
from the area \((n = 2\) zinc, \(n = 6\) placebo), refusals \((n = 8\) zinc, \(n = 2\) placebo) and infants who had received immunizations through another source \((n = 3\) zinc, \(n = 1\) placebo). There were no differences between treatment groups in reasons for lost-to-follow-up. A total of 176 infants \((80\) in zinc and \(96\) in placebo group) completed the course of three doses of the combined diphtheria, tetanus toxoid and pertussis (DTP) - *Haemophilus influenzae* type b (Hib) vaccine and provided pre- and post-vaccination serum samples for immune assays. Median ages at pre- and post-immunization blood sampling were 4.0 and 24.1 weeks respectively and median ages at the three rounds of diphtheria were 9.6, 13.9 and 18.1 weeks. Serum samples of 85 infants were insufficient for analysis resulting in a final sample of 91 infants \((38\) zinc, \(53\) placebo group) for analysis of Hib-response. Compared to infants who were lost-to-follow-up for the Hib assays, the 91 infants who were included in the Hib-trial had significantly higher gestational ages at birth (Table 1) and their mothers had significantly lower Body Mass Indexes (BMI) at baseline (i.e. 4 months gestation). We controlled for those variables in the multivariate analysis.

### Effect of zinc supplementation on cellular immune response

At 24 weeks of age, 60.9\% \((210\) of \(345\)) of infants showed a negative tuberculin skin response (induration of \(\leq 5\) mm) in response to the PPD skin test. There were no differences in proportion of negative responses between treatment groups (zinc: 58.9\%; placebo: 62.6\%; Table 2). A significantly higher percentage of infants born with low birth weights \((LBW; <2500 g)\) showed negative skin responses compared to infants born with normal birth weights \((NBW; 71.9\% vs. 52.7\% for LBW and NBW groups respectively, \(p < 0.0001\)). When data were analyzed separately for 139 LBW-infants, fewer infants in zinc compared to placebo group were not responsive to the tuberculin skin test \((66.2\% vs. 78.5\%)\). However, this difference was not statistically significant \((p = 0.07)\). No differences in response to the tuberculin skin test were observed between treatment groups for the 203 normal birth weight infants, or for infants born premature \((n = 50)\) or at-term \((n = 295)\). The mean size of induration among the positive responses was 11.8 mm (SEM 0.4) and did not differ for LBW and NBW infants of zinc and placebo groups (Table 2).

### Table 1

**Selected baseline and birth characteristics for mothers at 4 months gestation and infants included in analysis on immune response or lost-to-follow-up for analysis on immune response for 405 infants enrolled in BCG trial and 203 infants enrolled in DTP-Hib trial**

