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# Is Vitamin D Deficiency Associated with Disbiosis in Bowel Flora?

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**KEYWORDS** Absorption of Vitamin D. Gut Flora. Pediatrics. Probiotics. Vitamin D

**ABSTRACT** This retrospective study aimed to assess the increase in vitamin D levels after treatment of children with deficiencies of this vitamin diagnosed at Kagithane State Hospital ospital in winter and spring of 2014. Among 804 children below 18 years who were examined and detected to be vitamin D deficient by pediatricians in Kagithane State Hospital between 01.01.2014 and 30.06.2014, Researchers selected 113 patients meeting the necessary criteria of our study. After 6-8 weeks of treatment period, the increases in vitamin D levels were assessed.Vitamin D levels attained by probiotics without vitamin D were similar to solely vitamin D given subjects. Better results attained by Lactobacillus rhamnosus GG or Bifidobacterium BB 12 plus vitamin D treatment allowed us to think that probiotics elevate vitamin D absorbtion. This is the first study showing higher vitamin D levels in vitamin D+ probiotic treated group compared to only vitamin D treated group in children.

## INTRODUCTION

Many studies have shown the importance of vitamin D for human health, however vitamin D deficiency is still an ongoing worldwide health problem. In recent studies vitamin D inadequacy prevalence has been reported as 24 per cent in USA and severe vitamin D deficiency has been reported as 30 percent in Germany (Looker et al. 2011; Kramer et al. 2014). In Turkey its prevalence was reported to be 25 percent and 15 per cent for deficiency and insufficiency respectively in a group of 440 children and adolescents (Andiran et al. 2012). In another study its prevalence (deficiency) was reported 51.8 percent in 18-70 age group in Turkey (Ucar et al. 2012).

In recent decades there has been a reappearance of rickets due to its deficiency, despite its increased intake, sun exposure, fortification policies and usage of supplements (Kennel et al. 2010).Vitamin D is important for calcium and phosphorus metabolism. Now it is accepted to be important in homeostasis of a number of organs. Its deficiency causes rickets in children and results in osteoporosis and osteomalacia in adults. Low vitamin D levels are associated with increased incidence of autoimmune diseases, food allergy type 1 diabetes mellitus ,multiple sclerosis, hypertension, cardiovascular disease and some common cancers (Alhamad et al. 2014; Lips 1996 ; Garland et al. 2009; Vassallo et al. 2010).

Former researches supported an association between vitamin D deficiency and inflammatory bowel disease. Seasonal variations at the onset and exacerbation of IBD have also been reported with high incidence in the winter because of the lack of sunshine (Lim et al. 2005; Moum et al. 1996). Vitamin D and VDR have important roles in preserving the mucosal barrier of intestines by increasing production of tight junction proteins. It has been shown that VDR null mutant mice developed more severe colitis in mice models of IBD. It is also argued that vitamin D could reduce IBD risk by suppressing T cell mediated immune response in the colon (Lim et al. 2005; Froicu et al. 2007).

The intestinal microflora has important functions in regulating metabolism, intestinal epithelial function and its health, immunity, and inflammatory signaling (Kong et al. 2008; Neish 2009; Garrett 2010). Probiotic-induced modulation of anti-inflammatory VDR signaling in colitis needs further studies.VDR mediates the ac-

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tivity of 1-25 vitamin D resulting in junctional protein expression and strengthening of tight junction complex in intestinal mucosa (Adrian et al. 2005). Vitamin D deficiency may have harmful impact on the development of human intestinal microbial flora, resulting in gastrointestinal infections and skin inflammation by immunomodulatory effects (Toniato et al. 2015) and impairment in the immune system (Juan et al. 2007). There may be a vicious cycle between abnormal intestinal flora and vitamin D deficiency.

Researchers tried to unravel the factors leading to globally high deficiency rates of vitamin D in spite of widespread supplementation of foods with this vitamin.The researchers hypothesized that problem may originate from malabsorption rather than deficient supplementation, and we tried to focus on absorption of vitamin D. In this retrospective study, they aimed to assess the increase in vitamin D levels after treatment of children with this deficiency by adding probiotics.

## METHODOLOGY

Among 804 children below 18 years who were examined and detected to be vitamin D deficient by pediatricians in Kagithane State Hospital between 01.01.2014 and 30.06.2014, the researchers selected 113 patients meeting the necessary criteria of our study. Children with chronic diseseas and those lost to follow up are not included. Because of seasonal variation of vitamin D levels the study was performed in the same seasonal period and the same vitamin D (1500 IU) dosage was prescribed to all patients. After 6-8 weeks of treatment period, the increases in serum 25 (OH)D levels were measured by liquid chromotography (Spektra 2000Sr). Venous blood samples were obtained from the antecubital region between 8.00-8.30 am.

