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Original Research Article

Vitamin D status in children with recurrent acute diarrhea

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Keywords

Vitamin D, recurrent diarrhea, parasitic infection, children

A B S T R A C T

Over the past decade, interest has grown in the role of vitamin D in many non skeletal medical conditions. The immunemodulatory properties of vitamin D may influence susceptibility to infection. This cross sectional study aimed to study the association between vitamin D level and recurrent acute diarrhea. The study was conducted on 80 simple randomly selected children, aged from 4 to 12 years from November 2013 to May 2014, sixty patients were suffering from recurrent acut diarrhea and twenty were healthy, age and sex matched children taken as a control group. All children were subjected to complete history taking, clinical examination and Laboratory investigations in the form of hemoglobin level, stool analysis and estimation of the serum level of vitamin D by ELISA. There was highly significant decrease in vitamin D levels in patients group than control group. In patients with recurrent acute diarrhea, vitamin D deficiency was found in 58%, insufficient in 20 % and sufficient in 22%. Vitamin D deficiency was associated with increased rate of diarrheal attacks, vomiting and abdominal pain. Hemoglobin level was decreased below normal in 26.7% of children with recurrent diarrhea; most of them were vitamin D deficient children. Stool examination in children with recurrent diarrhea detected *Entameba histolytica* in 8.3%, *Giardia lamblia* in 13%, *Ascaris lumbricoides* in 1.7% and *Ancylostoma duodenal* in 1.7%, all parasites were detected in vitamin D deficient children, except *E. histolytica* detected also in vitamin D sufficient child. Recurrent acute diarrhea was associated with decreased serum level of vitamin D in preschool and school-age children. Vitamin D deficiency was associated with increased number of diarrheal attacks and *Giardia lamblia* parasitic infection.

Introduction

The immunoregulatory functions of Vitamin D have become prominent in the current medical literature. With respect to infectious diseases, there is growing evidence for vitamin D enhancing innate immunity (Bikle 2008 and White 2008). *In vitro* studies have

shown that 1,25-dihydroxyvitamin D_3, the active metabolite of vitamin D, is important for promoting and regulating immune responses (Cantorna, 2000; Pichler *et al.,* 2002). Vitamin D has numerous effects on cells within the immune system. It inhibits B cell proliferation and blocks B cell differentiation and immunoglobulin secretion (Chen *et al.,* 2007). Vitamin D additionally suppresses T cell proliferation and results in a shift from a Th1 to a Th2 phenotype (Boonstra *et al.,* 2001). Furthermore, it affects T cell maturation (Tang *et al.,* 2009) and facilitates the induction of T regulatory cells (Barrat *et al.,* 2002). These effects result in decreased production of inflammatory cytokines (IL-17, IL-21) with increased production of anti-inflammatory cytokines such as IL-10. Vitamin D also has effects on monocytes and dendritic cells (DCs). It inhibits monocyte production of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and TNFα (Almerighi *et al.,* 2009; Aranow, 2011). Previous studies have suggested a link between vitamin D deficiency and irritable bowel disease (IBD) risk (Lim *et al.,* 2005; Kong *et al.,* 2008). Few previous studies have reported on the potential effect of vitamin D on gastrointestinal infections in children (Finkelstein *et al.,* 2012). Thornoton *et al.,* (2013) suggested that Vitamin D deficiency is related to increased incidence of gastrointestinal and ear infections in school-age children. However, there is no clinical trial for the role of vitamin D in recurrent acute diarrhea in children. The present study represents the first trial in this direction. The aim of this study was to estimate the level of vitamin D in children with recurrent acute diarrhea.

Subjects and Methods

Subjects

-This case control study was carried out at pediatric department, Benha University hospitals, Qalubia Governorate, in the period from November 2013 to May 2014.
-It included two groups:

Group I (patients group): Sixty children that were selected randomly the ward, pediatric department, complaining of recurrent diarrhea. Recurrent diarrhea or repeated diarrhea is any large volume of stools, frequent bowel movements and/or watery loose stools that arise and resolve over and over again. Acute diarrhea is defined as an abnormally frequent discharge of semisolid or fluid fecal matter from the bowel, lasting less than 14 days, by World Gastroenterology Organization (Farthing *et al.,* 2013).

This group was divided into three subgroups according to vitamin D level (Thornoton *et al.,* 2013):

Group Ia: Vitamin D deficient group: was defined as vitamin D level below 20 ng/mL (50 nmol/L).

