



# Is There a Correlation Between Vitamin D Levels and Acute Diarrhea in Children?

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## Abstract

**Background:** Some studies have shown that low vitamin D is a risk factor for infectious diarrhea, but some have rejected it.

**Objectives:** Considering the high prevalence of infectious diarrhea among children, the high prevalence of vitamin D deficiency, and the possible mutual effect of these two, the present study aimed to measure vitamin D in children with acute diarrhea.

**Methods:** This study enrolled 222 children aged 2 - 14 in healthy control and acute diarrhea groups. The 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the Chemiluminescent Immunoassays (CLIA) method.

**Results:** The mean age of the participants was  $5 \pm 2.7$  years. Patients with acute diarrhea had lower vitamin D than healthy controls ( $P = 0.04$ ). The frequency of deficient and insufficient vitamin D levels was higher in the acute diarrhea group than in the healthy control group, but insignificantly ( $P = 0.146$ ).

**Conclusions:** The present study revealed an association between insufficient vitamin D and acute diarrhea. Hence, low vitamin D is a risk factor for acute diarrhea.

**Keywords:** Child, Diarrhea, Vitamin D, Vitamin D Deficiency

## 1. Background

Infectious diarrhea is one of the most prevalent diseases in underdeveloped nations. Despite improvements in hygiene and healthcare, the infectious diarrhea mortality rate remains high (1). Acute infectious diarrhea is classified as either inflammatory or non-inflammatory (2). Various organisms, including bacteria, viruses, and parasites, can cause acute infectious diarrhea. Viruses, parasites, and some bacteria are responsible for a significant proportion of non-inflammatory infectious diarrhea. In contrast, inflammatory diarrhea, an immense inflammation in the intestines, mainly results from bacteria and invasive pathogens such as *Shigella*, *Salmonella*, *Campylobacter*, Enteroinvasive *Escherichia coli* (EIEC), Shiga Toxin-producing *E. coli* (STEC), and *Entamoeba histolytica* (3).

A low vitamin D level is a risk factor for the occurrence or exacerbation of infectious diseases (4). Several studies have investigated the relationship between low vitamin D levels and infectious diseases such as pneumonia (5) and otitis media (6). On the other hand, it has been proven

that vitamin D supplementation plays a role in preventing some infectious diseases (7, 8). Several mechanisms have been proposed regarding the effect of vitamin D on the immune system. Synthesis of some bactericidal peptides activated in the innate immune system depends on sufficient vitamin D levels (9). Moreover, many functions in the adaptive immune system are inhibited by vitamin D, resulting in reduced inflammatory cytokines and increased anti-inflammatory cytokines (10, 11). On the other hand, in many cell types other than immune cells, such as keratinocytes, lung epithelial cells, and placental trophoblasts, an activated form of vitamin D regulates antibacterial proteins (12).

Several studies worldwide have reported the relationship between low vitamin D levels and diarrhea in children (13). Some studies have shown that low vitamin D level is a risk factor for infectious diarrhea (14), while some have rejected this association (15, 16). Therefore, there are contradictory studies in this field.

## 2. Objectives

Considering the high prevalence of infectious diarrhea among children, the high prevalence of vitamin D deficiency, and the possible mutual effect of these two, the present study aimed to measure vitamin D in children with acute diarrhea.

## 3. Methods

This case-control study was conducted at 17 Shahrivar Hospital in Rasht, Iran, a secondary and tertiary pediatric care center, between 15 May 2022 and 15 November 2022. We recruited 222 children aged 2 - 14 and assigned them to healthy control or acute diarrhea groups. They were sequentially included in each group if they fulfilled the inclusion criteria. The study procedure and possible risks were thoroughly explained, and informed consent was obtained.

Based on the WHO definition, we regarded acute diarrhea as the passage of three or more loose or liquid stools per day or more frequently than is normal for an individual for up to two weeks. According to this definition, patients aged 2 - 14 hospitalized due to acute diarrhea were included. The RBC should not be seen in the stool sample of any patient. Group matching was done for age and gender.

We excluded patients with underlying liver, kidney, or metabolic diseases, immunodeficiency, rickets, malnutrition, hypocalcemia, a history of cerebral palsy, drug complications such as phenytoin, phenobarbital, and rifampin complications, diarrhea for more than two weeks, children with known vitamin D deficiency, history of supplementation with vitamin D, and a history of intestinal resection surgery. Healthy children with no underlying disease admitted to checking their vitamin D level and consented to participate in the study were considered controls. The control group was matched with the other two groups for age, sex, and confounding factors.

