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Review

Vitamin D Modulates Intestinal Microbiota in Inflammatory Bowel Diseases

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Abstract: Inflammatory bowel disease (IBD) is a chronic disease in the gastrointestinal tract (GIT). IBD include ulcerative colitis (UC), which generally affects only the large intestine mucosa and submucosa, and Crohn's disease (CD), which may affect any part of the GIT by transmural inflammation. Both UC and CD are associated with an imbalance of the gut microbiota composition and injuries in the intestinal mucosa. The intestinal dysbiosis is related to a reduction in butyrate-producing species, impairing the anti-inflammatory response of the immune system, and is commonly associated with micronutrients deficiency, e.g. vitamin D hypovitaminosis. Vitamin D is involved in several critical functions, including immune cell differentiation, regulation of microbiota, gene transcription, and barrier integrity. Vitamin D supplementation in IBD patients showed promising results in reducing the disease activity and modulating gut microbiota. Vitamin D receptor (VDR) regulates the biological actions of the active vitamin D metabolite, 1α,25-dihydroxyvitamin D3. Evidence supports that the VDR signaling is involved in the genetic, environmental, immune, and microbial aspects of IBD. Low VDR expression and dysfunction of vitamin D/VDR signaling are reported in IBD patients. Vitamin D/VDR deficiency could be considered as a multifunctional susceptibility factor in IBD. Therefore, in this review, we will discuss the progress in clinical studies, mechanism studies on Vitamin D /VDR, and potential use of vitamin D supplementation as adjuvant therapy to restore gut microbiota balance, promote beneficial metabolites, and inhibit inflammation status in patients with IBD.

Keywords: Vitamin D; VDR; inflammation; microbiome; metabolites; nuclear receptor; probiotics; tight junctions

1. Introduction

Inflammatory bowel disease (IBD) is defined as a chronic inflammation of the gastrointestinal tract (GIT) that affects more than 6 million people worldwide [1,2]. The most common types are Crohn's disease (CD) and ulcerative colitis (UC), which will differ in the location and extension of the lesions throughout the GIT [1]. CD is a segmental, asymmetrical, and transmural inflammation that may affect the whole GIT, but is more frequently observed in the ileum and colon. On the other hand, UC is more related to mucosal inflammation from the rectum to the proximal colon [1,3,4]. In fact, IBD has a great impact on the physical, psychological, and social aspects of life, and depression and anxiety are usually increased in these patients. Thus, the management of these diseases is of utmost importance for the quality of life of the patients [2].

Studies have suggested that IBD may be triggered by an abnormal immune response to gut commensal bacteria in genetically predisposed individuals and is associated with an impaired intestinal barrier function and a less diverse gut microbiota composition [5-8]. Several factors are associated with the risk of IBD development, such as country development degree, smoking, sex, age, use of antibiotics or oral contraceptives, lower serum levels of vitamin D, and diet [2,9].

The gut microbiota is comprised of more than 2000 species of bacteria distributed throughout the GIT. The population density increases from the stomach to the colon, reaching 10^{10}-10^{12} CFU (colony forming units)/mL at the end of the large intestine. Innumerous functions are attributed to the gut microbiota, like metabolism of nutrients from the diet, fiber fermentation, SCFA (short-chain fatty acids) production, vitamin production, barrier function and tight junctions regulation, antimicrobial compounds secretion, immune regulatory, among others [10,11]. Microbial metabolites released by the gut microbiota circulate and may affect the proper function of other organs and systems of the body. Therefore, strategies that address the gut microbiota modulation, improvement of the gut barrier function, and decrease in the intestinal mucosa inflammation are of the greatest significance for IBD treatment [8,12].

Micronutrient deficiencies are often observed in IBD patients, and mostly low levels of vitamin D and zinc, even during disease remission [13]. Observational studies have reported that low levels of vitamin D are directly associated with increased disease activity, mucosal inflammation, clinical relapse, and quality of life. Thus, vitamin D deficiency might be both, the cause, and a consequence of IBD [13,14]. In fact, chronic diarrhea, nutrients malabsorption, low exposure to sunlight, and reduced consumption of vitamin D-fortified foods, like dairy products, are frequent in IBD patients, which may lead to vitamin D deficiency [15].

Additionally, several studies about epigenetic factors associated with IBD have been conducted. Indeed, epigenetics may explain how environment and genetics might be involved in the development, progression, pathogenicity, and response to treatments. Also, epigenetic markers related to immunoregulation, intestinal epithelial barrier, and autophagy are differently expressed among IBD and healthy controls, and between UC and CD patients as well, and miRNAs (microRNA) may be used as biomarkers for disease assessment in the future, as they are more convenient than endoscopy and biopsies, mainly for patients with active disease [16].

In this review, we will explore vitamin D deficiency and gut microbiota dysbiosis associated with IBD, and the potential use of vitamin D in the management of the disease. In addition, epigenetic factors involved in IBD and vitamin D mechanisms will also be discussed.

2. Pathogenesis of Inflammatory Bowel Diseases

2.1 Genetics

In the last decades, the understanding of the pathophysiology of IBD has markedly evolved. In addition to environmental, genetic, and microbial factors, the pathogenesis of IBD also involves the function of cells related to the inflammatory process, such as adipose, epithelial, and endothelial cells, together with regulatory RNAs and inflammasome. For a better elucidation of the disease, a broader approach of all these factors must be performed to clarify the underlying mechanisms that results in the abnormal immune response associated to these diseases [17]. Here we will focus on the

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main mechanisms related to genetic factors and intestinal microbiota that affect the immune response.

It is known that there is an important genetic component that predisposes the development of both UC and CD, and many of these variants are shared in these diseases, thus the mechanistic pathways may be similar. A meta-analysis regarding genome-wide association studies (GWAS) showed that, although 110 variants are shared in IBD, there are 23 specific for UC and 30 for CD. The identified loci are enriched for primary immunodeficiencies, reduced circulating T- cell levels, and mycobacterial diseases [18].