Group Ib: Vitamin D insufficient group: was defined as vitamin D level between 20 and 30 ng/mL (50 to 75 nmol/L).

Group Ic: Vitamin D sufficient group: was defined as vitamin D level above 30 ng/mL (75 nmol/L).

Group II (control group): Twenty healthy children matched for age and sex.

-Inclusion criteria:

1. Age: 4-12 years old
2. History of recurrent diarrhea.

-Exclusion criteria:

Children had history of rickets.

-A written informed consent was obtained from parents of all children before including in the study.

Materials and Methods

All children in the study were subjected to the following,

I. **Full history taking including**

- Personal history: name, age, sex, residence and social class (parents' education and crowding index), using Fahmy and El-Sherbini Scale (1983).

-History of diarrhea: number of episodes in last year, duration and associated symptoms, received treatment, previous hospital admission.

-Family history particularly of rickets or inflammatory bowel disease.

II. **Clinical examination**

Physical examination included weight and height. These values were plotted on international WHO Growth Charts of 2007 in percentiles. Children below the third percentile were considered underweight and short stature (and from 3^{rd} to97^{th}percentile were considered normal weight and height). Signs of nutrient deficiencies and abdominal distension were checked (Zella and Israel, 2012).

III. **Laboratory investigations:**

-CBC**:** Venous blood was collected and processed to detect complete blood count (Morris *et al.,* 1999).

-Stool analysis: Stool samples were examined for possible parasitic infection. Samples were collected in clean containers and then sent to the laboratory. Laboratory analysis included gross and microscopic examination by direct wet preparation, formol ether concentration technique and modified ZN stain. Stool was checked for color, consistency, amount, shape, odor, and the presence of mucus (Cheesbrough, 2005).

-Estimation of the serum level of 25 hydroxy-vitamin D in children with recurrent diarrhea as well as control by ELISA (Enzyme linked Immuno Sorbent Assay) technique. Blood was collected by venipuncture, allowed to clot. Serum was separated by centrifugation at room temperature. Specimens were frozen only once at -20°C till assay. Technique was performed according to manufacturer instructions (DRG International, Inc., USA).

Statistical analysis

Comparison between the different groups, the significance of difference was tested using one of the following tests:

1- Student's *t*-test: - Used to compare mean of two groups of quantitative data.
2- F test: - Used to compare mean of more than two groups of quantitative data of parametric and non-parametric data respectively.

3- Inter-group comparison of categorical data was performed by using chi square test (X^2-value) and fisher exact test (FET). *P* value <0.05 was considered statistically significant (S) while >0.05 statistically insignificant P value <0.01 was considered highly significant (HS) in all analyses.

Results and Discussion

A total of 80 children were collected: 60 with recurrent acute diarrhea (patients group) and 20 healthy (control group).

There were no significant differences between patients and controls as regard age (p=0.301), sex (p=0.69), residence (p=0.333), as showed in Table 1. Among patients group there were no significant differences between the three subgroups regarding age, sex, residence, and social class as p>0.05.

Children with recurrent acute diarrhea had significantly lower mean vitamin D level than controls (19.95±13.12 ng/ml vs. 47.36± 20.73 ng/ml, p=0.001), as showed in Table 2.

Thirty five children (58%) with recurrent diarrhea had vitamin D-deficient level (11.47±4.45 ng/ml), twelve children (20%) had insufficient level (25.06±2.71 ng/ml) and thirteen (22%) had sufficient level (38.07±13.85) ng/ml, with highly significant difference (P= 0.001) as showed in Table 3 and Figure 1 & 2. There was significant difference between the three subgroups as regard history of vitamin D supply (P < 0.05) but no significant difference as regard history of sun exposure, treatment received and history of dehydration (P > 0.05).

Numbers of diarrheal attacks increased with vitamin D deficiency, which was statistically significant (p=0.035) as showed in Table 4. Results of clinical examination in relation to vitamin D status, among 35 vitamin D deficient children, 6 were under weight, 3 had decreased height, 5 had pallor, one had history of dehydration and four had abdominal distention. In vitamin D insufficient group, one patient showed under weight and height and two patients had pallor. In vitamin D sufficient group 3 had underweight, 1 was under height, 2 had pallor,1 had abdominal pain and3 had distension with no significant difference (P> 0.05) as showed in Table 5.

