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Open Access Case Report

The LGS Protocol, validated through VDR Immunofluorescence and Microbiome Test Correlation, supports Vitamin D Receptor Renewal via an Anti-inflammatory Diet in a clinical case of Psoriasis Vulgaris.

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Abstract

This clinical case study investigates the relationship between the Vitamin D receptor (VDR) and psoriasis using Dr. Eduardo Beltran's "Leaky Gut Syndrome (LGS) Protocol." The intervention, combining an anti-inflammatory diet, high-dose vitamin D supplementation, and antimicrobial herbs (particularly oregano oil and turmeric), targeted small intestinal fungal overgrowth induced by Candida. Immunofluorescence analysis of VDR expression in skin biopsies was conducted before and after the intervention. Initial biopsy revealed reduced VDR expression correlating with persistent psoriasis. After four months, significant clinical improvement and increased VDR expression were observed in the follow-up biopsy. Significant improvements were also observed in a follow-up microbiome test. The study explores turmeric's integration for its anti-inflammatory and anti-microbial properties, emphasizing implications for managing chronic inflammatory disorders like psoriasis. The findings support the efficacy of an anti-inflammatory diet in reducing intestinal hyperpermeability, increasing VDR expression, and offer innovative insights for therapeutic strategies in resistant psoriasis.

Categories: Integrative/Complementary Medicine, Rheumatology, Dermatology
Keywords: coimbra protocol, small intestinal bacterial overgrowth, small intestinal fungal overgrowth, microbiome modulation, leaky gut syndrome, lgs protocol, herbal antimicrobials, vitamin d, anti-inflammatory diet, vitamin-d receptor

Introduction

In recent times, there has been a notable increase in the prevalence of psoriasis, impacting specific demographic groups at an alarming rate. This persistent inflammatory skin condition has gained significant attention due to its widespread occurrence and the complexities involved in its management [1]. Researchers are increasingly delving into potential connections between disruptions in the gut microbiome and the onset of psoriasis, aiming to understand the intricate interplay between gut health and immune function.

The expanding body of evidence emphasizes the crucial role of dysbiosis, an imbalance in the gut microbiota, as a potential catalyst for various health issues, including the well-known Leaky Gut Syndrome (LGS). The emergence of LGS, often linked to factors such as diets high in gluten, dairy, lectins, and excessive sugars, has gained attention for its involvement in triggering gut inflammation [2]. This inflammation, in turn, alters the diversity of the microbiome, setting the stage for the overgrowth of specific bacterial and fungal strains, leading to conditions like Small Intestinal Bacterial Overgrowth (SIBO) and Small Intestinal Fungal Overgrowth (SIFO) [3].

The maintenance of a diverse microbiome ecosystem is crucial as it contributes to the production of essential metabolites, including short-chain fatty acids (SCFA's) such as butyrate, acetate, and propionate [4]. These metabolites play a pivotal role in preserving the integrity of the gut lining and mucin layer, essential components for a well-functioning gastrointestinal system.

The disruption of this balance, fueled by inflammatory dietary components, contributes to the initiation of LGS. This condition opens the gateway for the entry of lipopolysaccharides (LPS's), mycotoxins, and foreign proteins such as lectins and bacteria into the bloodstream [5]. These substances interact with the immune system, a significant portion of which resides within the gut, triggering cascading immune responses. Over time, the gradual erosion of immunologic tolerance can lead to molecular mimicry, fostering the development of autoimmune disorders.

LPS's found in the outer membrane of gram-negative bacteria elicit a robust immune response upon entering the bloodstream. This interaction, primarily through toll-like receptor 4 (TLR4) activation, disrupts the functioning of vitamin D receptors (VDR), essential nuclear receptors that govern diverse physiological processes [6]. The activation of TLR4 by LPS induces the upregulation of nuclear factor kappa B (NF-κB) and the release of inflammatory factors, including interleukin-1 (IL-1) & Tumor Necrotic Factor Alpha (TNF-alpha), which inhibit VDR expression by binding to its promoter region. Additionally, LPS affects VDR activity by promoting the expression of gene CYP24A1, an enzyme responsible for converting active vitamin D into an inactive form, thereby diminishing the availability of calcitriol necessary for VDR activation [6].

