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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.

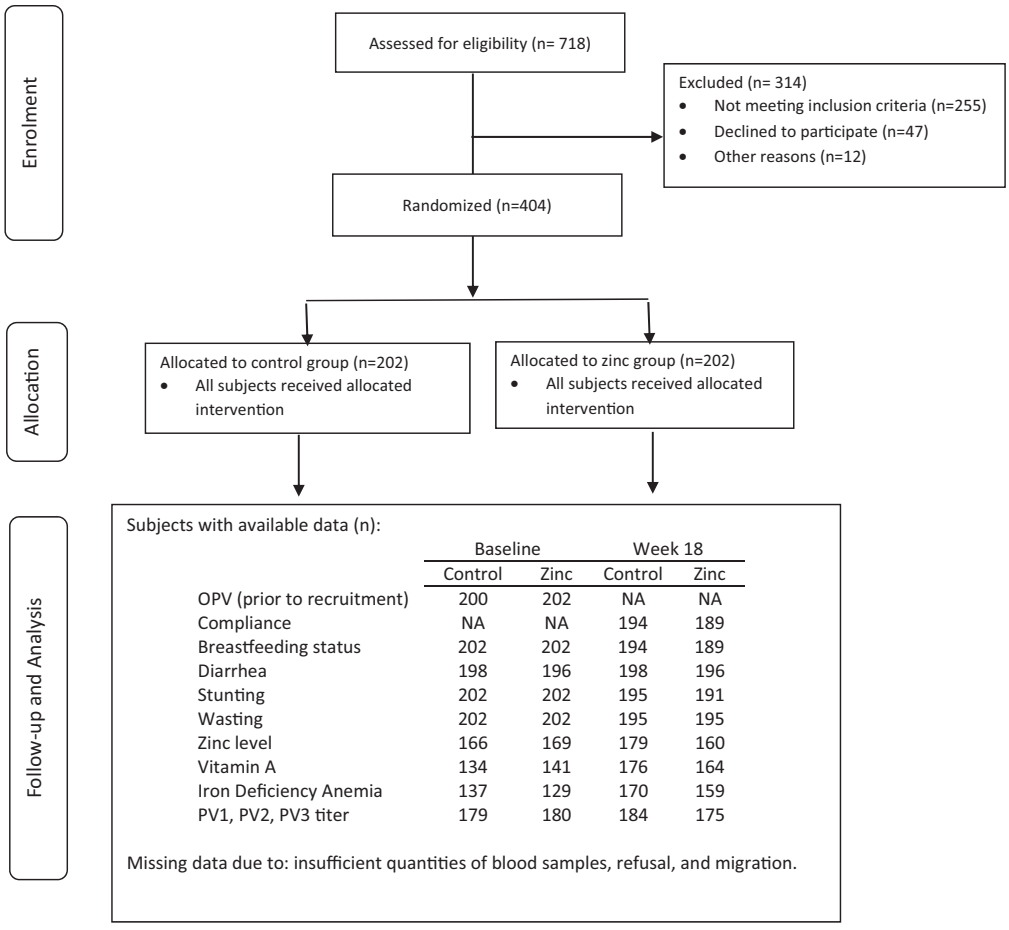
2.3. Data collection

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



	Baseline		Week 18	
	Control	Zinc	Control	Zinc
OPV (prior to recruitment)	200	202	NA	NA
Compliance	NA	NA	194	189
Breastfeeding status	202	202	194	189
Diarrhea	198	196	198	196
Stunting	202	202	195	191
Wasting	202	202	195	195
Zinc level	166	169	179	160
Vitamin A	134	141	176	164
Iron Deficiency Anemia	137	129	170	159
PV1, PV2, PV3 titer	179	180	184	175

Fig. 1. Consort flow diagram of study.

days [28]. For those who were seronegative at enrolment, a change to seropositivity (i.e. a reciprocal titer ≥ 8) was also considered to denote seroconversion. The non-parametric Wilcoxon rank-sum test was used to compare reciprocal median titers between groups and median zinc levels by seroconversion status. Pearson Chi-squared test was used to determine differences in the proportion of infants in each group. Student's *t*-test was used to test differences in compliance between groups. *p*-values ≤ 0.05 were considered as significant. Univariate analysis was undertaken to determine the nature of the relationship between seroconversion and potential explanatory variables. Factors significant in the univariate analysis were included in multivariate logistic regression models to determine the nature of the relationship between serum zinc status and optimal vaccine seroconversion. The remaining explanatory variables were incrementally added to the multivariable model if the Akaike's information criterion (AIC) reduced in value (i.e. stepwise addition approach) [29]. The most parsimonious yet adequate model was selected for each poliovirus serotype. All possible two-way interactions were evaluated in the multivariable models.