The strongest genetic risk associated with IBD is NOD2 (nucleotide binding oligomerization domain containing 2) [19]. The receptor belongs to the NOD-like receptor (NLR) family and encodes the primary receptor for muramyl dipeptide (MDP) present in all Gram-positive and negative bacteria. NOD2 is expressed in macrophages, Paneth cells, and lamina propria lymphocytes and is pivotal for bacterial recognition. Therefore, it acts in the innate immune response and regulation of commensal microbiota [20]. After binding to MDP, the NOD2 oligomer activates TAK1 (transforming growth factor beta activated kinase 1), which leads to activation of NF-κB (nuclear factor kappa B) and MAPK (mitogen-activated protein kinase), resulting in the production of inflammatory cytokines [21]. Changes in the microbiome with an abnormal NOD2 response can result in an exacerbated immune response and inflammation, which is usually present in CD. Still, NOD2 variants can reduce the transcription of IL (interleukin)-10 anti-inflammatory cytokine [22,23].

There are other genetic variants associated with autophagy identified by GWAS and related to CD, such as ATG16L1 (autophagy related 16 like 1) and IRGM (immunity related GTPase M). Activation of NOD-2 by bacterial MDP in epithelial cells leads to activation of autophagy and increases bacterial killing, a process that is impaired in individuals with CD associated with NOD-2 variants [24]. This further compromises the secretion of antimicrobial peptides, such as α-defensins and other cryptdins [23]. Cryptdins are antimicrobial peptides that are produced by Paneth cells, and their antimicrobial activity is important in reducing infection by pathogenic bacteria such as *Listeria monocytogenes* [22,23,25].

2.2 Microbiota and Immune response

The human intestinal microbiota holds approximately $3.8 \cdot 10^{13}$ bacterial cells and about 100-fold the number of human genes (microbiome), and the most representative phyla are Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [26-28]. Microbiota is shaped since before birth and is influenced by the mode of birth, the surrounding environment, breastfeeding, availability of nutrients, and other factors [29,30]. After the cessation of breastfeeding and introduction of food, the infant's microbiota becomes more similar to that of an adult, and its maturity occurs within 3 years of life [26,31]. Early colonization is essential for the development and maturation of the immune system. Children born by cesarean have delayed colonization and present lower diversity and reduced Th1 (T helper 1) response [32].

Immune receptors, such as Toll like receptor receiver (TLR) and NLR, recognize microbe-associated molecular patterns (MAMPs) and play a chief role in intestinal homeostasis [33]. The microbial composition is conditioned by the products of the immune and epithelial cells, such as IgA (Immunoglobulin A), mucus, and defensins. Regarding the mucosal immunity, it is regulated by the microbiota. *Bacteroides fragilis* promotes the differentiation of Th1 and Clostridia of T helper Reg (Treg), for example, in a symbiotic relation [34].

Increased intestinal permeability is frequent in CD and UC [35]. A defect in the intestinal barrier could be a primary cause of immunopathogenesis in IBD since increased permeability facilitates the absorption of food and microbial products able to induce an exacerbated immune response and lead to inflammation [36,37]. This is possibly due to a change in the mucus layer in the intestinal lumen. In patients with CD, a reduction in the expression of MUC3, MUC4, and MUC5B mRNA (MUC = mucin; mRNA = messenger RNA) in the ileal mucosa and MUC1 mRNA in the inflamed ileum has already been observed [38,39]. Also, the colon mucus of animals that develop UC spontaneously and

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patients with active UC has been shown to allow bacteria to penetrate and reach the intestinal epithelium [40]. The variants of the NOD2, JAK2 (tyrosine-Protein Kinase JAK2), MUC1, and MUC13 genes are associated with impaired intestinal barrier function and may predispose to infectious and inflammatory diseases and handle an abnormal immune response to luminal antigens [41-43].

In an experimental colitis model, it was demonstrated that mice with the gut epithelium vitamin D receptor (VDR) deletion developed a more severe clinical colitis and worsened epithelial cell apoptosis, leading to an increased intestinal mucosa permeability, and promoted the Th1 and Th17 (T helper 17) mucosal response [44]. This suggests that the downregulation of the colonic VDR observed in patients with IBD may be related to an increased intestinal mucosa permeability.

The immunological profile of IBD patients is a combination of Th1 and Th1/Th17 in CD and atypical Th2 (T helper 2) in UC [45]. An increase in the pro-inflammatory cytokine IL-17 is observed in the intestinal mucosa and blood, especially in patients with CD. Since Th17 cells also produce IL-22 and IL-21, it promotes IFN-γ (interferon gamma) production and Th1 response. In UC, an immune response Th-2 is characterized by the production of IL-5, IL-13, and IFN- γ. There is still disagreement regarding the pattern of cytokines secreted in different diseases and studies showed that the cytokine profile does not always match the type of immune response [46].

In IBD, there is an increased immune response against microbial antigens. This is noted by the circulating levels of antibodies against microbial antigens and glycans. Several studies have pointed out differences in the composition of the microbiota between the IBDs. The patients present an imbalance related to microbial diversity and relative abundance of specific bacteria, namely dysbiosis [47].

In comparison to healthy individuals, individuals with IBD show an increase in bacteria of the Proteobacteria phylum, such as Enterobacteriaceae and *Escherichia coli* [48]. Patients with CD usually present a reduction in the phylum Firmicutes, especially *Faecalibacterium prausnitzii*, which is reduced in relative abundance in the stool, and increased abundances of Bacteroidetes and Proteobacteria. In UC, the gut microbiota is characterized by the low abundance of butyrate-producing bacteria, and a high ratio of *B.fragilis/F. prausnitzii* is associated with a weaken anti-inflammatory response [47,49,50].