Moreover, LPS influences VDR activity by modulating the expression of co-regulators like histone deacetylases (HDACs), impacting chromatin accessibility for VDR binding. Chronic fungal infections, such as those caused by Candida albicans and other fungi armed with mycotoxins like candidalysin, Ochratoxin A, Aflatoxin B1, Zearalenone, Deoxynivalenol (DON), and T2 toxin, have been demonstrated to upregulate NF-κB, simultaneously downregulating VDR [6]. Prolonged exposure to LPS, bacterial toxins, and mycotoxins may contribute to sustained low-grade inflammation, potentially leading to autoimmune diseases through mechanisms involving autoreactive T cells, autoantibody production, and disruptions in regulatory T cells and B cells. This intricate interplay underscores the molecular intricacies through which bacterial toxins impact VDR function and immune regulation, offering insights into potential connections between the microbiota, inflammation, and autoimmune pathogenesis [7].

In the domain of psoriasis, a complex case unfolded, highlighting the nuanced connection between gut health and skin conditions. The patient, previously diagnosed with Psoriasis vulgaris, exhibited minimal improvement despite undergoing various allopathic treatments. Experiencing a serendipitous enhancement following treatment for a vaginal yeast infection, she ventured onto an unconventional path. Seeking an alternative approach, she decided to collaborate with our clinical practice employing an anti-inflammatory diet and targeted supplementation.

Case Presentation

The initiation of the patient's treatment regimen marked a crucial phase in her journey toward enhanced health. A comprehensive approach was set in motion, involving an anti-inflammatory diet (gluten, dairy, lectin and sugar free) tailored to address gut health. This was complemented by high-dose vitamin D supplementation at 50,000 IU of cholecalciferol, accompanied by essential cofactors.

This therapeutic strategy aligns with the principles and similar framework of High Dose Vitamin D (HDVD) therapy, also known as the Coimbra Protocol [7], recognized as an innovative intervention for various chronic autoimmune conditions.

To ensure patient safety and optimize outcomes, healthcare providers proficient in HDVD therapy adhere to a stringent monitoring protocol. This involves a thorough assessment of key biomarkers, including parathyroid hormone (PTH), serum and ionized calcium levels, 24-hour calciuria, 24-hour phosphorus excretion, and more. The objective is to prevent hypercalcemia, a potential complication arising from excessive vitamin D intake, despite its rarity among patients undergoing this type of treatment. The goal of suppressing PTH levels toward the lower reference range contributes to mitigating vitamin D resistance and enhancing its therapeutic effects.

Remarkably, our experience with the Leaky Gut Syndrome (LGS) Protocol has unveiled an interesting observation. In an extensive cohort of over 3000 patients over 5 years in our practice, we noted a significant reduction (>75%) in the demand for high-dose vitamin D, approximately by 50%, compared to other protocols.

This phenomenon is hypothesized to be closely connected to the resolution of heightened gut permeability, a characteristic feature of Leaky Gut Syndrome (LGS) [5]. By restoring the integrity of the gut and diminishing systemic inflammation, the immune system's workload is reduced, providing a rationale for the diminished requirement of vitamin D.

Utilizing a comprehensive research methodology, a pivotal measure was taken to substantiate the intricate relationship between psoriasis and gut health. With patient consent, a meticulous skin biopsy was conducted on March 22, 2019, providing crucial insights into the pathophysiology of the disease. Additionally, VDR immunofluorescence was employed as a potent investigative tool, offering valuable perspectives into the role of Vitamin D receptors in psoriatic pathology.

Initial Histopathological findings and VDR Immunofluorescence Analysis Report:

Clinical History: The patient has a known history of psoriasis, initially diagnosed in 2011. She presents with chronic erythematous scaly plaques on the left forearm, consistent with her previous diagnosis of psoriasis.

Specimen: Specimen Type: Skin biopsy Anatomic Location: Left forearm

Gross Description: The skin sample measures 1.5 cm x 1.0 cm in size. It consists of a raised erythematous plaque with well-defined borders. The surface of the plaque is covered with silvery-white scales. No signs of ulceration or bleeding are noted.

