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Zinc supplementation fails to increase the immunogenicity of oral poliovirus vaccine: A randomized controlled trial

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ABSTRACT

Background: Polio eradication remains a challenge in Pakistan and the causes for the failure to eradicate poliomyelitis are complex. Undernutrition and micronutrient deficiencies, especially zinc deficiency, are major public health problems in Pakistan and could potentially affect the response to enteric vaccines, including oral poliovirus vaccine (OPV).
Objective: To assess the impact of zinc supplementation among infants on immune response to oral poliovirus vaccine (OPV).
Methods: A double-blind, randomized placebo-controlled trial was conducted in newborns (aged 0–14 days). Subjects were assigned to either receive 10 mg of zinc or placebo supplementation daily for 18 weeks. Both groups received OPV doses at birth, at 6 weeks, 10 weeks and 14 weeks. Data was collected on prior immunization status, diarrheal episodes, breastfeeding practices and anthropometric measurements at recruitment and at 6 and 18 weeks. Blood samples were similarly collected to determine the antibody response to OPV and for micronutrient analysis. Logistic regression was used to determine the relationship between seroconversion and zinc status.
Results: Overall, 404 subjects were recruited. At recruitment, seropositivity was already high for poliovirus (PV) serotype 1 (zinc: 91.1%; control: 90.5%) and PV2 (90.0%; 92.7%), with lower estimates for PV3 (70.0%; 64.8%). By week 18, the proportion of subjects with measured zinc levels in the normal range (i.e. $\geq$60 μg/dL) was significantly greater in the intervention group compared to the control group (71.9%; 27.4%; $p < 0.001$). No significant difference in seroconversion was demonstrated between the groups for PV1, PV2, or PV3.
Conclusions: There was no effect of zinc supplementation on OPV immunogenicity. These conclusions were confirmed when restricting the analysis to those with measured higher zinc levels.

1. Background

Polio eradication in Pakistan has emerged as a public health challenge. In addition to routine immunizations, polio control in Pakistan is heavily dependent upon a strategy of administration of the vaccine in a campaign mode. There are several reasons for persistence of disease in Pakistan including poor polio program performance and coverage of routine immunization [1]. In addition, conflict and security issues affecting access, problems with community buy in, poor status of sanitation and hygiene, high burden of diarrhea and poor nutritional status are recognized as important determinants [2]. The recent National Nutrition Survey has underscored the importance of undernutrition among children with stunting rates exceeding 43% and widespread zinc (39%) and vitamin A (54%) deficiencies. In particular, zinc deficiency was identified as a public health issue as far back as 2001 and overall prevalence has not changed [2].

Zinc is an essential component of scores of enzymes in the human body and epidemiologic studies and micronutrient surveys indicate that zinc deficiency is widespread in socioeconomically deprived children in South Asia [2–5]. Reports have indicated that

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this trace element, along with other micronutrients, enhances the protective functions of immune cells [6]. Moreover, zinc deficiency leads to deregulation of balanced host responses to infection resulting in decreased antibody production and suppressed immunity. Zinc is also an essential cofactor for thymulin which is known to modulate cytokine release and induce immune cell proliferation [6]. Zinc deficiency has been found to impair an individual's epithelial barrier function [7,8], which may further hinder vaccine uptake by the mucosal cells and subsequent response. The role of zinc in the prevention of diarrheal diseases and other infections in children is also well documented [3,5,9–11]. Association between recent diarrheal history and increased vaccine failure in infants has been shown in a study from Brazil [11].

The recent Lancet nutrition series [12] has recommended regular zinc supplementation to address child undernutrition and stunting, and underscored the need to treat diarrheal episodes with zinc to expedite recovery. Other recent studies of zinc supplementation in low birth weight infants in South Asia have also shown significant improvement in diarrheal disease burden and mortality [13,14].

Vaccine failure in this region could be a consequence of compromised immunity and, hence, diminished response to oral polio vaccine (OPV). Understanding the synergistic role of zinc (if any) with OPV in enhancing immune response against polio and seroconversion rates would inform strategic action to address undernutrition and micronutrient deficiencies, to potentially reverse the immune response in infants to OPV. We conducted a randomized controlled trial among a cohort of Pakistani newborns to evaluate the impact of zinc supplementation on immune responses to OPV.