2.3 Therapies and adverse effects

Although IBDs are characterized by a chronic inflammatory disorder, there are several degrees in the severity of symptoms. Some patients may have the disease controlled with aminosalicylates and glucocorticoids treatments, while others require biological therapy and immunosuppressants [51].

Aminosalicylates have a long history of safe use. Their adverse effects are similar to placebo controls and are usually controlled with a dose reduction [52].

The most common immunomodulators are azathioprine (AZA), methotrexate (MTX), and mercaptopurine (MP), which are usually efficient in controlling symptoms without the need for corticosteroids. The most frequent adverse effects related to these medications are liver toxicity, nausea, vomiting, diarrhea, and fatigue. Anti-TNF-α (TNF-α = tumor necrosis factor alpha) is the first class of biological therapy created to treat moderate to severe IBD. They comprise a series of monoclonal antibodies that are effective for both maintenance and remission of CD and UC. Nonetheless, these therapies may increase the risk for autoimmunity, demyelinating disease, and opportunistic infections, and about a third of patients can develop infections during the first year of using the medication. Thus, some precautions are recommended before starting therapy, such as screening for tuberculosis and hepatitis B virus [51].

Additionally, there are the anti-integrin and anti-interleukin 12/23 therapies. Despite the short history of use, it is shown to be a promising and effective treatment due to their role in different molecules of the pro-inflammatory cascade [51].

The main reason for malnutrition in IBD is the reduced oral food intake due to symptoms of the disease, such as nausea and vomiting, fasting during hospitalization, or prolonged restrictive diets.

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Despite this, the use of medications may affect the absorption and use of micronutrients. Sulfasalazine, for example, is a folic acid antagonist, which may lead to anemia when used for a long period. On the other hand, glucocorticoids decrease the absorption and use of calcium, zinc, and phosphorus and impair vitamin D metabolism [53].

2.4 Diet and Quality of Life in IBD

IBD patients often report reduced quality of life (QoL) and have elevated levels of anxiety and depression when compared to healthy individuals. This may be due to the associated symptoms, disruption to usual life activities, employability, stigma, or disability [54]. In a recent systematic review, Knowles et al. verified the impacts of the disease on QoL when compared to healthy individuals. There is a significant reduction in both mental and physical health, similar to other medical conditions. QoL was also lower when the disease was active, and worse for those with CD compared to UC [55]. It has also been shown that the QoL of patients with IBD improves over time, which means that there is an adaptive process to the disease [54,55].

It is common for patients with IBD to self-impose dietary restrictions, which is generally associated with insufficient macro and micronutrients in the diet [56]. One study compared patients with inactive or average CD with healthy controls. An inadequate nutrient intake due to the exclusion of food groups, such as milk, vegetables, and grains in CD group was observed [57]. More than a third of the individuals with IBD had BMI (body mass index) above 25, showing malnutrition accompanied by obesity, which may be due to physical inactivity or treatment with corticosteroids. The main micronutrient deficiencies observed in patients with IBD are zinc, iron, vitamin B12, and vitamin D, contributing to a critical condition and influencing on well-being [58,59].

In the meta-analysis conducted by Gubatan et al., the relationship between low levels of vitamin D and the risks of clinically active disease, mucosal inflammation, clinical relapse, and low quality of life scores among 8316 IBD patients from observational studies was evaluated. Low levels of 25(OH)D were significantly associated with an increase in the clinically active disease [UC (pooled OR 1.47, 95% CI 1.03-2.09, P = .03, I2 = 0%); CD (pooled OR 1.66, 95% CI 1.36-2.02, P < .00001, I2 = 0%)] and clinical relapse [UC (pooled OR 1.20, 95% CI 1.01-1.43, P = .04, I2 = 0%); CD (pooled OR 1.35, 95% CI 1.14-1.59, P = .0004, I2 = 0%)]. Meanwhile, low vitamin D levels were associated with increased mucosal inflammation and low quality of life scores only in CD patients. In fact, mucosal inflammation may lead to malabsorption of vitamin D in CD, thus low levels of vitamin D could be considered as an inflammation biomarker for CD [14]. Accordingly, MacMaster et al. observed that around 30% of 93 IBD patients in remission presented vitamin D deficiency [13].

Hospitalization of IBD patients is associated with disease complications, surgical procedures, and a lack of specialized follow-up. Thus, the maintenance of UC and CD remission is of utmost importance. In fact, according to an integrative review conducted by Rocha et al., malnutrition is related to hospitalization of patients affected by the disease. Moreover, nutritional status may influence hospitalization in IBD, although no comparison with adequate nutritional status was evaluated [59]. Low or insufficient levels of vitamin D have already been linked to an increased need for hospitalization and surgery in IBD, when compared to normal serum levels [60,61]. This highlights the importance of maintaining levels considered as adequate for vitamin D, since its anti-inflammatory effect is very well studied, and these patients can benefit and improve their well-being.

3. Vitamin D as an alternative approach in IBD

3.1 Mechanisms of action of Vitamin D

Vitamin D is a fat-soluble vitamin that can be found in two different chemical structures: cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2). It can be obtained either by exposure of the skin to UVB (ultraviolet B) rays from the sun, when the 7-dehydrocholesterol, present in the skin, is converted to cholecalciferol, or by the consumption of some fatty fishes, sun-exposed mushrooms, fortified foods - mostly dairy products, or even by supplements. Vitamin D is transported to the liver

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by the circulation and transformed into 25(OH)D (25-hydroxyvitamin D), the main circulation form and vitamin D status marker, by the enzyme 25-hydroxylase (CYP2R1). Nonetheless, the 25-hydroxyvitamin D should have another hydroxylation in the kidneys by the enzyme 1-α-hydroxylase (CYP27B1), where it is converted to calcitriol or 1,25-$(OH)_2$D (1,25-dihydroxyvitamin D), the active form of the vitamin [62].