<table>
<thead>
<tr>
<th></th>
<th>Infants lost for PPD skin test ((n = 64))</th>
<th>Infants included for PPD skin test ((n = 345))</th>
<th>Infants lost for Hib-immune assay ((n = 112))</th>
<th>Infants included for Hib-immune assay ((n = 91))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2427 (484)</td>
<td>2558 (367)*</td>
<td>2534 (404)</td>
<td>2536 (351)</td>
</tr>
<tr>
<td>LBW (%)(^2)</td>
<td>54.1</td>
<td>40.6*</td>
<td>43.1</td>
<td>42.7</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>38.0 (2.8)</td>
<td>39.0 (2.1)*</td>
<td>38.6 (2.3)</td>
<td>39.7 (1.9)*</td>
</tr>
<tr>
<td>IUGR (%)(^3)</td>
<td>75.7</td>
<td>73.7</td>
<td>71.9</td>
<td>82.0*</td>
</tr>
<tr>
<td>Gender: male (%)</td>
<td>57.3</td>
<td>53.3</td>
<td>54.4</td>
<td>52.7</td>
</tr>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23.1 (5.6)</td>
<td>23.0 (5.5)</td>
<td>23.0 (5.5)</td>
<td>23.3 (5.7)</td>
</tr>
<tr>
<td>Nulliparous (%)</td>
<td>24.0</td>
<td>21.7</td>
<td>21.6</td>
<td>24.2</td>
</tr>
<tr>
<td><strong>Socioeconomic status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor/very poor (%)(^4)</td>
<td>81.3</td>
<td>76.5</td>
<td>79.0</td>
<td>71.5</td>
</tr>
<tr>
<td>BMI(^5)</td>
<td>18.9 (2.8)</td>
<td>19.0 (2.4)</td>
<td>19.1 (1.6)</td>
<td>18.4 (1.9)*</td>
</tr>
<tr>
<td>MUAC(^6)</td>
<td>224 (27)</td>
<td>227 (23)</td>
<td>228 (25)</td>
<td>229 (21)</td>
</tr>
<tr>
<td>Hemoglobin (g/l)</td>
<td>112 (13)</td>
<td>115 (13)</td>
<td>114 (13)</td>
<td>116 (12)</td>
</tr>
<tr>
<td>Serum zinc (μmol/l)</td>
<td>15.1 (3.8)</td>
<td>15.5 (4.6)</td>
<td>15.6 (4.7)</td>
<td>14.7 (3.1)</td>
</tr>
<tr>
<td>Treatment: zinc (%)</td>
<td>48.0</td>
<td>47.2</td>
<td>48.9</td>
<td>41.8</td>
</tr>
</tbody>
</table>

\(^1\)Values in mean (SD).

\(^2\)LBW = low birth weight \(<2500 g)\).

\(^3\)IUGR = Intra Uterine Growth Retarded \(<10% of fetal growth chart; 13)\).

\(^4\)Based on an index of household assets (13).

\(^5\)BMI = Body Mass Index.

\(^6\)MUAC = Mid Upper Arm Circumference.

*Different from infants dropped for PPD (purified protein derivate) skin test \((p < 0.05)\).

*Different from infants dropped for Hib (*Haemophilus influenzae* type b)-immune assays \((p < 0.05)\).
Effect of zinc supplementation on response to Hib conjugate vaccine

Geometric mean PRP antibody titres before and after immunization are shown in Table 3. There were no statistically significant differences in geometric mean PRP titres at 24 weeks of age (post-immunization) between infants from zinc (6.78 μg/ml; 95% CI: 3.36;12.01) and placebo groups (10.15 μg/ml; 95% CI: 6.29;16.14). Nearly all infants (95% in zinc and 98% in placebo supplemented group, difference NS) achieved protective antibody titres correlated with immediate protection (<0.15 μg/ml; Fig. 1). Furthermore, the large majority (81% in zinc and 89% in placebo group, difference NS) achieved titres ≥1 μg/ml associated with long-term protection (Fig. 2). No differences were observed between treatment groups in geometric mean PRP antibody titres for LBW (n = 38) and NBW (n = 51) infants but sample sizes were small.

Infants from zinc supplemented mothers had significantly fewer days of fever (>38.0°C) during post-immunization safety surveillance compared to infants from placebo supplemented mothers (means (SEM): 0.98 (0.11) vs. 1.23 (0.12); p < 0.05). No other differences in potential adverse effects following DTP-Hib immunization were observed between treatment groups (Table 4).

Discussion

There was a suggestion that zinc supplementation with 30 mg elemental zinc/day during the last two

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**Table 2**

*Cellular immune response to tuberculin (PPD) skin test at 24 weeks of age for infants by birth weight in zinc and placebo groups*

<table>
<thead>
<tr>
<th></th>
<th>Zinc group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anergic (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All infants</td>
<td>58.9</td>
<td>62.6</td>
</tr>
<tr>
<td>LBW</td>
<td>66.2</td>
<td>78.5*</td>
</tr>
<tr>
<td>NBW</td>
<td>52.3</td>
<td>53.0</td>
</tr>
<tr>
<td>Mean size of PPD-induration (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All infants</td>
<td>11.7 (0.4)</td>
<td>11.8 (0.4)</td>
</tr>
<tr>
<td>LBW</td>
<td>11.7 (0.6)</td>
<td>11.2 (0.9)</td>
</tr>
<tr>
<td>NBW</td>
<td>11.8 (0.5)</td>
<td>12.0 (0.4)</td>
</tr>
</tbody>
</table>

