# Serum levels of vitamins A and D, and zinc in children with acute diarrhea: A cross-sectional study

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## Abstract

**Background:** Diarrhea is a leading cause of mortality and morbidity during the early life period especially in developing countries. Micronutrients deficiencies have been proposed either as a risk factor or a consequence of diarrhea. Association studies highlight the relation of vitamins and minerals' deficiencies with acute diarrhea. In this regard we aimed to evaluate the status of vitamins A and D, and zinc serum levels in children with acute diarrhea.

**Methods**: In this cross sectional study performed in a referral teaching hospital, we measured and compared baseline vitamin A, 25-hydroxy vitamin D (25(OH)D), and zinc serum levels in 25 children admitted with acute diarrhea and 25 other children who were admitted for undergoing elective surgeries.

**Results**: 25-(OH)D levels were significantly lower in the diarrhea group (p=0.03). We were unable to demonstrate a significant difference in the levels of vitamin A and zinc between the case and control groups (p= 0.14 and p=0.07, respectively).

**Conclusion**: We observed lower serum 25(OH)D levels in children with acute diarrhea. Whether this finding indicates a premorbid risk factor or simply a consequence of diarrhea needs further studies. Regardless of the cause and effect relationship, supplementation with vitamin D in acute diarrhea remains as a plausible consideration.

**Keywords**: Acute diarrhea, Micronutrients deficiencies, Vitamin A, Vitamin D, Zinc, Children.

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## Introduction

Diarrhea is a major cause of malnutrition in children and ranked as the second cause of mortality among children younger than 5 years according to World Health Organization (WHO) report in 2009 (1). Almost one in every 5 deaths in children and a total of 1.5 million deaths annually were related to diarrhea (2). On the other hand, malnutrition is one of the main risk factors for diarrheal disease and diarrhea confers a high mortality rate in malnourished children (1).

Combination of micronutrient deficiency and diarrhea still is a leading cause of mortality in the early life globally and remains one of the greatest health challenges in developing countries (3).

Adequate intake of vitamins and micronutrients are essential for proper function of immune system. Micronutrient deficiency may affect both innate cell-mediated immunity and adaptive antibody response and has shown to increase diarrhea related morbidity and mortality (4). It is claimed that

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overtreatment of diarrhea with antibiotics is a main reason for drug resistance in developing countries and increases the need for alternative treatments and preventive methods (5). Besides, there is an abundant literature on the specific role of zinc and vitamin A in the outcome of diarrheal illness particularly in developing countries. In fact, zinc and vitamin A supplementation have been already recognized as simple and affordable measures to reduce incidence and severity of diarrhea (5-7). Zinc supplementation reduces the duration of diarrhea by 25% and is associated with a 30% reduction in stool volume (8). Furthermore, the unique and critical function of vitamin D and its receptors in gastrointestinal (GI) mucosal protective barrier have been investigated and emerging evidence support the role of vitamin D in the integrity and repair of colonic epithelium (9).

Updated information on the frequency of micronutrients in childhood diarrheal illness in a local setting provides new guides for further research in this area. Accordingly, in this study we intended to compare the serum levels of zinc, vitamins A and D in children with infectious diarrhea with a control group.

### Methods

We conducted this cross sectional study in the pediatric department of Hazrat-e-Rasoul Akram hospital, a tertiary care center, located in Tehran, Iran between July and November 2012. We recruited 25 children with acute infectious diarrhea and 25 children as control group. The cases were included sequentially if they fulfilled the inclusion criteria and consented by proxy before participating into the study.

The enrolled children aged between 6 m and 15 y admitted to the hospital with a clinical diagnosis of acute infectious diarrhea. In our study we defined acute diarrhea according to WHO definition (i.e. the passage of three or more loose or liquid stools per day or more frequently than is normal for the individual for less than 2 weeks duration), regardless of the infectious etiology

of diarrhea (1). An infectious cause of diarrhea was ascertained either by identification of the responsible organism by stool smear and culture or if other etiologies for acute diarrheal illness were effectively ruled out.

We excluded subjects with growth problems (i.e. weight for age, height for age or weight for height <-2 z-score in WHO Multicenter Growth Reference Study growth curves), underlying diseases, and associated infection out of GI tract or diarrheal illness of more than 2 weeks duration (10,11).

A questionnaire was applied to participants to collect data on demographic variables, medical history and laboratory results including stool smear and culture.

The control subjects were recruited from the children aged in the same range selected for the patients' group who were admitted to the hospital for elective surgeries and lacked any concurrent infections.

After fulfilling the inclusion criteria and taking proxy consent, a sample of additional 5 milliliters of clotted blood was obtained from each participant simultaneously with the same venipuncture attempt to collect blood for the desired tests. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored in -20 °C. The Zinc, vitamin A and 25-hydroxyvitamin D (25(OH)D) levels were measured in all samples. The normal cut off points were adopted from the kits' information sheets.