The functions of calcitriol in the body are mediated by the VDR. VDR is expressed in various tissues (e.g. skin, parathyroid gland, adipocyte, small intestines, and colon). The VDR bounded to 1,25-$(OH)_2$D forms an heterodimer with the retinoic acid receptor (RXR), which migrates to the cell nucleus and binds to the vitamin D-response element (VDRE) in the promoter regions of target genes, acting as a nuclear transcription regulator [62-64]. The VDRE is found in many genes, explaining the mechanisms associated with vitamin D, like autophagy [65], cell proliferation [66], intestinal barrier function [67,68], gut microbiota modulation [65,69,70], and immune functions [71,72], besides the most well-known mechanism, regarding calcium homeostasis and bone health [63,64,69].

Vitamin D immunomodulatory effects are directly related to antigen presenter cells (e.g. macrophages and dendritic cells) and T-cells functions. It seems that 1,25-$(OH)_2$D modulates the T-cell differentiation, shifting from a pro-inflammatory Th1 immune response to an anti-inflammatory Th2 immune response, increasing the secretion of IL-4 while decreasing the secretion of IL-2 and IFN-γ. Moreover, 1,25-$(OH)_2$D may inhibit dendritic cell differentiation and IL-12 production while increasing IL-10. Also, the lack of 1,25-$(OH)_2$D harms regulatory T-cells (Tregs) differentiation and weakens its functions, which may trigger autoimmune diseases [73-75].

There is no consensus about the ideal circulating level of vitamin D. According to the Institute of Medicine (IOM), for the majority of the population, a minimum 25(OH)D serum level of 20 ng/mL (50 nmol/L) is considered enough, in case of a minimum sun exposure. Meanwhile, the risk of vitamin D deficiency is considered when the 25(OH)D serum level is below 12 ng/mL (30 nmol/L) [76]. Nevertheless, the Clinical Practice Guideline from the Endocrine Society defined vitamin D deficiency as serum level of 25(OH)D below 20 ng/mL (50 nmol/L) and values between 21-29 ng/mL (525-725 nmol/L) are considered as vitamin D insufficiency [77]. These thresholds of vitamin D serum levels were established for bone health. However, it is known that vitamin D deficiency may also be related to certain types of cancer, cardiovascular diseases and hypertension, type 2 diabetes and metabolic syndrome, autoimmune diseases (e.g. type 1 diabetes, rheumatoid arthritis, IBD, CD, systemic lupus erythematosus, and multiple sclerosis), and infectious diseases (e.g. tuberculosis and upper respiratory infections), autism, depression, among others [62,75-77]. Furthermore, it is important to point out that the exposure to sunlight is the most effective natural source of vitamin D. However, people usually avoid sunlight exposure or use sunscreen due to skin cancer risk and it is difficult to reach the minimum required through the diet, thus supplementation is often necessary [62,76,77].

3.2 Vitamin D as an alternative treatment for gut microbiota modulation and improvement of inflammation in IBD

Low levels of circulating vitamin D are related to increased IBD disease activity and relapses, in addition to gut microbiota dysbiosis. In fact, IBD is characterized by an abnormal immune response to gut commensal bacteria in genetically predisposed individuals. There are few studies with humans exploring the impact of vitamin D in IBD management and gut microbiota modulation, which will be discussed hereafter and are summarized in **Table1**.

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Table 1: Summary of Vitamin D studies in Inflammatory Bowel Disease

Group	Type of IBD	Treatment/Condition	Duration of study	Outcome	Ref.
Adults N = 90	UC	Single intramuscular injection: Vitamin D_3: 300,000 IU	Follow up after 3 months	↑ 25(OH)D ↓ TNF-α, IFN-γ, IL-12p70, hs-CRP, ESR ↓ Th1 immune response	88, 89
Children and adolescents N= 61	UC and CD	Oral liquid preparation: Arm A - Vitamin D_2: 2,000 IU daily Arm B - Vitamin D_3: 2,000 IU daily Arm C - Vitamin D_2: 50,000 IU weekly	Follow up after 6 weeks	↑ 25(OH)D	84
Adolescents N= 40	UC and CD	Oral pills: Vitamin D_3: 5,000 IU/10 kg of body weight (max. 25,000 IU weekly) or 10,000 IU/10 kg or body weight (max. 50,000 IU weekly)	6 weeks (follow up after 2, 8, and 12 weeks)	↑ 25(OH)D	85
Adults N= 10	UC and CD	Oral liquid preparation: Vitamin D_3: 5,000-10,000 IU daily	12 weeks (follow up at week 16)	↑ 25(OH)D ↓ CD clinical disease activity (HBI)	90
Adults N= 25	UC	Oral pills: Vitamin D_3: 4,000 IU weekly	8 weeks	↑ 25(OH)D ↓ clinical disease activity ↓ fecal calprotectin ↓ inflammation in active UC Trend in reducing mucolytic species in fecal microbiota	91

CD = Crohn's Disease; ESR = erythrocyte sedimentation rate; GIT = gastrointestinal tract; HBI = Harvey-Bradshaw Index; HE = high sunlight exposure; hs-CRP = high-sensitivity C-reactive protein; IBD = inflammatory bowel disease; IU = International Units; LE = low sunlight exposure; UC = Ulcerative colitis.