1Anergic = size of PPD induration <5 mm.
2All infants (n = 163/182).
3Low birth weight infants (<2500 g, n = 74/65).
4Normal birth weight infants (n = 87/116).
5Mean size of induration of positive responses (SEM).
6Different from zinc supplemented group (p = 0.07; Logistic regression controlling for birthweight and gestational age).

PPD = purified protein derivate tuberculosis.

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**Table 3**

*Geometric mean PRP antibody titres pre- and post-immunization for infants in zinc and placebo group*

<table>
<thead>
<tr>
<th></th>
<th>Zinc group (n = 38)</th>
<th>Placebo group (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-immunization (μg/ml)</td>
<td>0.22 (0.15;0.33)</td>
<td>0.21 (0.15;0.29)</td>
</tr>
<tr>
<td>Post-immunization (μg/ml)</td>
<td>6.78 (3.36;12.01)</td>
<td>10.15 (6.29;16.14)</td>
</tr>
</tbody>
</table>

1Geometric mean titres calculated as the antilog of the mean of the logarithms of values (values in brackets: 95% confidence intervals).
2Pre-immunization at 4 weeks of age.
3Post immunization at 24 weeks of age.
PRP = polyribosylribitol phosphate.
trimesters of pregnancy resulted in a positive albeit small effect on the response to the delayed hypersensitivity skin test on BCG vaccine at 24 weeks of age in infants born with low birth weight (LBW). No differences were observed among infants born with normal birth weights (NBW). There were no differences in antibody responses to the *Haemophilus influenzae* type b (Hib) vaccine between the groups. However, fewer days with fever were observed in the 5 days post-Hib-vaccination in infants born to zinc-supplemented compared to placebo-supplemented mothers.

We previously reported results from the same cohort of infants demonstrating that maternal zinc supplementation during pregnancy did not improve birth weight but reduced episodes of acute diarrhea, dysentery and impetigo during the first six months of life among LBW infants [17]. The prevalence of LBW in Bangladesh is among the highest in the world and in our study a total of 43% of live births were LBW [16]. LBW infants are known to be at-risk for impaired immunity and increased morbidity later in life [23]. The results of this current study, although not conclusive, suggest that an improved cellular immunity might be a possible mechanism for the observed reductions in morbidity among LBW infants after maternal zinc supplementation.

Beneficial effects of zinc supplementation on indicators of cellular immune response such as the delayed hypersensitivity skin response to PPD or CMI, number or functioning of circulating T-lymphocytes and thymus size have been reported previously in studies supplementing infants and children [13, 24–27]. Animal models of zinc deficiency suggest that improved delayed-type hypersensitivity after zinc repletion may be caused by a restored interaction between effector T-cells and macrophages [7]. Zinc deficiency is also known to be associated with atrophy of lymphoid organs and a reduced number of T-lymphocytes in animal models, all of which respond positively to a repletion of zinc [9].

Zinc deficiency has in addition been shown to affect elements of the immunologic memory as reduced T-dependent and, to lesser extent, T-independent antibody responses have been observed in zinc deficient mice [11]. Antenatal zinc deprivation in rats resulted in lower immunoglobulin serum concentrations, in particular IgA, IgG2 and IgM in the off-spring at 6 months of age [9]. Evidence for an effect of zinc supplementation on humoral immune response in humans is less and not conclusive. Increased serum IgA and salivary IgA concentrations have been observed after zinc supplementation in a study on Bangladeshi infants [24]. However, serum IgG and IgM concentrations were not affected by zinc supplementation among malnourished infants in Bangladesh [24]. Similarly, no effects of zinc supplementation on salivary IgA were observed in young rural Gambian children [28].