3. Results

Overall, 404 subjects were recruited for the study. Of the 202 subjects in each group, 175 and 184 subjects in the zinc and control groups had complete titer information for PV1, PV2, and PV3 over the 18 weeks period (Fig. 1). Over the study time period, up to 21% of subjects were missing complete zinc level, vitamin A, ferritin, hemoglobin, diarrhea and/or breastfeeding information. There were no significant differences in baseline demographic characteristics when comparing subjects with complete information with those missing data.

Baseline characteristics of study participants at recruitment are shown in Table 1. Both groups were similar for all variables. There was a high proportion of exclusive breastfeeding at recruitment and a low proportion of diarrhea. Wasting was more frequent than stunting in both groups. Approximately half of subjects received OPV prior to recruitment, based on respondent recall. SES quintiles were similar between the two groups. Seropositivity was high for PV1 (zinc: 91.1%; control: 90.5%) and PV2 (90.0%; 92.7%), with much lower proportions for PV3 (70.0%; 64.8%). Median reciprocal titers were lowest for PV3 (zinc: 14.2; control: 11.3) and higher for both PV1 (56.9; 45.3) and PV2 (45.3; 72.0). When comparing subjects with a prior dose of OPV to those without a history of OPV vaccination, there were no significant differences between seropositivity or median reciprocal titer at baseline (Supplemental Table 1). The proportion of subjects with normal zinc levels were 47.6% and 45.6% in the control and zinc groups, respectively, while the normal vitamin A levels were 26.1% and 21.3%. No subjects had iron deficiency anemia at recruitment.

At week 18, there were no differences between groups for PV1, PV2 or PV3 seropositivity, with similar proportions of all other variables across groups, with the exception of zinc (Table 2). The proportion of subjects with measured zinc levels in the normal range (i.e. ≥ 60 μg/dL) was significantly greater in the intervention group (71.9%) compared to the control group (27.4%) ($p < 0.001$). A significant difference in proportion of subjects in the normal range for zinc was evident by week 6 ($p < 0.001$). The proportion of diarrhea, stunting and wasting was not significantly different between the 45 subjects with deficient zinc when compared to

Table 1
Baseline characteristics of pediatric patients receiving zinc supplementation or placebo, Pakistan, 2010.

Variable	Control group *N* (median)	Zinc group *N* (median)	*p*-value
Age (days)	202 (9.0)	202 (8.0)	0.602
PV1 reciprocal titer	179 (45.3)	180 (56.9)	0.422
PV2 reciprocal titer	179 (72.0)	180 (45.3)	0.247
PV3 reciprocal titer	179 (11.3)	180 (14.2)	0.158
	n/*N* (%)	*n*/*N* (%)	*p*-value
PV1 seropositivity	162/179 (90.5)	164/180 (91.1)	0.842
PV2 seropositivity	166/179 (92.7)	162/180 (90.0)	0.356
PV3 seropositivity	116/179 (64.8)	126/180 (70.0)	0.294
OPV (prior to recruitment)	101/200 (50.5)	97/202 (48.0)	0.619
Zinc level (normal (≥60 μg/dL))	79/166 (47.6)	77/169 (45.6)	0.710
Stunting[a]	25/202 (12.4)	19/202 (9.4)	0.338
Wasting[b]	98/202 (48.5)	100/202 (49.5)	0.842
Breastfeeding status (exclusive)[c]	175/202 (86.6)	168/202 (83.2)	0.331
Diarrhea[d]	6/198 (3.0)	8/196 (4.1)	0.573
Vitamin A (normal)[e]	35/134 (26.1)	30/141 (21.3)	0.345
Iron deficiency anemia (anemia)[f]	0/137 (0.0)	0/129 (0.0)	NA
SES Q1	37/202 (18.3)	45/202 (22.3)	0.322
SES Q2	46/202 (22.8)	34/202 (16.8)	0.134
SES Q3	39/202 (19.3)	46/202 (22.8)	0.393
SES Q4	42/202 (20.8)	36/202 (17.8)	0.449
SES Q5	38/202 (18.8)	41/202 (20.3)	0.707