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Table 1: continued

Group	Type of IBD	Treatment/Condition	Duration of study	Outcome	Ref.
Adults N= 17	CD in clinical remission	Oral: Vitamin D_3: Day 1 – 3: 20,000 IU Day 4 – 28 (alternated): 20,000 IU	4 weeks	↑ 25(OH)D ↑ week 1: *Alistipes*, *Barnesiella*, *Roseburia*, *Anaerotruncus*, *Subdoligranulum* ↑ week 2: *Faecalibacterium*, *Veillonella*, *Blautia*, *Fusicatenibacter*, *Intestinibacter* ↑ week 4: *Lactobacillus*, *Megasphera* ↓ reduced diversity	78
Adults N= 16	Healthy volunteers	Oral drops: Vitamin D_3: First 4 weeks: 980 IU/ kg of body weight weekly (max. 68,600 IU weekly) Last 4 weeks: 490 IU/ kg of body weight weekly (max. 34,300 IU weekly)	8 weeks	↑ 25(OH)D Upper GIT: ↓ Gammaproteobacteria – Pseudomonas spp., Escherichia/Shiguela spp. ↑ bacterial richness Terminal ileum: ↑CD8+ T cell	71
Adults N= 87	CD and UC active or in remission	Comparison between Seasonal 25-(OH)D circulating levels (supplemented or not)	Summer/autumm (HE) vs winter/spring (LE)	25(OH)D levels were correlated with changes in microbiome ↓ 25(OH)D → balanced microbiome composition	80

CD = Crohn's Disease; ESR = erythrocyte sedimentation rate; GIT = gastrointestinal tract; HBI = Harvey-Bradshaw Index; HE = high sunlight exposure; hs-CRP = high-sensitivity C-reactive protein; IBD = inflammatory bowel disease; IU = International Units; LE = low sunlight exposure; UC = Ulcerative colitis.

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3.2.1 Vitamin D and gut microbiota modulation

Schaffler et al. reported that vitamin D_3 supplementation altered the gut microbiota composition only in remission CD patients, and no changes were noted in the healthy controls. Throughout 4 weeks, an increase in the abundance of beneficial bacteria like *Alistipes*, *Parabacteroides*, *Roseburia*, and *Faecalibacterium* was observed, even though it was transient. The authors suggested that 4 weeks might have been a too short intervention period to detect a greater change. However, these results suggest that vitamin D administration has potential as an adjuvant therapy for CD patients [78]. It is noteworthy that the reduced abundance of the *Faecalibacterium* genus is commonly associated with both diseases, UC and CD. Its characteristic of producing butyrate has already been shown to be a way to reduce inflammation and promote a balance between Th17 and Treg [79].

In an interesting cohort study, the possible connection between the seasonality of serum vitamin D levels and changes in the microbiome was evaluated. The target population was composed by adults (n = 87) with IBD (CD or UC), who lived in regions far from the equator, and both the intestinal mucosa and the fecal samples microbiome were evaluated. After confirming the differences in the concentrations of 25(OH)D, which were higher in periods with higher sun exposure (summer / autumn), some changes in the microbial composition were also observed. In the summer / autumn period, an increase in the abundance of *Pediococcus* spp., *Clostridium* spp., and *Escherichia* / *Shigella* spp. was observed. In contrast, inflammation-related bacterial genera such as *Eggerthella lenta*, *Fusobacterium* spp., *Helicobacter* spp., and *Faecalibacterium prausnitzii* showed lower relative abundance. Unlike other studies, low levels of vitamin D were associated with a more balanced composition of the microbiome. It should be noted that it was not a randomized controlled trial (RTC), but vitamin D levels were correlated with changes in the microbiome in individuals with IBD [80].

Vitamin D supplementation in healthy individuals has also been shown to alter the microbiome. In a study conducted in healthy adults (n = 16) supplemented with vitamin D_3 (first 4 weeks: 980 IU / kg of body weight; last 4 weeks: 490 IU / kg of body weight) for 8 weeks, the supplemented group had the upper GIT (gastric corpus, antrum, and duodenum) microbiome composition changed [71]. The abundance of Proteobacteria was reduced in the gastric corpus (GC) and antrum (GA), along with an increase in Bacteroidetes in the GC and the descending part of the duodenum, while no changes were observed in the microbiome of the lower GIT and fecal samples. The supplemented group also showed an increase in bacterial richness and significant changes in Gammaproteobacteria in the upper GIT, such as a reduction in *Pseudomonas spp*. and *Escherichia* / *Shigella*. The authors suggested that the increase in the phylotype richness and the microbial changes in the upper GIT, mainly due to the reduction in typically opportunistic pathogens, supports the beneficial effects of vitamin D on the human gut microbiome, especially in the upper GIT [71]. Still, similarly to what had been previously observed by Veldman et al., a trend in the increase of CD8+ T cell, the immune cell with the highest expression of VDR, was observed in almost all regions of the gastrointestinal tract evaluated [71,72].

In another interventional study conducted with healthy individuals, higher circulating levels of 25(OH)D (above 20 ng/mL) were related to a higher abundance of the beneficial bacteria *Akkermansia muciniphila* and a reduced abundance of the pathogen *Porphyromonas* spp. Moreover, after supplementation with vitamin D_3 for 8 weeks (600, 4,000 or 10,000 IU/day), an increase in the relative abundances of *Bacteroides* spp. and *Parabacteroides* spp. was observed. This fact is usually associated with an improvement in the IBD activity, and a decrease in the Firmicutes:Bacteroidetes ratio [81]. Accordingly, Luthold et al. reported that 25(OH)D circulating levels were inversely correlated with the fecal abundances of the Gram-negative genera *Haemophilus* and *Veillonella*, in addition to higher LPS (lipopolysaccharides) levels, suggesting the vitamin D role in the intestinal homeostasis and inflammation [82].

3.2.2 Anti-inflammatory potential of vitamin D in IBD

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Supplementation of vitamin D in IBD patients is challenging due to nutrients malabsorption issues, and higher doses are often necessary to achieve the recommended circulating level (above 20 ng/L, according to IOM). A meta-analysis conducted by Guzman-Prado et al. indicated that the administration of vitamin D to IBD patients might improve the vitamin status while reducing the disease activity index and the levels of hs-CRP (high-sensitivity C-reactive protein) [83]. Nevertheless, these benefits seemed to be more pronounced in CD cases when compared to UC [14,83].