After obtaining informed consent, demographic information, medical history, drug history, and the severity of dehydration at admission were recorded in pre-designed forms. Then, 5 mL of clotted blood was taken from each participant (the sample was collected from a superficial vein in the upper limb, generally the median cubital vein). The serum was separated by centrifugation at 3,000 rpm for 10 minutes and kept at  $-20^{\circ}\text{C}$  until sufficient samples were collected for a batch test. Finally, the 25-hydroxyvitamin D (25(OH)D) level was measured in all samples by the chemiluminescent immunoassays (CLIA) method using the Architect Abbott i2000 immunoassay

analyzer (USA). The person in charge of the test was unaware of group assignments and only reported the results. A more than 30 ng/mL serum vitamin D level was sufficient, between 20 and 30 was insufficient, and less than 20 was deficient.

### 3.1. Ethical Considerations

The research was accepted by the Faculty of the Medicines Ethics Committee of Guilan University of Medical Sciences (number: [IR.GUMS.REC.1401.065](#) date: 2022-5-18). Informed consent was obtained from all participants or legal guardians in this study, and the study procedure and possible risks were clearly explained. Patient information remained utterly confidential. Honesty in data collection and reporting was fully accomplished. No coercion or promotion was applied for the patients to participate in this study, and no fees were charged.

### 3.2. Sample Size

Based on the Mahyar et al. study (14), the frequency of vitamin D deficiency was 11.6% in the case group and 3.3% in the control group. We found that 74 cases for the control group and 148 for the acute diarrhea group are needed to determine the relationship between vitamin D levels and acute diarrhea (power = 85%,  $\alpha = 0.05$ ).

### 3.3. Statistical Analysis

The quantitative variables are shown as mean (standard deviation), and the qualitative variables as frequency percentages. The Kolmogorov-Smirnov test was used to check the assumption of normality of the vitamin D level. Kruskal-Wallis's non-parametric analysis compared the average vitamin D level between three groups of children with acute inflammatory diarrhea, acute non-inflammatory diarrhea, and healthy children. Also, the prevalence of vitamin D deficiency among the two groups of children was compared using the chi-square test. The data were analyzed using IBM SPSS Statistics version 24 and MedCalc version 20.011 software. The significance level was set at 0.05.

## 4. Results

The current study enrolled 222 children aged 2 - 14, including 148 children with acute diarrhea and 74 healthy controls. The baseline characteristics of patients are shown in [Table 1](#). The mean age of the participants was  $5 \pm 2.7$  years, and 114 (51.4%) were female. The mean age of healthy controls and acute diarrhea children were  $5.53 \pm 3.18$  and  $5.31 \pm 2.31$ , respectively.

**Table 1.** Baseline Characteristics of Participants

Variables	Healthy Controls	Acute Inflammatory Diarrhea	Acute Non-Inflammatory Diarrhea	P-Value
Age (y)	5.53 (3.18)	5.36 (2.14)	5.26 (2.74)	0.753 <sup>a</sup>
Female (%)	52.7	48.6	52.7	0.85 <sup>b</sup>

<sup>a</sup> based on Kruskal-Wallis test.<sup>b</sup> based on chi-square test.

Patients with acute diarrhea had lower vitamin D levels than healthy controls ( $P = 0.04$ ). In the qualitative assessment, the frequency of deficient and insufficient vitamin D levels was higher in the acute diarrhea group than in the healthy control group, but insignificantly ( $P = 0.146$ ) (Table 2).

## 5. Discussion

The present study evaluated the impact of vitamin D deficiency on acute diarrhea in children aged 2 to 14. This study included 222 patients in two groups, including acute diarrhea and healthy controls, and the results indicated that patients with acute diarrhea had lower vitamin D levels than healthy controls.

Studies in this field have contradictions. Some studies believe that low levels of vitamin D are related to diarrhea. Bener et al. (17) and Thornton et al. (6) indicated that the incidence of diarrhea was significantly higher in children with vitamin D deficiency. Talachian et al. noted that low vitamin D levels were associated with acute infectious diarrhea (18). Bucak et al. also concluded that low vitamin D was a predisposing factor for rotavirus diarrhea (19). Mahyar et al. proved a significant correlation between acute bacterial diarrhea and serum vitamin D levels, and vitamin D might play a role in the pathogenesis of diarrhea (14). Other studies also concluded that low vitamin D has a role in exacerbating and prolonging *Clostridium difficile* diarrhea (20, 21). In line with these studies, we showed that insufficient and deficient vitamin D levels were associated with acute diarrhea.