[a] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) stunting.
[b] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) wasting.
[c] Breastfeeding defined as exclusive breastfeeding from birth to recruitment, based on responder report.
[d] Diarrhea defined as the passage of ≥3 loose or watery stools in a 24 h period from birth to recruitment, based on responder report.
[e] Normal vitamin A classified as serum retinol levels ≥20 μg/100 ml.
[f] Iron deficiency anemia defined as hemoglobin level <11 g/dL and ferritin < 12 ng/dL. SES = socio-economic status. Q = quintile; *p*-value calculated using Chi-squared or Wilcoxon rank-sum tests for categorical and continuous variables, respectively; *n* = number of subjects in numerator; *N* = total number of subjects; NA = not available.

those with normal levels at week 18. Similar results were found when using zinc cut-off values of ≥50 μg/dL, ≥55 μg/dL, ≥65 μg/dL and ≥70 μg/dL, and continuous zinc (μg/dL) (Supplemental Table 2A).

In the univariate analysis, no significant difference in seroconversion was established between the groups for PV1, PV2, or PV3 (Table 3). The proportion of subjects seroconverting by week 18 was lower for PV3 (zinc: 60.3%; control: 60.7%) than PV1 (87.2% and 85.3%) and PV2 (81.4% and 77.3%). No significant association was found between seroconversion and zinc level at any cut-off and median zinc levels were similar for subjects that did and did not seroconvert (Supplemental Table 2B). A significant inverse association was found between stunting and PV1 seroconversion ($p = 0.001$). Additionally, at least one episode of diarrhea was significantly associated with a decrease in seroconversion for both PV2 ($p = 0.003$) and PV3 ($p = 0.005$). In the multivariate analysis ($N = 319$), no association was found between seroconversion and zinc supplementation for PV1, PV2, or PV3 considering the most parsimonious yet best fitting model (Table 4). Vitamin A and iron deficiency anemia were not significant in the univariate analysis and were excluded from the stepwise approach to maximize sample size. No difference was found in the relationship between zinc group and PV1, PV2, and PV3 seroconversion when including these two variables to identify the most parsimonious yet best fitting model ($N = 274$) (Supplemental Table 3). In this model, PV3 seroconversion was significantly associated with the absence of wasting ($p = 0.016$). However, of the 45 subjects missing vitamin A and iron deficiency anemia data, there was a significantly lower

Table 2
Characteristics of pediatric patients receiving zinc supplementation or placebo at week 18, Pakistan, 2010.

Variable	Control group *n*/*N* (%)	Zinc group *n*/*N* (%)	*p*-value
PV1 seropositivity	167/184 (90.8)	161/175 (92.0)	0.676
PV2 seropositivity	160/184 (87.0)	149/175 (85.1)	0.620
PV3 seropositivity	114/184 (62.0)	108/175 (61.7)	0.962
Zinc level (normal (≥60 μg/dL))	49/179 (27.4)	115/160 (71.9)	<0.001
Stunting[a]	31/195 (15.9)	36/191 (18.8)	0.444
Wasting[b]	60/195 (30.8)	69/195 (35.4)	0.333
Breastfeeding (≥80% exclusive)[c]	125/194 (64.4)	128/189 (67.7)	0.496
Diarrhea (≥1 episode)[d]	59/198 (29.8)	50/196 (25.5)	0.341
	N (median)	*N* (median)	*p*-value
Compliance (% of total days)	194 (87.5)	189 (87.5)	0.881

[a] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) stunting.
[b] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) wasting.
[c] Breastfeeding defined as ≥80% exclusive breastfeeding over 18 weeks, based on responder report.
[d] Diarrhea defined as the passage of ≥3 loose or watery stools in a 24 h period in 18 weeks, based on responder report; *p*-value calculated using Chi-squared or Student's *t* tests for categorical and continuous variables, respectively; *n* = number of subjects in numerator; *N* = total number of subjects.

Table 3
Poliovirus type 1, 2, and 3 seroconversion by various factors, univariate analysis, Pakistan, 2010.