Supplementation of vitamin D_2 (2,000 IU daily or 50,000 IU weekly) or vitamin D_3 (2,000 IU daily) were able to increase vitamin D level in children and adolescents with IBD and vitamin D insufficiency. However, the higher vitamin D_2 dose (50,000 IU weekly) was the most successful treatment, achieving a serum level of 25(OH) above 32 ng/mL in 75% of the patients enrolled in this group [84]. Likewise, adolescents with IBD and vitamin D deficiency that received oral vitamin D_3 supplementation during 6 weeks (5,000 IU or 10,000 IU per 10 g of body weight) showed an improvement in the vitamin D status up to 12 weeks of follow up [85]. Nonetheless, none of these clinical trials evaluated inflammatory markers or reported the impact on the disease status.

An observational study with 206 IBD patients showed that vitamin D deficiency and insufficiency were observed in both CD and UC patients, but were more frequent in CD patients. In addition, moderate and severe clinical disease activities reported were significantly associated with vitamin D deficiency in CD [86]. Similarly, Mechie et al. reported that the majority of IBD patients had vitamin D deficiency and the serum levels of 25(OH)D may be considered an important marker for IBD disease activity, in addition to hs-CRP and fecal calprotectin [87].

In a RCT with 90 adults with UC, a single dose injection of 300,000 IU of vitamin D_3 significantly increased the serum levels of 25(OH)D while it decreased inflammatory markers hs-CRP and ESR (erythrocyte sedimentation rate) and suppressed the Th1 immune response in UC patients [88,89]. Similarly, in the study conducted by Garg et al., UC and CD patients that received 5,000-10,000 IU of vitamin D_3 daily for 12 weeks showed a significant increase in the 25(OH)D serum levels [90]. At the same time, CD patients reported a decrease in the clinical disease activity index, even though the biomarkers did not confirm this anti-inflammatory effect [91].

In summary, vitamin D plays a critical role in the proper immune responses, and its status should be monitored in individuals from risk groups, such as IBD patients. Moreover, a limited number of interventional studies evaluating the impact of vitamin D in the inflammation pathways and in the gut microbiota modulation in IBD were conducted. However, the outcomes are hopeful but not consistent, and future studies are encouraged.

4. Epigenetics and IBD, vitamin D/VDR

Genetics is popularly known as the study of heredity, evaluating the changes in nucleic acids and their performance in organisms. On the other hand, epigenetics is defined as changes in gene expression or reversible hereditary changes without altering the DNA sequence [92]. A central goal of epigenomics is to understand the gene expression alteration by dietary molecules [93], and it makes a joint focus with nutrigenomics and epigenomics [94].

Epigenetics changes occur in the following ways: DNA methylation, histone modifications, chromatin remodeling, and noncoding RNAs regulation [95].

The most well-known epigenetic modification is DNA methylation, which is characterized by the addition of a methyl group covalently to the C5 carbon of a cytosine [96]. The degree or number of methylations defines the expression of a gene. High degree of methylations (hypermetilation) silences the promoter of tumor suppressor genes, while under-methylated DNA (hypomethylation) induces proto-oncogenes, for example [97].

The second group of epigenetic changes is the histone modifications, which either activate or repress gene expression. Based on post-translational modifications in the histone tail at lysine, arginine, and serine residues [98], such as acetylation, methylation, and phosphorylation [93].

Chromatin remodeling is the third group of epigenetics modification. Chromatin is modulated by a group of enzymes that catalyze changes in histone residues, such as the addition or removal of

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acetyl or methyl groups. Acetylation (addition of acetyl) is generally associated with transcriptional activation. These reactions are performed by two classes of enzymes, histone acetyltransferase (HAT) and histone deacetylase (HDAC), while for histone methylation there are two classes of enzymes with opposite functions, histone methyltransferase (HMT) and histone demethylase (HDM) [93].

There are several classes of RNA, including the noncoding RNAs (ncRNAs). This class of molecules are grouped in the fourth group of epigenetics modification. NcRNA is a group of RNA that has several other smaller groups, and neither their production nor their functions can be generalized. Many of them regulate post-transcriptional processes, and others are involved in transcriptional regulation. They can be divided in long ncRNAs (lncRNAs), with up to more than 100 kilobases, and short ncRNAs, with less than 30 nucleotides, such as microRNAs (miRNAs), short interfering RNAs (siRNAs), and PIWI-interacting RNAs (piRNAs) [95].

4.1 Epigenetics and IBD

For a long time, a genetic susceptibility in IBD pathogenesis was suggested, and the technological progress in DNA/RNA sequencing allowed many GWAS, and thus, the single nucleotide polymorphisms (SNPs) identification [99-101].

The identification of markers for the diagnosis of IBD is of utmost importance, and DNA methylation and miRNAs are special biomarkers for diagnosis at the molecular level. Indeed, studies have shown a strong sensitivity, specificity, and precision of these markers in the diagnosis of IBD [16].

Compared with a healthy control group, patients with IBD showed different changes in the mucosa methylation of the THRAP2, FANCC, GBGT1, DOK2, and TNFSF4 markers. Differences were also observed between patients with CD and UC. CD patients had hypermethylated GBGT1, IGFBP4, and FAM10A4 and hypomethylated IFITM1 when compared to UC patients. Thus, enabling them as markers for differentiating CD and UC [102]. Recently, Kim et al. identified that the fragile histidine triad (FHIT) gene was hypermethylated in patients with CD, therefore a possible biomarker for this disease [103].

4.2 Vitamin D/VDR epigenetic role in IBD

It is common to associate vitamin D with skeletal homeostasis [104]. However, VDR is linking at hundreds of sites in the genome [105] and is associated with the regulation of more than 60 genes [106,107]. VDR regulates the opening of ion channels, as well as the activity of various enzymes such as kinases, phosphatases, and phospholipases [64].

The role of vitamin D/VDR in the secretion of intestinal mucus may be regulated by the expression of CYP27B1 [108]. In both UC and CD, VDR expression is down-regulated while CYP27B1 is up-regulated [109-111]. This reduced VDR expression can also be attributed to the miRNA-346 [109], one of the post-transcriptional mechanisms explained further.