### Statistical Analysis

The researchers used NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah USA) to analyze therapy results. To compare small groups they used Pearson Chi-Square test, Fisher-Freeman-Halton exact and Kruskarr Wallis test. Mann Whitney U test was used to determine the group making the difference . P<0.01 and p<0.05 levels were considered to be significant.

## RESULTS

The study included 52 males (46 per cent) and 61 female (54 percent) (age range 1 to 18 years;mean age 8.5-+3.8 years) vitamin D deficient children visiting the hospital from January first to June 30th 2014. There was no statistically significant difference as to age and gender among groups (Table 1). When the researchers noticed differences in various subgroup of patients as to vitamin D levels, they scrutinized treatment modalities. Some patients were noted to use vitamin D prescription while others used both vitamin D and a probiotic formula. Another group used probiotics for medical reasons but omitted or refused to take vitamin D. The difference in the increase of vitamin D levels prompt-

**Table 1: Evaluation of gender and age**

| | *N* | *Age (year) Mean±SD (Median)* | *Gender* | | | |
|---|---|---|---|---|---|---|
| | | | *Male(n=52)* | | *Female (n=61)* | |
| | | | *n* | *(%)* | *n* | *(%)* |
| [1]Lactobacillus rhamnosus GG Bifidobacterium BB -12 + vitamin D | 7 | 7.2± 5.3 (6.0) | 5 | (71.4%) | 2 | (28.6%) |
| [2] Vitamin D | 34 | 8.7± 3.8 (8.5) | 18 | (52.9%) | 16 | (47.1%) |
| [3]Multivitamin- Lactobacillus rhamnosus + Vitamin D | 34 | 8.4± 2.9 (8.0) | 13 | (38.2%) | 21 | (61.8%) |
| [4]L.reuteri | 20 | 8.3± 4.6 (7.7) | 10 | (50.0%) | 10 | (50.0%) |
| [5]No vitamin D user | 18 | 9.1± 4.11 (9.0) | 6 | (33.3%) | 12 | (66.7%) |
| | P | [a]0.725 | | [b]0.344 | | |

[a]*Kruskal Wallis Test* [b]*Fisher-Freeman-Halton Test*

ed us to analyze results in terms of statistical significance. The researchers identified with respect to treatment response five groups 1- high responder group who took Lactobacillus rhamnosus GG Bifidobacterium BB -12 + vitamin D showed a mean 242.9 percent increase in serum vitamin D levels after 8 weeks of treatment. 2- responder group who took only Vitamin D for 8 weeks showed a mean 112.3 per cent increase in serum vitamin D levels.3- responder group who took Multivitamin- Lactobacillus rhamnosus + Vitamin D after 8 weeks treatment showed a mean 143.8 per cent increase in serum vitamin D levels.4- responder group who used solely Lactobacillus reuteri and after 8 weeks of usage, vitamin D levels rose 97.2 per cent when compared to pretreatment levels. Fifth group consisted of vitamin D deficient children who refused or neglected to take vitamin D for one reason or another; in this group vitamin D levels didn't change, in fact showed a minor fall.

The interesting point was that vitamin D levels increased significantly in the probiotic group without addition of a vitamin D prescription. But the best results were in the probiotic plus vitamin D group.

There were no significant differences in vitamin D levels in pretreatment measurements among groups (Table 2). After treatment there was a highly significant difference-increase in the second vitamin D measurements made 6-8weeks later in the devit 3 group, probiotic plus devit 3 group and multivitamin plus devit 3 group, when compared to pretreatment levels and also when each one separately compared with non-vitamin D taking group (Table 2) but also there was a statistically significant increase in vitamin D levels in solely probiotic using group.

There was a statistically significant difference among groups as to increase rate between first and second measurements (Table 2). According to double comparisons done to determine the group creating the difference ;the increase rate of only vitamin D and only probiotic taking groups were significantly lower than vitamin D plus probiotic group. At the end of treatment period , all treatment groups had significantly higher vitamin D levels when compared to the group not taking vitamin D (Table 2).

## DISCUSSION

Since vitamin D deficiency is a serious problem even in sunny tropical regions of the world it makes us think different factors for its deficiency and high prevalence (Gebreegziabher et al. 2013; Djennane et al. 2014). The researchers suggest that failure to eradicate vitamin D defi-