2. Methods

2.1. Study population

The study was conducted in the rural district of Hala and Matiari located about 200 km north-east of Karachi. Healthy newborns aged between 0 and 14 days were enrolled into the study. Infants beyond this age or preterm infants (<37 weeks gestation or <2 kg birth weight) [15–17] or having any major congenital abnormalities were excluded. The number of subjects required per group was calculated assuming a conservative estimate of seroconversion among infants of 50%. To detect a 20% improvement in seroconversion rates upon zinc administration at 5% significance and 90% power, the number of subjects required in each group was estimated to be 121. Assuming a 20% maximum dropout or refusal rate, we required 145 subjects in each group; for a total of 290 subjects.

2.2. Study design

This was a double-blind, placebo-controlled clinical trial. To identify newborns for recruitment, a cohort of pregnant women in their third trimester were identified through a baseline census and visited to inform them about the study. Within 24 h following notification of birth, a written informed consent was sought and subjects were randomly assigned to one of two treatment groups: (1) the zinc group received a 10 mg zinc sulfate liquid preparation daily for 18 weeks and (2) the control group received an identical placebo preparation daily for 18 weeks. Both groups received standard OPV doses at birth, at 6 weeks, at 10 weeks, and at 14 weeks of age, as recommended by the Expanded Program of Immunization (EPI) [18]. The subjects were randomized using a computerized block randomization strategy with groups matched in blocks of 20, with the codes maintained by an independent pharmacist at the pharmacy of Aga Khan University (AKU). The health workers replenished zinc or placebo supplies every week (in 60 ml bottles) and monitored compliance to the assigned intervention three times a week by observing the empty bottles of zinc and placebo suspension. The study protocol was approved by the Ethical Review Committees of the World Health Organization and AKU. The trial was registered in clinical trials with the reference number of NCT01229579.

2.3. Data collection

A study team comprised of medical officers and data collectors was hired and trained on research protocol, field operations and various project activities. Data was collected on birth history, immunization status, medical history and management, number of diarrheal episodes, breastfeeding practices, laboratory nutritional indices, anthropometric measurements (i.e. weight for height, and height for age), physical examination and vital signs on structured instruments. The anthropometry data was characterized for moderate (z-score: -2 to -3) or severe (z-score: <-3) stunting, and moderate (z-score: -2 to -3) or severe (z-score: <-3) wasting. Moderate and severe were combined for both stunting and wasting. Diarrhea was defined as the passage of three or more loose or watery stools in a 24 h period [19] and was considered between birth and recruitment or over the 18 weeks period. Breastfeeding was defined as exclusive breastfeeding from birth to recruitment or ≥80% exclusive breastfeeding over the 18 week study period. Diarrhea and exclusive breastfeeding were based on responder report and were recorded for biweekly intervals. Data was collected at recruitment, and at 6 and 18 weeks.

A 3 ml blood sample was collected from every infant as per WHO standards [20] for the assessment of poliovirus antibodies and the analysis of micronutrient deficiencies. The blood sample was centrifuged, and the serum was separated and stored in two separate aliquots. Both aliquots were transported under cold chain conditions to the Nutrition Research Lab of the Women and Child Health Division of Aga Khan University (AKU), Pakistan. A 1 ml blood sample was then transported to the Centers for Disease Control and Prevention laboratory in Atlanta, USA, where the assessment of poliovirus antibodies was performed using the WHO standard procedure [21]. The maximum dilution of sera that still protected at least 50% of test cells from viral lysis was determined positive. Seroprevalence, defined as the proportion of subjects with titers ≥3 [1/dil], were calculated for each poliovirus (PV) serotype (i.e. PV1, PV2, and PV3). The remaining 2 ml sample was analyzed for micronutrient deficiencies at AKU. Flame Atomic Absorption Spectroscopy (FAAS) was used to estimate zinc concentration. Micronutrient deficiency was characterized for vitamin A (retinol < 20 μg/100 ml) [22–24] and zinc (<60 μg/100 ml) [2]. Zinc deficiency was also explored as a continuous variable and at <50 μg/100 ml, <55 μg/100 ml, <65 μg/100 ml and <70 μg/100 ml, as there is no commonly accepted standard at this age group. Iron deficiency anemia was defined as hemoglobin levels <11 g/dL and ferritin levels <12 ng/dL [25]. Blood samples were collected at recruitment, and at 6 and 18 weeks.

2.4. Statistical analyses

The collected data was dual entered in a database developed using FoxPro, and was further analyzed using STATA version 11.1. All subjects with available data were included in the analysis. The analysis was carried out at week 18 to allow adequate amount of zinc supplementation and multiple doses of OPV. Additionally, zinc levels in both groups were assessed at week 6. Seropositivity was defined as a reciprocal titer ≥ 8 [26,27]. Seroconversion was defined as a fourfold or higher increase over expected decline in maternal antibody. The half-life of antibody decay was assumed to be 28