Microscopic Description: Microscopic examination of the skin biopsy reveals characteristic features compatible with psoriasis. The epidermis shows marked hyperplasia with elongation and thickening of the rete ridges. There is focal parakeratosis, regular acanthosis, and elongated epidermal projections referred to as "regular acanthosis." Munro microabscesses, collections of neutrophils within the epidermis, are also observed. The underlying dermis demonstrates a mild perivascular lymphocytic infiltrate.

Comment: The microscopic findings of the skin biopsy are consistent with the clinical diagnosis of psoriasis. The presence of epidermal hyperplasia, parakeratosis, regular acanthosis, and Munro microabscesses is indicative of classical psoriatic skin lesions as depicted in Figure *1*.

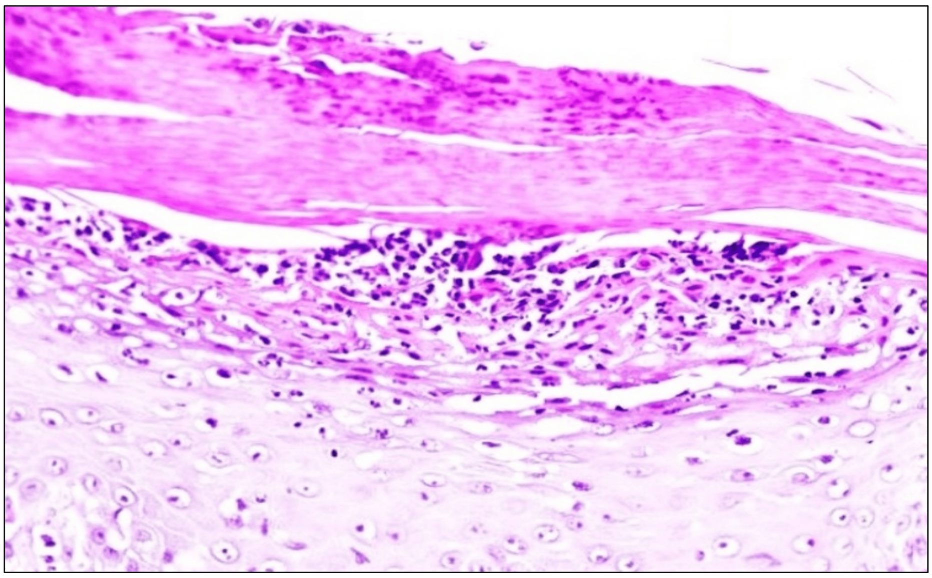


FIGURE 1: Skin biopsy Anatomic Location: Left forearm

The skin biopsy under microscopic examination displays distinctive features consistent with psoriasis. The epidermis exhibits significant hyperplasia, characterized by elongation and thickening of the rete ridges. Notable findings include focal parakeratosis, uniform acanthosis, and elongated epidermal projections known as "regular acanthosis." Additionally, Munro microabscesses, aggregations of neutrophils within the epidermis, are evident. In the underlying dermis, there is a mild perivascular lymphocytic infiltrate.

Immunofluorescence Examination - VDR Expression

Immunofluorescence staining for vitamin D receptor (VDR) was performed to assess its expression in the lesional skin of the left forearm.

Results: The immunofluorescence examination demonstrates decreased expression of VDR in the epidermis of the lesional skin. Compared to adjacent non-lesional skin, there is a reduction in VDR immunoreactivity in the epidermal cells.

Interpretation: The findings of the skin biopsy indicate typical features of psoriasis, including epidermal hyperplasia, parakeratosis, regular acanthosis, and Munro microabscesses. The immunofluorescence examination reveals decreased expression of vitamin D receptor (VDR) in the epidermis, suggesting altered VDR signaling in the pathogenesis of psoriasis as shown in Figure *2*.