E. histolytica, *Giardia lamblia*, *Ancylostoma dudenale* and *Ascaris lumbricoides* were detected in stool samples of 5, 8, 1, 1 child, respectively. All parasites were detected in vitamin D deficient children except for *E. histolytica* detected in one vitamin D sufficient child, with no significant difference (P > 0.05) as showed in Table 6.

CBC results detected microcytic hypochromic anemia in 16 children, ten of them were vitamin D deficient. One was vitamin D insufficient and five were vitamin D sufficient. Data was statistically insignificant.

Inadequate vitamin D status is highly prevalent in children worldwide, even in equatorial regions where it had not been previously suspected. In Latin America, prevalence of vitamin D insufficiency and deficiency among children and adolescents ranges from 28% in Costa Rica (Brehm *et al.*, 2009) to 56% in Bogota, Colombia (*Gilbert-Diamond et al., 2010*) and 62% in Sao Paulo, Brazil (Peters *et al.*, 2009). Emerging evidence suggests that the consequences of vitamin D deficiency (VDD) extend beyond its well-known effects on bone metabolism and calcium homeostasis, and also include alterations of specific arms of immunity. The immunomodulatory properties of vitamin D may influence susceptibility to infection (Van Belle *et al.*, 2011).

In our study Vitamin D deficiency was found in 58%, insufficient in 20 % and sufficient in 22% of children with recurrent diarrhea. In other studies, Vitamin D deficiency was found in 22–70% of patients with chronic diarrhea has been proposed to play an important role in its pathogenesis (Pappa *et al.*, 2006).

In this study of preschool and school-age children, VDD was associated with increased rates of vomiting, diarrhea, abdominal pain.

Few previous studies have reported on the potential effect of vitamin D on gastrointestinal infections in children. Tanzanian children born to mothers with serum 25(OH) D <80 nmol/L low level during pregnancy had no increased risk of diarrhea over a median follow-up time of 58 months (Finkelstein *et al.,* 2012). Vitamin D supplementation in a randomized controlled trial in school-age children did not reduce the incidence of gastroenteritis, a secondary outcome of the trial (Urashima *et al.,* 2010). Other cross-sectional study of 458 Qatari children reported a significantly higher prevalence of gastroenteritis among those who were vitamin D-deficient (Bener *et al.,* 2009). However, in this cross-sectional study, vitamin D status was measured concurrently with disease diagnosis; thus, the potential for reverse causation in which infection may have affected 25(OH) D concentrations cannot be excluded. Immune cells are able to alter vitamin D metabolism in several diseases (*Hewison, 2011*).

In this study, no age limited behavior for vitamin D deficiency was detected. Most of the literature describing the effects of vitamin D on infection in children has been limited to age groups less than 4 years. But Bener *et al.* (2009) reported that Vitamin D deficiency increased with age. As they found vitamin D deficiency was highly prevalent in Qatari adolescents 11–16 years old (61.6%), followed by the 5–10 year olds (28.9%) and those below 5 years old (9.5%).

Most cases of vitamin D deficiency in this study were from rural areas (78.3%) and 12.7% were from urban areas. This may be related to mother education, socioeconomic status and vitamin supply behavior. Thornoton *et al.* (2013) in prospective study of school-age children, found that VDD was associated with increased rates of vomiting, diarrhea and earache or ear discharge, they detected associations with age, sex and socioeconomic status.

The weight and height were lower in vitamin D-deficient children. 17.1 % showed under weight, 8.6% children showed decreased height agree with Bener *et al.* (2009). While Thornoton *et al.* (2013), detect no difference in weight and height between vitamin D deficient and sufficient children groups. Gilbert-Diamond *et al.* (2010), reported that Vitamin D deficiency was related to slower linear growth. The mechanisms by which vitamin D may influence adiposity are unknown, and possible explanations are still speculative. In vitro experiments suggested that vitamin D may prospectively influence the risk of obesity by modulating the catabolic and anabolic activity of adipocytes (Wood, 2008).

There was significant difference between the three subgroups as regard history of vitamin D supply ($P < 0.05$) but no significant difference as regard history of sun exposure, treatment received and history of dehydration ($P > 0.05$).

Bener *et al.* (2009) showed that, most of the vitamin D-deficient children had no physical activity (60.6%) and no exposure to sunlight (57.5%).