Variable	Category	PV1 seroconversion		PV2 seroconversion		PV3 seroconversion	
		n/total *N* (%)	*p*-value	*n*/total *N* (%)	*p*-value	*n*/total *N* (%)	*p*-value
Zinc group	Zinc group	136/156 (87.2)	0.622	127/156 (81.4)	0.365	94/156 (60.3)	0.930
	Control group	139/163 (85.3)		126/163 (77.3)		99/163 (60.7)	
Zinc level	Normal (≥60 μg/dL)	126/145 (86.9)	0.682	118/145 (81.4)	0.494	90/145 (62.1)	0.747
	Deficient (<60 μg/dL)	133/156 (85.3)		122/156 (78.2)		94/156 (60.3)	
Stunting[a]	Normal	238/267 (89.1)	0.001	216/267 (80.9)	0.112	165/267 (61.8)	0.283
	Stunting	37/52 (71.2)		37/52 (71.2)		28/52 (53.8)	
Wasting[b]	Normal	190/218 (87.2)	0.470	177/218 (81.2)	0.223	139/218 (63.8)	0.080
	Wasting	85/101 (84.2)		76/101 (75.2)		54/101 (53.5)	
Breastfeeding[c]	≥80% exclusive	195/220 (88.6)	0.061	175/220 (79.5)	0.877	135/220 (61.4)	0.639
	<80% exclusive	80/99 (80.8)		78/99 (78.8)		58/99 (58.6)	
Diarrhea[d]	≥1 episode	71/84 (84.5)	0.602	57/84 (67.9)	0.003	40/84 (47.6)	0.005
	0 episodes	204/235 (86.8)		196/235 (83.4)		153/235 (65.1)	
Vitamin A[e]	Normal	150/169 (88.8)	0.173	140/169 (82.8)	0.095	108/169 (63.9)	0.211
	Deficient	110/132 (83.3)		99/132 (75.0)		75/132 (56.8)	
Iron deficiency anemia[f]	Normal	220/255 (86.3)	0.666	201/255 (78.8)	0.157	154/255 (60.4)	0.687
	Anemic	32/36 (88.9)		32/36 (88.9)		23/36 (63.9)	

[a] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) stunting.
[b] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) wasting.
[c] Breastfeeding defined as ≥80% exclusive breastfeeding over 18 weeks, based on responder report.
[d] Diarrhea defined as the passage of ≥3 loose or watery stools in a 24 h period in 18 weeks, based on responder report.
[e] Normal vitamin A classified as serum retinol levels ≥ 20 μg/100 ml.
[f] Iron deficiency anemia defined as hemoglobin level < 11 g/dL and ferritin < 12 ng/dL; *p*-value calculated using Chi-squared test; *n* = number of subjects in numerator; *N* = total number of subjects.

proportion of wasting in those that did not seroconvert ($p = 0.004$). No significant two-way interactions were found.

4. Discussion

The most important finding of our study is that zinc supplementation, despite evidence of improvement in serum zinc concentration, was not associated with any impact on immune response to OPV, as tested by seroconversion. Furthermore, these results remained basically unchanged, regardless of the cut-off values for zinc deficiency selected or when considering a continuous measure of zinc. In the multivariate logistic regression models, no association was found between seroconversion and zinc supplementation for PV1, PV2, or PV3, after adjusting for potential confounders. These results reveal that, among infants, zinc supplementation does not have an impact on immune response to OPV in our study. This is the first study to show a definitive lack of effect of zinc supplementation or higher zinc levels on the immune response to OPV.

Seroconversion was significantly associated with absence of diarrhea for PV2 and PV3, indicating that diarrhea may have an impact on seroconversion. Previous studies suggest that the presence of diarrheal illness at the time of administration of OPV significantly reduces the likelihood of seroconversion [11,30]. Association between recent diarrheal history and increased vaccine failure in infants has been shown in a study from Brazil [11]. Further, studies suggest that competing enteric virus infections, which are a common cause of diarrhea, may interfere with the OPV [31]; however, findings are inconsistent [32,33]. No association was found when considering PV1. This lack of association could be due, in part, to the low proportion of subjects with multiple diarrheal episodes. As a result, it was not possible to analyze frequency of events in relation to OPV vaccination. Additional information regarding duration and severity of each episode may have provided clarity on the relationship between seroconversion and diarrhea.

It is well documented that zinc plays a critical role in the prevention of diarrheal diseases and other infections in children through its central role in the immune system [3–5,9,10,34,35]. This is contradictory to the lack of difference in proportion of diarrhea between zinc and control groups found in this study. However, although zinc supplementation has proven effective for the treatment of diarrhea in studies with children >6 months of age [5,9,36], studies assessing the efficacy of zinc in <6 month olds have failed to find any relationship [37,38].

Our study failed to find an association between seroconversion and normal vitamin A levels for PV1, PV2 and PV3. There is evidence to suggest a relationship between adequate vitamin A status and optimal antibody responses [39]. However, findings remain inconclusive as some studies have shown an effect of vitamin A supplementation on OPV seroconversion [40] while others have failed to find an association [41–43].

There was no clear pattern between stunting or wasting and seroconversion. The only significant association was between stunting and a decrease in PV1 seroconversion. When considering the model with reduced sample size ($N = 274$) (i.e. assessing for vitamin A and iron deficiency anemia), the absence of wasting was

Table 4
Relationship between factors and poliovirus type 1, 2, and 3 seroconversion, multivariate odds ratios, Pakistan, 2010.