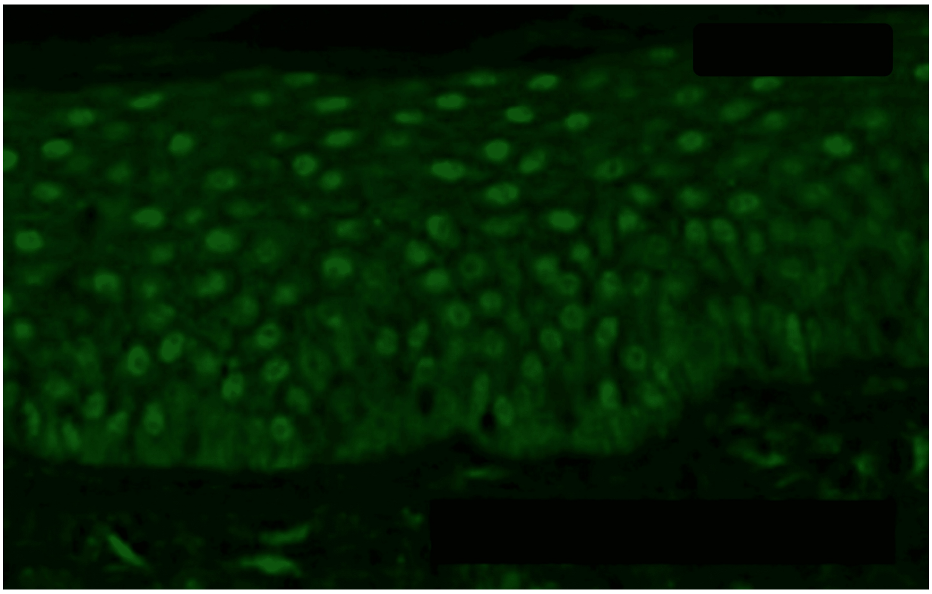


FIGURE 2: First Immunofluorescence staining for vitamin D receptor (VDR)

The immunofluorescence examination reveals a diminished expression of VDR in the epidermis of the affected skin. In comparison to the neighboring non-lesional skin, there is a decrease in VDR immunoreactivity within the epidermal cells.

2 Month Follow-up

After a duration of two (2) months of treatment, the patient displayed initial mild improvements in her psoriasis. While observing mild progress, a comprehensive diagnostic assessment utilizing the Gastrointestinal Microbial Assay Plus (GI Map) test provided further clarity on the underlying factors contributing to her condition. The identification of Small Intestinal Fungal Overgrowth (SIFO) triggered by Candida albicans revealed a crucial aspect of her clinical presentation.

In response to these findings, a targeted therapeutic strategy was implemented, introducing a herbal antimicrobial agent - oregano oil on 05/20/2019. Recognized for its potent activity against Candida albicans, oregano oil was administered at a dosage of 500 mg twice a day for a period of 3 weeks with 1 week rest repeating the same process in 4 cycles. Our in vitro studies conducted by SPIMS validated its efficacy, indicating substantial zones of inhibition against Candida albicans.

Turmeric (Curcumin longa), another potent natural agent, was integrated into her protocol. Recognized for its multifaceted therapeutic attributes, turmeric provides a strategic approach to modulating the activity of Vitamin D receptors (VDRs). A prescribed dose of 2000 mg twice a day (total 4000 mg/day) was recommended.

Evidence of Remarkable Recovery and Immunofluorescence Analysis:

The course of therapeutic interventions led to a convergence of positive outcomes, emphasizing the substantial impact of the integrated approach. Throughout a span of five (5) months, the patient's advancement demonstrated significant efficacy, illustrating a strong synergy between anti-inflammatory strategies and targeted interventions.

Clinical Progress and PASI Score Improvement

Two months after starting the herbal antimicrobials a significante improvement was becoming evident through the Psoriasis Area and Severity Index (PASI) score evaluation, a widely recognized metric for assessing the severity of psoriasis. The initial PASI score of 38, indicating a significant disease burden, underwent a remarkable transformation to a PASI score of 5, indicating a state of nearly complete remission. This notable shift emphasized the effectiveness of the integrated therapeutic regimen in alleviating the clinical manifestations of psoriasis.

Second Biopsy and Immunofluorescence Analysis Report

Clinical History: The patient has a documented history of psoriasis, initially diagnosed in 2011. She presents with a prior diagnosis of psoriasis and reports significant improvement in her skin lesions, which now appear almost normal.

Specimen: Specimen Type: Skin biopsy Anatomic Location: Left forearm

Gross Description: The skin sample measures 2.0 cm x 1.5 cm in size. It consists of an area of skin with subtle erythema and minimal scaling. The previously observed plaques have significantly flattened and show remarkable resolution. No signs of ulceration or bleeding are noted.