Briefly, to measure the zinc levels we used the atomic absorption spectrophotometer AVANTA ∑ (GBC Scientific Equipment Pty Ltd, Melbourne, Australia). Serum zinc levels equal or higher than 65µg/dL were considered normal.

Vitamin A levels were measured by high performance liquid chromatography (HPLC) (Craft technologies, Inc). The normal levels were expressed according to the age. In our study vitamin A levels between 0.2 and 0.49 mg/mL were considered normal.

We measured 25(OH)D levels by a radioimmunoassay kit (Diasorin, Stillwater, MN, USA) and considered a 25(OH)D level ≤20

ng/mL (50 nmol/L) as deficient and a higher level of 25(OH)D up to 30 ng/mL (75 nmol/L) as insufficient (12-14).

This study was approved by the Ethics Committee of Research Center of Infectious Diseases in Iran University of Medical Sciences. For all participants, informed consents were obtained from parents or legal guardians. No obligations were imposed if parents were unwilling to enter their child into the program. Strict considerations were applied to protect all personal information. No additional expenses were imposed on participants for the study laboratory tests.

We used SPSS 19 for statistical operations. The data were presented using descriptive statistics. We employed Chi square and student's t-tests where appropriate to compare the two groups. All p values were calculated two-sided and values lower than 0.05 were considered significant.

## Results

Of 50 participants, divided equally into case and control groups, 31 (62%) were male and 19 (38%) female. The average (±SD) age was 25.9 ± 25.6 months. The weight, height, and body mass index (BMI) were 11.05 ± 6.69 kg, 84.60 ± 23.69 cm, and 15.64 ± 7.00, respectively. Table 1 demonstrates the baseline characteristics of case and control groups.

Table 2 indicates that serum vitamin A levels were not significantly different between the two groups (p= 0.14), however serum 25(OH)D levels were lower in patients with diarrhea (p= 0.03). Nobody was diagnosed with vitamin A deficiency in either group.

According to our reference values, no one in control group but 9 children (36%) in case group had 25(OH)D levels below 20 ng/mL which defined as deficient. There were 5 individuals in control group with 25(OH)D levels between 20 and 30 ng/mL indicating a rate of 20% for vitamin D insufficiency. The rate for vitamin D insufficiency in diarrhea group was 24% (6 cases) rising the cumulative frequency of vitamin D deficiency/insufficiency to 60% (15 cases) in this group.

A trend for lower zinc levels was detected in the patient group though this trend was not statistically significant (p= 0.07). Zinc level in the range of deficiency was only detected in a single child in case group.

## Discussion

Gastrointestinal (GI) tract mucosa is a part of innate immune system and acts as a defensive barrier against offending intraluminal organisms. Vitamin A and its derivatives play a critical role in maintaining the integrity of GI mucosa through regulating growth and development of intestinal epithelial cells. Therefore, patients with vitamin A deficiency are susceptible to various GI infections (15, 16). Nojilana et al. studied the role of vitamin A in mothers and their children in South Africa in 2000 and found that 28% diarrhea related mortality in children younger than 4 years were due to vitamin A deficiency (17). Inversely, diar-

Table1. Baseline characteristics of case and control groups

| | Case group (n=25) | Control group (n=25) | p |
|---|---|---|---|
| Male/female ratio | 1.7 | 1.5 | 1 |
| Age (years) | 2.4 | 6.7 | <0.001 |
| Weight (kg) | 10.2 | 22.8 | <0.001 |
| Height (cm) | 83.3 | 115.6 | <0.001 |
| BMI (kg/m$^2$) | 14.6 | 16.4 | 0.1 |

Table 2. Micronutrient levels in patients with diarrhea compared to control group

| Micronutrient | Control | | Patient | | p |
|---|---|---|---|---|---|
| | Average | Standard deviation | Average | Standard deviation | |
| 25(OH)D (ng/mL) | 52.80 | 25.39 | 36.01 | 26.28 | 0.03 |
| Vitamin A (µg/mL) | 0.57 | 0.14 | 0.47 | 0.15 | 0.14 |
| Zinc (µg/dL) | 118.05 | 17.29 | 102.77 | 38.35 | 0.07 |

rheal illness may cause vitamin A deficiency (18, 19).

Likewise, there may be a role of vitamin A supplementation in decreasing the rate of GI infections. Lima et al. showed vitamin A supplementation in patients with Giardia prevented subsequent parasitic infestations (20). Lang found that vitamin A and zinc supplementations were effective in primary prevention of Giardia infection (21). Based on such data, many believe that vitamin A deficiency has an important role in parasitic diarrhea incidents especially Giardiasis and may even cause chronic diarrhea (18, 21).