On the other hand, Urashima et al. concluded that the diarrhea incidence risk did not decrease with vitamin D supplementation (15). Ahmed et al. also indicated no correlation between vitamin D deficiency and diarrhea caused by Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and Enteroaggregative *E. coli* (EAEC). They asserted that this result might be specific to enterotoxin-producing bacteria. Different sample sizes, exposure to the sunlight, climatic conditions, nutritional status, age, and race of patients can justify these contradictions (16).

Besides the main role in regulating calcium metabolism, vitamin D also has other functions, such

as anti-inflammatory, antibacterial, and immunomodulatory effects (22). For example, it can affect the synthesis of bactericidal peptides (23) and regulate the adaptive immune system through IgA upregulation (24). In our study, the higher prevalence of vitamin D insufficiency and deficiency in the acute diarrhea group possibly shows a more significant role of vitamin D against viral diarrheagenic pathogens. Bucak et al. also stated that low vitamin D is a predisposing factor for rotavirus diarrhea (19). An in vivo study by Blutt et al. indicated the effect of IgA on the protection against viral infections. This study also showed the vulnerability against rotavirus diarrhea in the absence of IgA (25). More studies are needed to prove the definitive effect of vitamin D on viral diarrhea and investigate the underlying mechanism.

It should be mentioned that climate conditions and sunlight exposure can affect vitamin D levels (26). In the current study, no significant differences were observed among the study groups regarding vitamin D sufficiency, insufficiency, and deficiency. According to the climatic and cultural conditions of the study city, Rasht, deficient and insufficient vitamin D are common (27). As seen in the control group, 74.3% had deficient and insufficient vitamin D. It was one of our study's limitations. Therefore, more studies in settings where vitamin D deficiency and insufficiency are not common are needed to prove the more precise effects of vitamin D on viral diarrhea.

Nonetheless, our study had several limitations to consider in interpreting the data. First, this study had a small sample size, and the findings need confirmation in larger studies with adequate sample sizes. Second, it would be better to measure vitamin D in patients with diarrhea after recovery, which was not measured in our study. It is suggested to consider this in future studies.

### 5.1. Conclusions

The present study revealed the association between insufficient vitamin D levels and acute diarrhea. Low vitamin D is a risk factor for acute diarrhea. More studies are needed to clarify this issue.

**Table 2.** Comparison of Age, Gender, and 25-Hydroxyvitamin D by Groups

Variables	Healthy Controls; (N = 74) (%)	Acute Diarrhea; (N = 148) (%)	P-Value
Age	5.53 (3.18)	5.31 (2.44)	0.576 <sup>a</sup>
Female	39 (52.7)	75 (50.7)	0.776 <sup>b</sup>
25 (OH) D (quantitative)	25.26 (10.55)	22.92 (6.25)	0.04 <sup>a</sup>
25 (OH) D (qualitative)			0.146 <sup>b</sup>
Deficient	22 (29.7)	49 (33.1)	
Insufficient	33 (44.6)	77 (52)	
Sufficient	19 (25.7)	22 (14.9)	

<sup>a</sup> Kruskal-Wallis test<sup>b</sup> Chi-square test

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## Footnotes

**Authors' Contribution:** H. H.: Study concept and design. S. E. S.: Acquisition of data. S. S.: Critical revision of the manuscript for important intellectual content. S. M.: Analysis and interpretation of data. N. D.: Critical revision of the manuscript for important intellectual content. A. H.: Statistical analysis. M. A. E.: Drafting of the manuscript. S. D.: Study supervision.