There was no significant difference between group I subgroups as regard to age, sex, residence, order of birth, social class and history of exposure to sunlight

Stool examination detected *Entameba histolytica, Giardia lamblia, Ascaris* and *Ancylostoma duodenale* parasites. Parasitic

infections in children are an important public health issue, particularly in developing countries. Parasites lead to malabsorption and chronic blood loss in children, with long-term effects on their physical (height-weight) and cognitive development (Pezzani *et al.,* 2009; Koroma *et al.,* 2005). *Giardia lamblia* represented the highest incidence (22.9%) among studied cases of diarrhea. It is kite shaped flagellates. In parasitic infection, parasites live in the upper intestine and cause damage by the action of its suction discs and by paving the surface of intestinal mucosa. *Giardia* develops into cysts which are intermittently passed in the feces (Issenman 1987). So it can affect vitamin absorption from small intestine, as detected in our study all cases of *Giardia lamblia* infection were vitamin D deficient cases.

Anemia was detected in 26.7% of children, 62.5% of them were vitamin D deficient. This can be due to recurrent acute diarrheal attacks or vitamin and iron deficiency (Stephan and Gerhard, 2012). They found a significant correlation between low serum vitamin D and markers of iron deficiency anemia in these patients. Many authors suggested several explanations for this relationship, including: decreased vitamin D absorption following impairment of intestinal fat absorption due to iron deficiency; or conversely, induction of anemia due to increased inflammation; and marrow myelofibrosis associated with vitamin D deficiency (Mehta *et al.,* 2010).

The etiology of decreased vitamin D level in recurrent acute diarrhea is multi-factorial and partly due to anorexia (such as pain-related decrease in food intake), or malabsorption i.e. decreased absorption of vitamins by the inflamed mucosa. Furthermore intestinal losses may play a role (Filippi *et al.,* 2006). So regular nutritional monitoring in patients with recurrent diarrhea patients is warranted and requires special attention of treating physicians and dieticians (Stephan and Gerhard, 2012).

In conclusion, recurrent diarrhea was associated with decreased serum level of vitamin D in preschool and school-age children. These results supporting role for vitamin D in increase the susceptibility to infection-related illness in children. We recommend further studies to be done to elucidate the mechanisms for the role of vitamin D in the diarrheal etiology, severity and morbidity. Randomized intervention trials are needed to ascertain whether vitamin D supplementation reduces the risk of gastrointestinal morbidities experienced in children.

Table.1 Groups of patients

		Group I patients		**Group II controls**		**Total**		**FET**	**P value**
		No	%	No	%	No	%		
Sex	**male**	31	51.7	16	80.0	47	58.8	0.155	0.69
	female	29	48.3	4	20.0	33	41.2		
Residence	**Rural**	47	78.3	18	90.0	65	81.2	2.02	0.333
	Urban	13	21.7	2	10.0	15	18.8		

Variable	**Groups**	**Group I**	**Group II**	**Student t test**	**P value**
Age	Mean± SD	6.83±2.44	9.05±2.33	3.38	0.301

Table.2 Vitamin D level in studied groups

Variable	Groups	Group I	Group II	Student t test	P value
Vitamin D level ng/ ml	Mean± SD	19.95±13.12	47.36±20.73	6.93	**0.001 HS**
	Range	3.34-80.4	30-81.16		

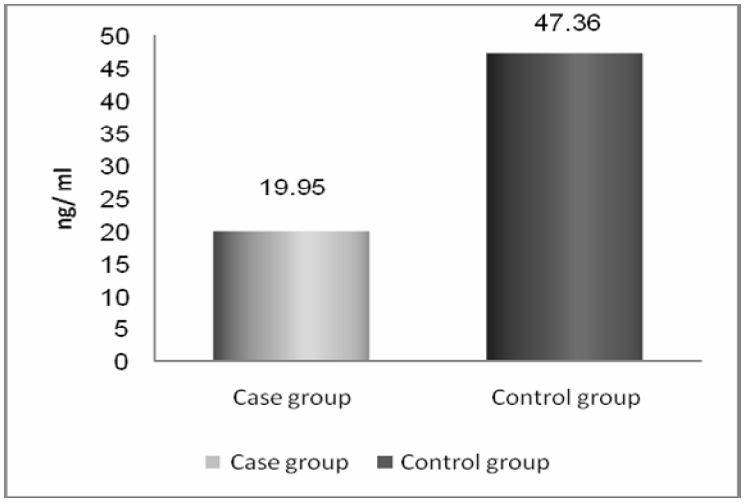

Figure.1 Vitamin D level in studied groups

Table.3 Vitamin D status in patients groups

Variable	Vitamin D-deficient	Vitamin D-insufficient	Vitamin D-sufficient	F test	P value
NO.	35	12	13	66.28	**0.001 HS**
%	58%	20%	22%		
Vit D level (ng/ml)	11.47±4.45	25.06±2.71	38.07±13.85		