In our trial no difference was noticed between the serum levels of vitamin A in children with or without diarrhea, although no discrimination was imposed based on the causative pathogen.

Zinc plays a multi-aspect role in immune system and children with zinc deficiency are prone to many infections. Its mechanism has been an interesting subject for research for a long time (22). It is clear that zinc deficiency affects the immune system in many ways ranging from problems with skin protection to lymphocyte gene expression. Zinc is essential for development and function of immune cells such as neutrophils and natural killer cells, growth and function of T and B lymphocytes, and cytokines and immunoglobulin production. This micronutrient also acts as an antioxidant and support stability of cell membranes (23).

Diarrheal diseases occur less frequently in children receiving supplementary zinc. The studies on pediatric age group have shown that zinc supplementation reduced the incidence of diarrhea and improved treatment outcome in comparison to the controls (24-35).

There are two studies from Iran, reported from Babol and Ahvaz cities in which frequency of zinc deficiency was assessed in diarrhea and control groups showing lower serum zinc levels in diarrhea patients and a correlation of zinc deficiency with diarrhea duration (36, 37). In our study, despite observing a trend, we were unable to demonstrate a significant reduction in zinc levels in the case group. That could be attributed to the time of blood sampling. We obtained the blood samples at the time of hospital admission, based on a restricted policy to detect the baseline levels of micronutrients. However, in the two former studies a more liberal sampling policy was employed so that sampling had temporally happened in a wide range over hospital stay. It is wise to postulate that low serum zinc levels in those studies may be due to late sampling in diarrhea course when there is a higher chance for zinc deficiency. Moreover, we selected our sample case from Tehran -a more developed metropolitan area- where a higher density of advantaged population with a favorable premorbid nutritional status is more expected.

Vitamin D is involved in inflammatory processes and immune regulations. Receptors for vitamin D are extensively distributed in the majority of body tissues including all immune cells (38- 40).

Few studies have been investigated the effects of vitamin D on diarrhea in children (41). Available data suggest a relation between vitamin D deficiency and increased rate of GI and ear infections (42).

In our study serum vitamin D levels were lower in the case group compared to the controls. Whether this finding was just an epiphenomenon or indeed representing a co-risk factor for diarrhea is unclear. As we obtained the blood samples early during the hospital course, the authors are in favor of the latter explanation. Longitudinal studies are required to address and explore this issue.

Interestingly, no one in control group was diagnosed with vitamin D deficiency. This is in contrast to current literature which suggests a high rate of vitamin deficiency in general population (43). Our contradictory results may be related to the controversy surrounding the cut off point for defining low 25(OH)D levels (44, 45). The contemporary literature tends to accept a cut off level of 20 ng/mL, the same as we adopted for our study. Nevertheless, some authors

believe a level between 20 and 30 ng/mL still signifies a mild level of deficiency (insufficiency) as it is associated with a rise in PTH level. This confusion in recognizing vitamin D deficiency/insufficiency has been extended to the literature and caused different reports on the prevalence of vitamin D deficiency. Thus, true severe vitamin D deficiency is not as common as it is supposed. In fact, we identified 5 individuals (20%) with insufficient serum vitamin D levels in our control population. Similarly, a survey on children population aged 6 and 7 years in Isfahan, Iran demonstrated that while 26% of the children had a 25(OH)D level below 33 ng/mL, only 3% had a level below 20 ng/mL (46).

There are several limitations in our research. As this was a single center study, we were unable to rule out the bias of local population specific characteristics which hinder the extrapolation of the results to reference population. The limited numbers of our case and control groups could have affected the power of the study. In fact, we found a non-significant trend toward lower levels of vitamin A and serum zinc levels in diarrhea group. We cannot reject the possibility of type II error due to under sampling. We enrolled the control subjects from admitted children expecting elective surgeries. Their mean age was 2.4 years versus 6.7 years in the case group. It is probable that the age difference may have interacted as a confounding variable in comparing micronutrient serum levels between the two groups. The design of our study was cross sectional. This design is unable to demonstrate a cause and effect relationship. Longitudinal studies are more appropriate to show whether there is a higher frequency of diarrheal illness in children with micronutrient deficiencies or not.

### Conclusion

We found lower serum vitamin D levels in children with acute diarrhea. That was not the case for vitamin A and zinc levels. Our observation may signify a lower premorbid vitamin D reserve with a resultant susceptibility to vitamin D deficiency in children affected by diarrhea. The implication of vitamin D deficiency on pathogenesis of diarrhea and the role of supplementation of vitamin D in improving diarrhea outcome are two major issues that require further investigation.

### *Conflict of interest*

The authors declare no competing interests.

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