Microscopic Description: Microscopic examination of the skin biopsy reveals findings compatible with significant improvement in the skin lesions, which now appear almost normal. The epidermis shows regular thickness and architecture, with no evidence of hyperplasia or elongation of rete ridges. There is no parakeratosis or acanthosis observed. The absence of regular acanthosis and Munro microabscesses previously observed indicates near-complete resolution. The underlying dermis appears unremarkable, with no significant inflammation or infiltrates.

Comment: The microscopic findings of the skin biopsy demonstrate significant improvement in the skin lesions compared to prior tests. The near-normal epidermal architecture, absence of hyperplasia, and resolution of characteristic features of psoriasis suggest a substantial response to treatment or natural remission of the psoriatic lesions as seen on Figure *3*.

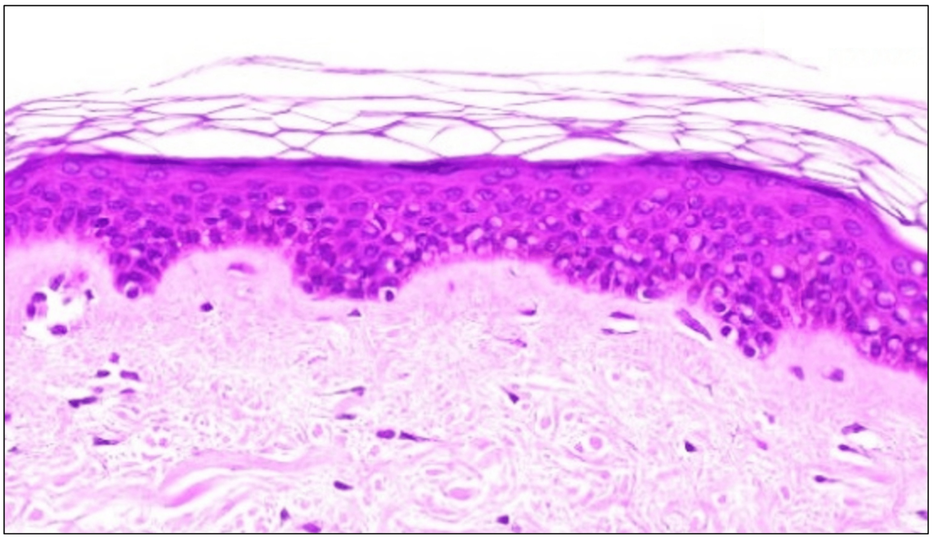


FIGURE 3: Second Skin Biopsy - Anatomic Location: Left forearm (Near original site)

Microscopic Description: Upon examining the skin biopsy, the findings indicate substantial improvement in the skin lesions, now displaying an almost normal appearance. The epidermis exhibits regular thickness and architecture, with no signs of hyperplasia or elongation of rete ridges. Parakeratosis and acanthosis are not present. The absence of regular acanthosis and previously noted Munro microabscesses suggests near-complete resolution. The underlying dermis appears unremarkable, showing no significant inflammation or infiltrates.

Immunofluorescence Examination - VDR Expression:

Immunofluorescence staining for vitamin D receptor (VR) was performed to assess its expression in the lesional skin of the left forearm. Near prior site of initial biopsy.

Immunofluorescence Examination - VDR Expression: Immunofluorescence staining for vitamin D receptor (VDR) was performed to assess its expression in the lesional skin of the left forearm.

Results: The immunofluorescence examination reveals increased expression of VDR in the epidermis of the lesional skin. Compared to prior tests, there is a notable enhancement of VDR immunoreactivity in the epidermal cells.

Interpretation: The findings of the skin biopsy indicate significant improvement in the psoriasis lesions, with the skin now appearing almost normal. The absence of characteristic psoriatic features on microscopic examination suggests substantial resolution. The immunofluorescence examination shows increased expression of vitamin D receptor (VDR) in the epidermis, suggesting a potential role of VDR signaling in the improved skin condition as observed on Figure *4*.