Table.4 Duration of diarrhea and number of recurrence

Variable	Vitamin D-deficient (35)	Vitamin D-insufficient (12)	Vitamin D-sufficient (13)	F test	P value
Duration of diarrhea	2.77±0.73	2.67±0.65	2.85±0.69	0.203	0.817
No of recurrence	5.71±2.23	6.0±1.91	3.31±1.49	9.4	0.035

Table.5 Clinical examination results

		Vitamin D-deficient (35)		Vitamin D-insufficient (12)		Vitamin D-sufficient (13)		FET	P value
		No	%	No	%	No	%		
Weight	**Under weight**	6	17.1	1	8.3	3	23.1	0.972	0.648
	Normal	29	82.9	11	91.7	10	76.9		
Hight	**Under height**	3	8.6	1	8.3	1	7.7	0.311	1.0
	Normal	32	91.4	11	91.7	12	92.3		
Pallor	**+ve**	5	14.3	2	16.6	2	15.4	2.38	0.757
	-ve	30	85.7	10	83.3	11	84.6		
Abdominal pain	**-ve**	0	0.0	0	0.0	1	7.7	3.09	0.417
	+ve	35	100	12	100	12	92.3		
Dehydration	**+ve**	1	2.9	0	0.0	0	0.0	1.11	1.0
	-ve	34	97.1	12	100	13	100		
Distension	**+ve**	4	11.4	0	0.0	3	23.1	2.86	0.206
	-ve	31	88.6	12	100	10	76.9		

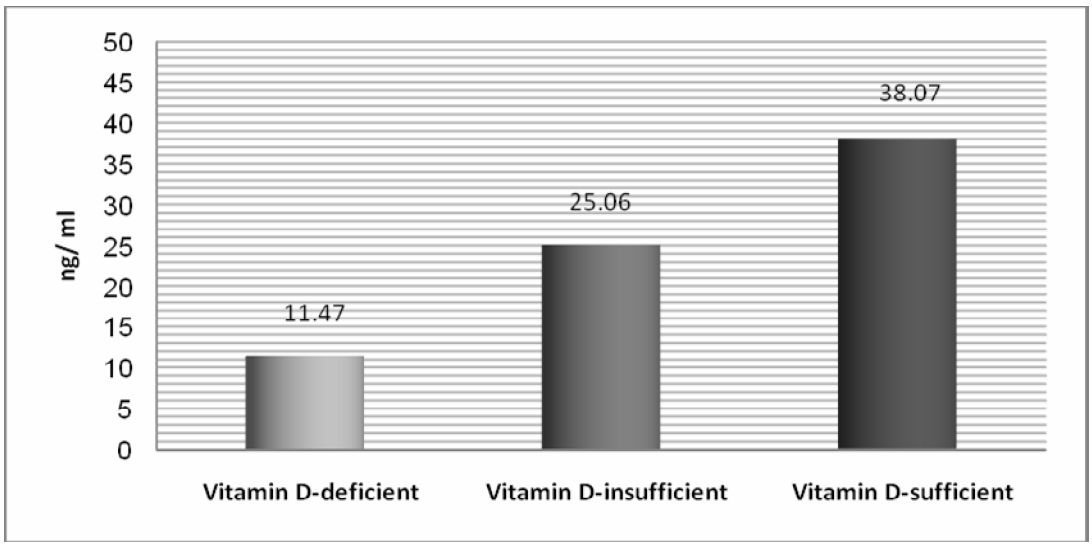

Figure.2 Vitamin D level in patients groups

Table.6 Stool examination results

Parasite	Vitamin D-deficient (35)		Vitamin D-insufficient (12)		Vitamin D-sufficient (13)		FET	P value
	No	%	No	%	No	%		
E. histolytica	4	11.4	0	0.0	1	7.7	10.35	0.138
Giardia lamblia	8	22.9	0	0.0	0	0.0		
Ancylostoma dudenale	1	2.9	0	0.0	0	0.0		
Ascaris lumbricoid	1	2.9	0	0.0	0	0.0		

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