We observed a lower number of febrile days during the first five days post-immunization after maternal zinc supplementation. Negative correlations between plasma zinc and number of febrile days have been
concentrations post-immunization were 7.89 mg/ml in PRP titres at 24 weeks or a difference of 74% in proportion of post-immunization titres above the protective threshold (≥1 mg/ml) between treatment groups assuming 80% power and a type I error of 5%.

In our study we did not observe effects of maternal zinc supplementation on infants’ antibody response to the Hib vaccine. The polysaccharide Hib-conjugate vaccine that we used in our study contains a T-dependent antigen (HbOC) that induces an anamnestic response after repeated infections and is predominantly of the IgG class. We had anticipated a beneficial effect of zinc supplementation for this specific antigen since it is known that T-dependent B-lymphocytes are more affected by zinc deficiency than T-independent ones. Therefore our findings are somewhat unexpected. Our sample sizes were small and only sufficient to detect a difference of 4.6 μg/ml in PRP titres at 24 weeks or a difference of 74% in proportion of post-immunization titres above the protective threshold (≥1 mg/ml) between treatment groups assuming 80% power and a type I error of 5%.

The Hib-conjugate vaccine that was used in this study has been shown to be more immunogenic than the unconjugated pure polysaccharide vaccine. In addition, the Hib conjugate vaccines, unlike the pure polysaccharide vaccines are known to reduce Hib carriage in the upper respiratory tract, especially among younger children in the US and Gambia. The highly potent conjugate vaccine may have induced immune response in all our infants and therefore overcome any blunting of immune response resulting from zinc deficiency.

In our population, geometric mean PRP antibody concentrations post-immunization were 7.89 μg/ml which is substantially higher than levels observed in infants in developed countries such as the UK (3.65 μg/ml) after immunization with a similar conjugate vaccine [32]. Although our sample sizes were small, it is possible that frequent exposure to the Haemophilus influenzae type b-antigen even at this young age, may have caused an anamnestic response if the child came in contact with the organism after receiving the primary series of the vaccine. Published rates of invasive Haemophilus influenzae type b (Hib) diseases in Asia range from less than 10 per 100,000 to 50 per 100,000 compared to 22–109 per 100,000 in pre-vaccination USA and Europe [33]. Moreover, a 700% increase in Hib incidence over the period 1987–1994 has been reported from a hospital in Bangladesh [34]. We hypothesize, therefore, that high infection rates of Haemophilus influenzae type b in our population may have contributed to the humoral immune response to the vaccine in nearly all our infants which may have masked any potential beneficial effects of zinc supplementation.

Prior to immunization at the age of 4 weeks, 58% of our infants had PRP antibody concentrations of ≥0.15 μg/ml compared to only 30% observed in UK infants of similar age [32]. At 4 weeks of age, concentrations primarily reflect maternal Hib-specific immunoglobulins and the high maternal antibody concentrations in our population are another indication of a high degree of exposure to Hib organism.

In conclusion, in our cohort of poor Bangladeshi infants we were not able to demonstrate an effect of maternal zinc supplementation on infants’ immune response to vaccines, but our findings seem to be in contrast with the observed improvements in neonatal humoral immunity by increased cord-blood immunoglobulin concentrations after zinc supplementation during pregnancy in a similar trial in Peru [15]. Additional research is required to define the exact role of zinc in the development of the immune system in infants in developing countries.

Finally, the findings of our current study suggest that an improved cellular immune response may have caused observed reductions in morbidity from dysentery, acute diarrhea and impetigo among infants born with LBW after maternal zinc supplementation during the last two trimesters in pregnancy [17]. LBW infants are known to have impaired immunity and an increased burden of infectious diseases during infancy [19], and maternal zinc supplementation might be an effective way to reverse some of these disadvantageous outcomes.