**Table 2: The increasing rates of vitamin D following its usage( ng/mL)**

| | *n* | *First assay* | *Second assay* | *Increasing rates* |
|---|---|---|---|---|
| | | *Mean±SD (Median)* | *Mean±SD (Median)* | *Mean±SD (Median)* |
| [1]Lactobacillus rhamnosus GG Bifidobacterium BB -12 + vitamin D | 7 | 9.9± 4.0 (9.9) | 29.6± 8.1 (27.2) | 242.9± 182.8 (174.7) |
| [2] Vitamin D | 34 | 12.3± 4.6 (12.2) | 23.7± 6.8 (24.2) | 112.3± 82.9 (93.1) |
| [3]Multivitamin- Lactobacillus rhamnosus + Vitamin D | 34 | 12.1± 4.8 (11.0) | 26.6± 7.4 (26.6) | 143.8± 105.5 (104.0) |
| [4]L.reuteri | 20 | 11.3± 4.2 (11.2) | 20.6± 8.5 (20.5) | 97.2± 107.3 (63.3) |
| [5]No vitamin D user | 18 | 13.50± 6.2 (12.4) | 12.7± 4.6 (12.9) | -0.5± 19.7 (0.9) |
| [a]p | 0.605 | 0.001** | 0.001** | |
| Pairwise comparisons | | | | |
| [b]$p_{1-2}$ | 0.160 | 0.111 | 0.017* | |
| $p_{1-3}$ | 0.323 | 0.396 | 0.103 | |
| $p_{1-4}$ | 0.407 | 0.025* | 0.008* | |
| $p_{1-5}$ | 0.183 | 0.001** | 0.001** | |
| $P_{2-3}$ | 0.778 | 0.116 | 0.194 | |
| $P_{2-4}$ | 0.425 | 0.147 | 0.165 | |
| $P_{2-5}$ | 0.597 | 0.001** | 0.001** | |
| $P_{3-4}$ | 0.635 | 0.013* | 0.016* | |
| $P_{3-5}$ | 0.525 | 0.001** | 0.001** | |
| $P_{4-5}$ | 0.342 | 0.003** | 0.001** | |

ciency probably stems from disrupted microbial flora of modern human beings. Recent two studies pertaining to absorption of vitamin D state that its mechanism is not yet clear and to fully understand this absorption mechanism there is still a need for further studies (Borel et al. 2015; Reboul 2015). A research observing the therapeutic response and attained serum vitamin D levels after fixed dose vitamin D supplementation showed substantial variations probably originating from intestinal absorption dynamics (Binkley 2015).

The researchers analyzed the achieved vitamin D levels in five groups as to efficiency of treatment strategies Vitamin D levels attained by probiotics were almost equal to those with only vitamin D treatment. Better results attained by Lactobacillus rhamnosus GG or Bifidobacterium BB 12 +vitamin D treatment allowed the researchers to think that probiotics elevate vitamin D absorbtion. An original research showed that administration of only a probiotic formula increased serum 25 hydroxyvitamin D levels significantly (Jones et al. 2013).

Latest treatment protocols have recommended increasing vitamin D dosages. (Management of Vitamin D Deficiency in Children 2012 ). However, they draw attention to toxicity caused by higher doses (National Institutes of Health. Vitamin D 2011). If probiotics ensure adequate levels by decreasing dosage, in place of using higher dosages of a fat soluble vitamin, combined treatment may be more appropriate. Increasing body of evidence shows that vitamin D pathway may be important in gut homeostasis and it has immune modulator effect on host (Kong et al. 2008). Vitamin D receptor is a nuclear receptor that mediates action of active form of vitamin D. VDR activated by 1-25 dihidroxy D3, possibly regulates intestinal homeostasis by preventing pathological bacterial invasion and inhibits inflammation and maintains cell integrity in the intestinal system (Diane et al. 2010; Joyce et al. 2009; Juan 2008; Adrian et al. 2005; Lagishetty et al. 2010). The researchers also know that vitamin D and microbiota have similar physiologic effects on gut ; so there may be an interaction between them. The effect of probiotics on gut has been assessed in pig models and found to have caused increased VDR expression invivo in pigs (Yoon 2011). VDR is activated by 1-25 vitamin D, probiotics augment effects of vitamin D by increasing VDR expression; so similar effects of vitamin D and probiotics emerge on VDR expression.

The main limitation of this study originated from small sample size but it is highly valuable since this is the first study showing higher serum vitamin D levels in vitamin D plus probiotic treated group compared to only vitamin D treated group in children. Further studies are needed (especially placebo controlled–double- blind) in this field. The mechanism how probiotics increase vitamin D absorption remains to be elucidated.

## CONCLUSION

This research showed that probiotics had favorable affects on intestinal absorption of vitamin D. Despite ongoing efforts to reduce vitamin D deficieny it still has a high prevalence so addition of probiotics to vitamin D therapy regimen may be useful. This research showed beneficial preliminary results of adding probiotics to vitamin D deficiency regimen**.**.

## RECOMMENDATIONS

To eradicate hypovitaminosos D it is imperative to increase its absorbtion. For the treatment of global vitamin D deficiency it may be a safer alternative adding probiotics to the therapy regimen with the aim of increasing absorption and reducing toxicity of higher doses. Perhaps simply probiotic administration may solve the deficiency without resorting to vitamin D prescription.

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