Variable	PV1 seroconversion OR (*p*-value)	PV2 seroconversion OR (*p*-value)	PV3 seroconversion OR (*p*-value)
Zinc group	1.15 (0.677)	1.25 (0.424)	0.97 (0.907)
Stunting[a]	0.30 (0.001)	0.59 (0.134)	NA
Wasting[b]	NA	NA	0.66 (0.097)
Diarrhea[c]	NA	0.42 (0.004)	0.49 (0.006)

[a] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) stunting.
[b] Includes moderate (*z*-score: −2 to −3) and severe (*z*-score: <−3) wasting.
[c] Diarrhea defined as the passage of ≥3 loose or watery stools in a 24 h period in 18 weeks, based on responder report. NA = not included in final model. Breastfeeding removed because not included in final models; number of subjects (*N*) = 319.

associated with PV3 seroconversion. Of the 45 subjects removed for this analysis, there was a significantly lower proportion of wasting in those that did not seroconvert ($p = 0.004$). As fewer wasted compared to non-wasted subjects that did not seroconvert to PV3 were removed from the analysis, the association between these variables were inflated in the reduced sample size analysis. The model in Table 4 likely better reflects the true relationship between PV3 seroconversion and wasting. Research suggests that undernutrition related to diarrhea, helminth infections, or micronutrient deficiency is more likely to reduce vaccine response in older children [32]. This indicates that prolonged undernutrition is likely more predictive of vaccine response. Our study was restricted to young infants, which may explain the lack of clear association.

This study found no evidence for a hypothesized augmentation of the immune response to OPV by zinc supplementation. However, important study limitations must be mentioned. The study population may not be representative of the entire population of Pakistan, as the subjects were from the rural settlement of low socioeconomic status. Therefore, these findings may not be generalizable. Moreover, of greater concern was the issue of missing data as up to 21% of subjects were missing lab data on included variables (e.g. ferritin and vitamin A). This may have prevented the adequate adjustment of key covariates and biased the association between seroconversion and factors included in the multivariate analysis. However, less than 13% of subjects were missing data on the primary outcome (i.e. PV1, PV2, PV3 titer) and the number of subjects in each group fulfilled the sample size requirement of 121, providing enough power to find an association between zinc supplementation and immune response to OPV. This missing data was mainly due to insufficient quantities of blood samples, which is a challenge encountered when collecting samples from infants in community settings. Additionally, the study lacked information regarding whether children with diarrhea were given additional zinc in combination with oral rehydration salts, as recommended by the WHO/UNICEF in the clinical management of diarrhea [44]. However, the proportion of subjects with multiple diarrheal episodes was quite low. Further, not all subjects receiving zinc supplementation had normal zinc levels by week 18. In those receiving zinc, the proportion of diarrhea, stunting and wasting was not significantly different between subjects with deficient and normal zinc levels at week 18. There are likely underlying factors (e.g. genetics, sub-clinical infections) contributing to the lack of effect of zinc supplementation in this population. Finally, since our study was restricted to young infants, the findings cannot be generalized to all children, as infants may not display the same patterns of zinc deficiency as older children (i.e. >6 months).

This study demonstrates that zinc supplementation alone does not impact the immune response to OPV in infants; however, further research assessing multiple forms of intervention (e.g. zinc plus vitamin A) as well as details on frequency, duration and severity of nutritional markers (e.g. diarrhea) is required to understand the complex role between malnutrition and micronutrient deficiencies and the immune response to OPV. It is undisputed that improved nutrition improves the immune response to vaccines; however, identifying the components relevant to improving this immune response must be uncovered. Finally, a clearer understanding of whether infants in this age group actually develop clinically significant zinc deficiency is required.

In Pakistan, the continuing circulation of both PV1 and circulating vaccine-derived poliovirus type 2 is a major threat to the global polio eradication initiative. Improving the immune response to OPV in this population will help Pakistan stop poliovirus transmission by enabling the country to achieve its target of >95% population immunity in children under five. Addressing risk factors such as malnutrition and micronutrients deficiencies particularly play an important role in achieving this goal. Further research is required to understand that role micronutrients play in enhancing the immune response to OPV to shape strategic action. However, as reported in our study, notwithstanding the potential health benefits of zinc supplementation, there is no imperative to justify the implementation of zinc supplementation to accelerate polio eradication in Pakistan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.12.001.

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