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## References

- Shin HJ, Kang SH, Moon HS, Sung JK, Jeong HY, Kim JS, et al. Serum procalcitonin levels can be used to differentiate between inflammatory and non-inflammatory diarrhea in acute infectious diarrhea. *Medicine (Baltimore)*. 2018;**97**(32). e11795. [PubMed ID: [30095641](https://pubmed.ncbi.nlm.nih.gov/30095641/)]. [PubMed Central ID: [PMC6133584](https://pubmed.ncbi.nlm.nih.gov/PMC6133584/)]. <https://doi.org/10.1097/MD.00000000000011795>.
- Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. *Harrison's principles of internal medicine*. 19 ed. New York, USA: McGraw Hill Professional; 2015.
- Kim DH, Kang SH, Jeong WS, Moon HS, Lee ES, Kim SH, et al. Serum C-reactive protein (CRP) levels in young adults can be used to discriminate between inflammatory and non-inflammatory diarrhea. *Dig Dis Sci*. 2013;**58**(2):504-8. [PubMed ID: [22926497](https://pubmed.ncbi.nlm.nih.gov/22926497/)]. <https://doi.org/10.1007/s10620-012-2365-3>.
- Hassam I, Kisenge R, Aboud S, Manji K. Association of vitamin D and diarrhoea in children aged less than five years at Muhimbili national hospital, Dar es Salaam: An unmatched case control study. *BMC Pediatr*. 2019;**19**(1):237. [PubMed ID: [31307425](https://pubmed.ncbi.nlm.nih.gov/31307425/)]. [PubMed Central ID: [PMC6628469](https://pubmed.ncbi.nlm.nih.gov/PMC6628469/)]. <https://doi.org/10.1186/s12887-019-1614-4>.
- Oduwole AO, Renner JK, Disu E, Ibitoye E, Emokpae E. Relationship between vitamin D levels and outcome of pneumonia in children. *West Afr J Med*. 2010;**29**(6):373-8. [PubMed ID: [21465443](https://pubmed.ncbi.nlm.nih.gov/21465443/)]. <https://doi.org/10.4314/wajm.v29i6.68261>.
- Thornton KA, Marin C, Mora-Plazas M, Villamor E. Vitamin D deficiency associated with increased incidence of gastrointestinal and ear infections in school-age children. *Pediatr Infect Dis J*. 2013;**32**(6):585-93. [PubMed ID: [23340562](https://pubmed.ncbi.nlm.nih.gov/23340562/)]. <https://doi.org/10.1097/INF.0b013e3182868989>.
- Jolliffe DA, Camargo CJ, Sluyter JD, Aglipay M, Aloia JF, Ganmaa D, et al. Vitamin D supplementation to prevent acute respiratory infections: A systematic review and meta-analysis of aggregate data from randomised controlled trials. *Lancet Diabetes Endocrinol*. 2021;**9**(5):276-92. [PubMed ID: [33798465](https://pubmed.ncbi.nlm.nih.gov/33798465/)]. [https://doi.org/10.1016/S2213-8587\(21\)00051-6](https://doi.org/10.1016/S2213-8587(21)00051-6).
- Ganmaa D, Uyanga B, Zhou X, Gantsetseg G, Delgerekh B, Enkhmaa D, et al. Vitamin D supplements for prevention of tuberculosis infection and disease. *N Engl J Med*. 2020;**383**(4):359-68. [PubMed ID: [32706534](https://pubmed.ncbi.nlm.nih.gov/32706534/)]. [PubMed Central ID: [PMC7476371](https://pubmed.ncbi.nlm.nih.gov/PMC7476371/)]. <https://doi.org/10.1056/NEJMoai915176>.
- Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006;**311**(5768):1770-3. [PubMed ID: [16497887](https://pubmed.ncbi.nlm.nih.gov/16497887/)]. <https://doi.org/10.1126/science.1123933>.
- Tang J, Zhou R, Luger D, Zhu W, Silver PB, Grajewski RS, et al. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. *J Immunol*. 2009;**182**(8):4624-32. [PubMed ID: [19342637](https://pubmed.ncbi.nlm.nih.gov/19342637/)]. [PubMed Central ID: [PMC2756755](https://pubmed.ncbi.nlm.nih.gov/PMC2756755/)]. <https://doi.org/10.4049/jimmunol.0801543>.
- Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, et al. Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. *J Immunol*. 2007;**179**(9):6273-83. [PubMed ID: [17947703](https://pubmed.ncbi.nlm.nih.gov/17947703/)]. <https://doi.org/10.4049/jimmunol.179.9.6273>.
- Ismailova A, White JH. Vitamin D, infections and immunity. *Rev Endocr Metab Disord*. 2022:1-13.
- Marginean CO, Melit LE, Borka Balas R, Vasiesiu AM, Fleseriu T. The crosstalk between vitamin D and pediatric digestive disorders. *Diagnostics (Basel)*. 2022;**12**(10). [PubMed ID: [36292016](https://pubmed.ncbi.nlm.nih.gov/36292016/)]. [PubMed Central ID: [PMC9600444](https://pubmed.ncbi.nlm.nih.gov/PMC9600444/)]. <https://doi.org/10.3390/diagnostics12102328>.