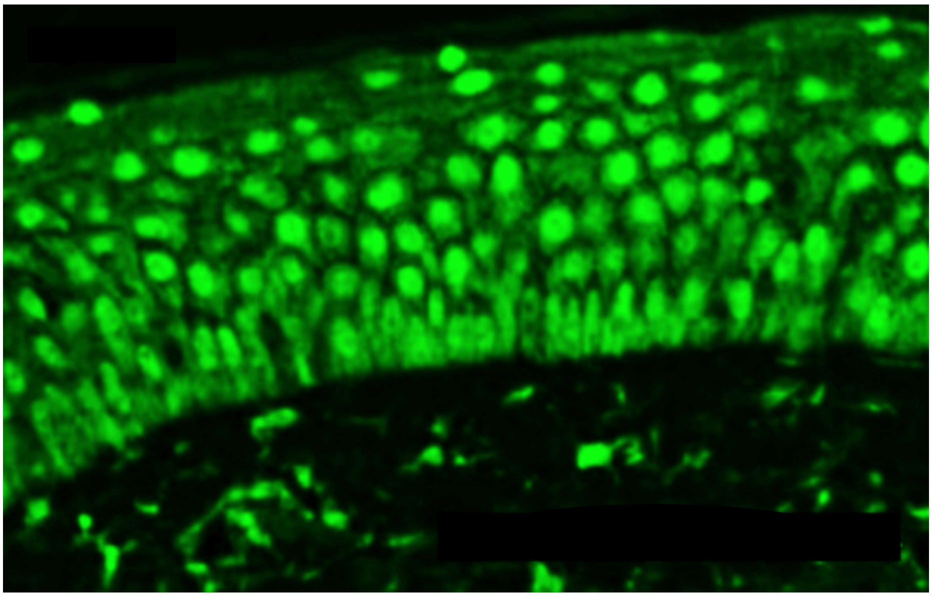


FIGURE 4: Second Immunofluorescence staining for vitamin D receptors (VDR)

The examination through immunofluorescence indicates a heightened expression of VDR within the epidermis of the affected skin. In comparison to previous assessments, there is a significant and discernible increase in VDR immunoreactivity observed in the epidermal cells. This notable enhancement suggests a positive trend in VDR expression, highlighting a potential correlation with improvements or changes in the skin condition. The augmented VDR immunoreactivity underscores the dynamic nature of this parameter in the context of the skin's health and may signify a positive response to interventions or changes in the course of treatment.

Laboratory Findings:

Table *1* illustrates a series of laboratory results over three different dates, providing insights into various biochemical parameters. Notable trends include the progression of vitamin D levels, where the 25OHD3 (Calcifediol) showed an increase from 46 ng/ml to 123 ng/ml and eventually to 148 ng/ml, indicating a positive response to supplementation. Similarly, the active form of vitamin D, 1,25OHD3 (Calcitriol), demonstrated a consistent range within the normal reference (NR) range across the three time points.

Parathyroid hormone (PTH) levels exhibited a downward trend from 81 pg/ml to 41 pg/ml and finally to 24 pg/ml, suggesting a potential regulatory response to the changes in vitamin D status. Serum calcium (Ca+) levels remained within the normal range, with slight fluctuations from 1.24 to 1.20 and back to 1.23 mmol/L.

Ionized calcium (Ionized Ca+) levels showed an increasing trend from 8.9 to 9.3 and eventually to 9.6 mg/dL, indicating adequate calcium ionization. Renal function markers, urea, and creatinine remained within the normal reference range, suggesting stable kidney function.

Liver enzyme levels (ALT and AST) showed improvement, with both ALT and AST decreasing over the three time points. This may signify a positive response to interventions targeting liver health and through diet modifications.

Notably, insulin levels exhibited a decreasing trend from 28.2 μU/mL to 10.4 μU/mL and further down to 7.7 μU/mL, implying enhanced insulin sensitivity and potential improvement in metabolic health. Overall, the interpreted results suggest positive changes in vitamin D metabolism, parathyroid function, calcium homeostasis, renal function, liver health, and insulin sensitivity over the observed period.