Another way to prevent intestinal inflammation is by regulation of junctional proteins [112]. Although there is a variety of functions and routes that vitamin D / VDR plays a role, there are few studies exploring gene regulation of junctional proteins [113]. Liu et al. showed that VDR binds to histone inhibiting transcription of ZO-1, claudin-5, and occludin genes [114]. Zhang et al. have identified that VDR increases the tight junction protein *claudin-2* as a direct target of the VDR signaling pathway [68]. Interestingly, inflammatory cytokines could also increase the expression of Claudin-2 and enhance intestinal permeability. Thus, lacking intestinal epithelial VDR regulation in inflamed intestine leads to hyperfunction of Claudin-2 and exaggerates the inflammatory responses in intestine [115].

MiRNA are a class of small non-coding RNA (17-22 nucleotides) that regulates gene expression post-transcriptionally. There is a growing interest in understanding and exploring the contribution of miRNAs in common diseases, including IBD [116]. Liu et al. performed a meta-analysis exploring the association of SNPs from miRNAs and IBD. They reported three polymorphisms (rs11614913, rs2910146, and rs3746444) in miRNA-196a2, miRNA-146a, and miRNA-499 in patients with IBD

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[117]. In addition, other miRNAs expression profiles changed during IBD. Among them, are the following: miRNA-21, miRNA-122a, miRNA-155 or miRNA-150, which have been associated to intestinal epithelial permeability [118-121]; and miRNA-126, miRNA-146a or miRNA-155, which are linked to innate and adaptive immune response in intestinal inflammation [122-124]. Moreover, miRNA-146a and miRNA-155- are directly related to the communication between the GIT microbiome and the brain, and miRNA-155 acts on 3 proteins, for which down-regulation is related to Alzheimer's Disease [125].

The most studied miRNAs in IBD are miRNA-21, miRNA-155, and miRNA-31 [126-132]. The association of miRNA-21 and IBD has been the subject of several studies [133]. MiRNA-21 is upregulated in the serum and colonic mucosa in UC and is related to the impairment of tight junctions in intestinal epithelial cells by inducing the degeneration of RhoB mRNA [134]. Moreover, the overexpression of miRNA-31 in tissues of patients with IBD and in mice with colitis induced by dextran sulfate sodium (DSS), reduced IL7R and IL17RA inflammatory cytokine receptors and signaling proteins [135]. Meanwhile, in another study using a mice DSS colitis model, it was demonstrated that miRNA-155 binds directly to SHIP-1 mRNA, responsible for regulating cell membrane traffic [136]. However, these miRNAs act directly at IBD, and none of them related to VDR functions. In fact, James et al. (2020) reported several miRNAs associated with IBD, however, none of them target VDR [133].

Using TargetScan (http://www.targetscan.org), a miRNA database, we can verify that VDR is regulated by miRNA-23, miRNA-124, miRNA-125, miRNA-302, miRNA-372, miRNA-373, and miRNA-506 [137]. Among these miRNAs, only miRNA-124 was previously associated with IBD, where it has been shown that the reduced levels of this miRNA in colon tissues of children with UC increased the expression of STAT3, as miRNA-124 acts by regulating this protein [138]. On the other hand, Yang et al. (2017) showed that there is a positive regulation of miRNA-23 when cells were treated with high glucose (HG) and miR-23 affected the process of HG-induced epithelial-mesenchymal transition (EMT) in HPMCs by targeting VDR. Although not directly related to IBD, this study is a pioneer in elucidating the role of a miRNA that targets VDR in intestinal epithelial cells, that is, starting from a search on TargetScan and identifying miRNA-23 targets, in this case, VDR, the authors aimed to study the relationship of this miRNA with VDR in the process of intestinal fibrosis [139].

Chen et al. (2017) investigated the role of the lncRNA H19 in the tumorigenesis of many types of colon cancer. They reported that VDR signaling was able to inhibit the expression of H19 through regulating C-Myc/Mad-1 network, and H19, in which turn, was able to inhibit the expression of VDR through miRNA-675. These data showed not only the interaction of a miRNA and VDR but also of another class of ncRNA [140]. To date, there is no study that shows the role of a miRNA by regulating VDR directly in the pathogenesis of IBD, however, miRNA-23 and miRNA-675 seem to be the most promising for studying the role of miRNAs in VDR interaction in IBD.

4.3 Probiotic role regulated by Vitamin D/VDR

The potential use of probiotics for microbiota modulation and IBD management have been studied by researchers [141-143]. Ryan et al. reported that inflamed and non-inflamed colonic segments in CD and UC differ in microbiota composition and epigenetic profiles [6]. Moreover, it has been suggested that probiotics may modulate the expression of miRNAs [144].

Importantly, the proper function of VDR is crucial for probiotic anti-inflammatory effects, while probiotic consumption may improve the VDR status as well [145]. Recently, Lu et al. reported that the anti-inflammatory and anti-infectious activity of lactobacilli strains isolated from Korean kimchi depends on the VDR expression [146]. Yet, in an earlier study, Lu et al. investigated the tissue-specific role of intestinal epithelial VDR apoptosis and autophagy. The authors concluded that VDR loss impairs autophagy and enhances cell death through apoptosis. They suggested that this mechanism is mediated by the action of vitamin D in ATG16L1 and Beclin-1, which promotes cell survival and thus an anti-inflammatory role in the intestine [147]. Likewise, *Saccharomyces boulardii*

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revealed to be a promising probiotic specie for the management of IBD, increasing the expression of miRNA-155 and miRNA-223, while decreasing the expression miRNA-143 and miRNA-375 [148].