14. Mahyar A, Ayazi P, Saffari Rad M, Dalirani R, Javadi A, Esmaeily S. The correlation between vitamin D and bacterial diarrhea in children. *Arch Pediatr Infect Dis.* 2019;**In Press**(In Press). <https://doi.org/10.5812/pedinfect.84382>.
15. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr.* 2010;**91**(5):1255-60. [PubMed ID: 20219962]. <https://doi.org/10.3945/ajcn.2009.29094>.
16. Ahmed AM, Soares Magalhaes RJ, Long KZ, Ahmed T, Alam MA, Hossain MI, et al. Association of vitamin D status with incidence of enterotoxigenic, enteropathogenic and enteroaggregative *Escherichia coli* diarrhoea in children of urban Bangladesh. *Trop Med Int Health.* 2016;**21**(8):973-84. [PubMed ID: 27253178]. <https://doi.org/10.1111/tmi.12731>.
17. Bener A, Al-Ali M, Hoffmann GF. Vitamin D deficiency in healthy children in a sunny country: A ssociated factors. *Int J Food Sci Nutr.* 2009;**60 Suppl 5**:60-70. [PubMed ID: 18946796]. <https://doi.org/10.1080/09637480802400487>.
18. Talachian E, Bidari A, Noorbakhsh S, Tabatabaei A, Salari F. Serum levels of vitamins A and D, and zinc in children with acute diarrhea: A cross-sectional study. *Med J Islam Repub Iran.* 2015;**29**:207.
19. Bucak IH, Ozturk AB, Almis H, Cevik MO, Tekin M, Konca C, et al. Is there a relationship between low vitamin D and rotaviral diarrhea? *Pediatr Int.* 2016;**58**(4):270-3. [PubMed ID: 26287796]. <https://doi.org/10.1111/ped.12809>.
20. Wong KK, Lee R, Watkins RR, Haller N. Prolonged clostridium difficile infection may be associated with vitamin D deficiency. *JPEN J Parenter Enteral Nutr.* 2016;**40**(5):682-7. [PubMed ID: 25623479]. <https://doi.org/10.1177/0148607114568121>.
21. Abdelfatah M, Nayfe R, Moftakhar B, Nijim A, El Zoghbi M, Donskey CJ, et al. Low vitamin D level and impact on severity and recurrence of *Clostridium difficile* infections. *J Investig Med.* 2015;**63**(1):17-21. [PubMed ID: 25479065]. <https://doi.org/10.1097/JIM.0000000000000117>.
22. Hewison M. Antibacterial effects of vitamin D. *Nat Rev Endocrinol.* 2011;**7**(6):337-45. [PubMed ID: 21263449]. <https://doi.org/10.1038/nrendo.2010.226>.
23. Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M, Wheelwright M, et al. Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. *PLoS One.* 2009;**4**(6). e5810. [PubMed ID: 19503839]. [PubMed Central ID: PMC2686169]. <https://doi.org/10.1371/journal.pone.0005810>.
24. Shirakawa AK, Nagakubo D, Hieshima K, Nakayama T, Jin Z, Yoshie O. 1,25-dihydroxyvitamin D3 induces CCR10 expression in terminally differentiating human B cells. *J Immunol.* 2008;**180**(5):2786-95. [PubMed ID: 18292499]. <https://doi.org/10.4049/jimmunol.180.5.2786>.
25. Blutt SE, Miller AD, Salmon SL, Metzger DW, Conner ME. IgA is important for clearance and critical for protection from rotavirus infection. *Mucosal Immunol.* 2012;**5**(6):712-9. [PubMed ID: 22739233]. [PubMed Central ID: PMC3461240]. <https://doi.org/10.1038/mi.2012.51>.
26. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. *Dermatoendocrinol.* 2013;**5**(1):51-108. [PubMed ID: 24494042]. [PubMed Central ID: PMC3897598]. <https://doi.org/10.4161/derm.24494>.
27. Ettehad H, Asadi K, Mirbolook MR, Soleimanha M, Adeli A, Haghparast Ghadim Limudahi Z, et al. Evaluation of 25-hydroxy vitamin D blood levels in patients with musculoskeletal pain. *J Guilan Univ Med Sci.* 2014;**23**(89):51-6. Persian.