Furthermore, in a study conducted by Chatterjee et al., it became clear that the VDR signaling affects both the microbiome and the metabolomics profile. Indeed, impaired VDR together with a high fat diet (HFD), promoted a significant impact on bile acid metabolism, which was more pronounced in female mice. In addition to microbiome regulation, long chain acylcarnitines (LCACs), tocopherol, and equol metabolisms were also influenced by VDR function and HFD. Thus, it can be concluded that both, diet and VDR status, play a role in metabolic diseases, inflammation, risk of colon cancer, and epigenetic pathways [149].

In summary, emerging studies have pointed out the role of vitamin D/VDR in regulating proteins that are related to IBD, especially promoting transcription factors, such as miRNAs. There is also evidence that probiotics play a role in these modulations. Our recent study has shown that VDR promotes healthy microbial metabolites and microbiome in a tissue specific and gender specific manner [149]. However, more studies are necessary to elucidate this influence and all the metabolic steps associated.

5. Conclusions and Future Directions

In this review, we addressed the immunomodulatory effects of vitamin D. The knowledge about the particularities of IBD has advanced substantially in recent years, and the anti-inflammatory and modulating effect of vitamin D and VDR expression has been studied. However, despite the fact that the chronic inflammation profile in IBD is the key role that the microbiota plays in this relationship, studies in humans are still scarce.

Vitamin D immunomodulatory effects seem to be related to the suppression of the pro-inflammatory Th1 immune response, while lower levels of 1,25-$(OH)_2$D impair Tregs, triggering autoimmune diseases. Indeed, the supplementation of vitamin D improved the hs-CRP and fecal calprotectin status, while reduced the disease activity index and relapses, predominantly in CD patients.

So far, it is possible to observe that vitamin D supplementation contributes to the reduction of inflammation in individuals with IBD and can promote changes in the human microbiota. However, the studies reported have several limitations, such as the small sample, the unmatched methodology, or even the lack of definition of what would be the composition of a healthy microbiota. Surely, VDR is a crucial factor for gut microbiota homeostasis, having a great impact on the metabolome profile as well. In addition, its proper functions influence several genes associated with inflammation, barrier function, cancer, autophagy, among others. Therefore, more studies to assess the microbiota at the metabolic level are needed, which would be more appropriate than in the taxonomic level, although alterations in genera and species have already been associated with the disease.

Finally, a different profile of miRNA is expressed in CD, UC, or healthy control individuals and epigenetics markers revealed to be a highly sensitive, specific, and precise tool for IBD diagnosis, therefore a promising and less invasive alternative when compared with endoscopy and biopsies, which are employed nowadays. Moreover, vitamin D also plays a role in IBD regulating transcription factors associated with barrier function and immune responses. The exact mechanisms are not well understood and more studies are needed to explore the therapeutic potential of vitamin D/VDR in the gut microbiota modulation and anti-inflammatory effects in IBD at the metabolic, immunological, and epigenetic levels.

Vitamin D/VDR deficiency could be considered as a multifunctional susceptibility factor in IBD (Figure 1). Evidence has demonstrated that vitamin D deficiency is a critical factor in the pathology associated with IBD. Vitamin D administration leads to a shift of the intestinal bacterial composition in CD patients, but not in healthy controls [78]. To move forward, we need well-designed therapeutic studies to examine whether enhanced vitamin D will restore functions of VDR and microbiome in inhibiting chronic inflammation.

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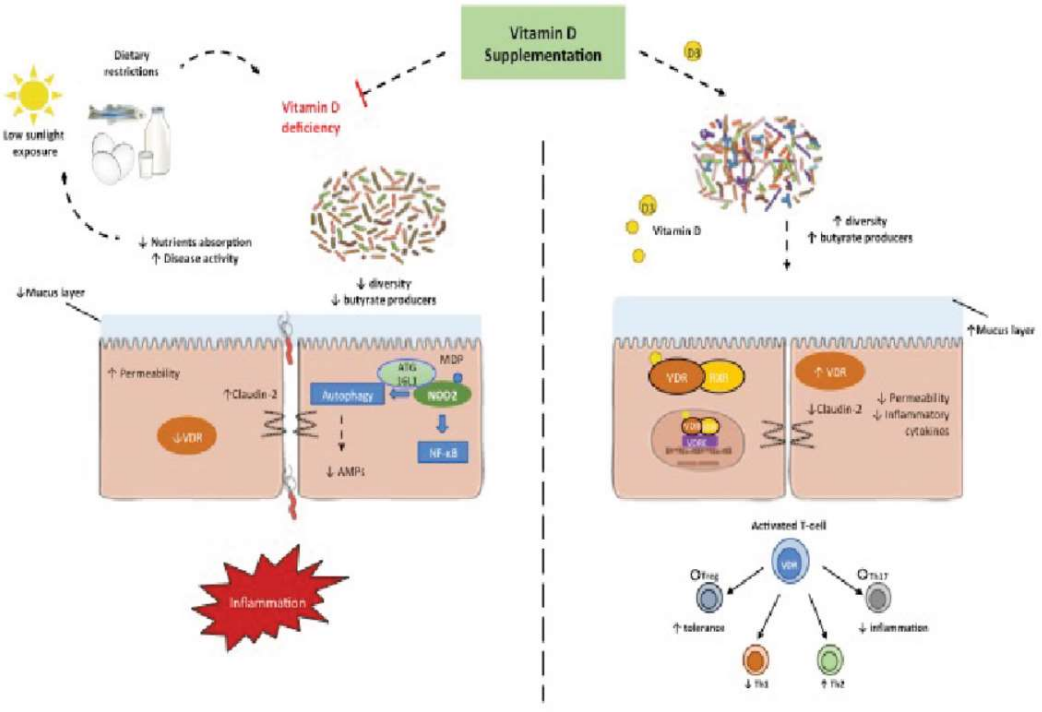

Figure 1. Vitamin D/VDR is involved in the genetic, environmental, immune, and microbial aspects of IBD. Thus, the vitamin D supplement and activation of VDR could be considered as a multifunctional factor in IBD treatment.

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