Lab Results	03/18/2019	07/16/2019	10/23/2019	Normal Reference Values
25OHD3 - Calcifediol	46 ng/ml	123 ng/ml	148 ng/ml	20-100 ng/ml
1,25OHD3 - Calcitriol	72 pg/ml	78 ng/ml	76 ng/ml	19.9-79 pg/ml
PTH	81 pg/ml	41 pg/ml	24 pg/ml	13.6 – 85.8 pg.ml
Serum Ca+	1.24 mmol/L	1.20 mmol/L	1.23 mmol/L	1,00 a 1,35 mmol/L
Ionized Ca+	8.9 mg/dL	9.3 mg/dL	9.6 mg/dL	8,4 a 10,2 mg/dL
Urea	23 mg/dL	28 mg/dL	26 mg/dL	15,0 a 36,0 mg/dL
Creatinine	1.0 mg/dL	0.9 mg/dL	1.0 mg/dL	0,7 a 1,2 mg/dL
ALT (Alanine Aminotransferase)	44 U/L	24 U/L	17 U/L	<35 U/L (women)
AST (Aspartate Aminotransferase)	41 U/L	26 U/L	15U/L	14,0 a 36,0 U/L
Insulin	28.2 μU/mL	10.4 μU/mL	7.7 μU/mL	2,0 a 25,0 μU/mL

TABLE 1: Laboratory Results with Follow-ups

The individual undergoing high-dose vitamin D therapy (50,000 IU D3) demonstrates positive outcomes with increased 25OHD3 (Calcifediol) levels, indicating a robust response to supplementation. Notably, the levels of 1,25OHD3 (Calcitriol) remain within the normal range, suggesting controlled activation of vitamin D. The decreasing trend in PTH levels suggests a suppressive effect on parathyroid hormone secretion, aligning with expected therapeutic responses. Importantly, Serum Ca+ and Ionized Ca+ levels remain within the normal range, indicating the absence of hypercalcemia. Kidney function, as reflected by Urea and Creatinine levels, appears unaffected. Liver enzymes (ALT and AST) and insulin levels are also within normal limits, indicating no apparent adverse effects. Overall, the laboratory findings suggest a well-tolerated and beneficial response to high-dose vitamin D therapy without observable toxicity.

Vitamin D Metabolism Graphical Interpretation:

The following graphs (Figure 5) present a comprehensive overview of the patient's laboratory results at three different dates, shedding light on various biochemical parameters. A significant trend is observed in vitamin D levels, particularly the 25OHD3 (Calcifediol), which exhibited a progressive increase from 46 ng/ml to 123 ng/ml and eventually to 148 ng/ml. This positive response aligns with the patient's adherence to a regimen involving 50,000 IU of cholecalciferol, following the LGS Protocol recommendations.

It's noteworthy that the initial vitamin labs indicated a lower range for vitamin D (25OHD3) compared to calcitriol (1,25OhD3), a characteristic finding in patients with ongoing active disease processes, such as autoimmunity. This phenomenon is explained by the body's response to an inflammatory state, where the demand for active vitamin D (calcitriol) increases to sustain normalcy, leading to the faster conversion of the "burning" calcifediol (vitamin D) reserve.

Following the initiation of 50,000 IU supplementation, a progressive increase in 25OHD3 levels coupled with a decrease in 1,25OH3 was observed. The last vitamin D result highlighted a notable conversion difference between both metabolites, suggesting a potential CYP27B1 SNP, correlating with genetic findings.

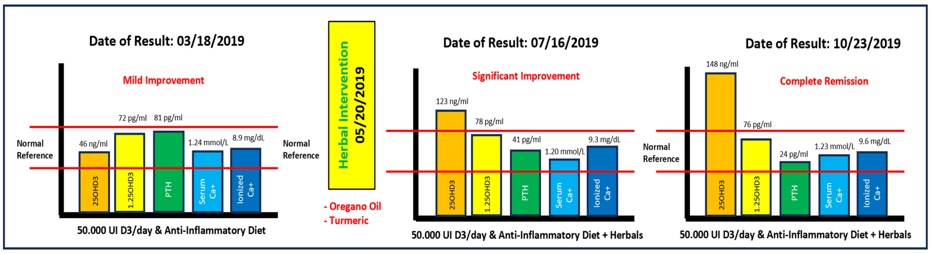


FIGURE 5: Graphical Interpretarion of Vitamin D Metabolites, PTH and Serum/Ionized Ca+

Genetic Testing (SNP's Findings):

Genetic polymorphisms were identified (Table2) in the specified single nucleotide polymorphisms (SNPs) holding substantive implications for the intricate interplay between vitamin D and B vitamin metabolism.

Within the CYP27B1 gene, the allelic variants rs10877012 and rs4646536 were present, which confer potential regulatory effects on the enzyme responsible for catalyzing the conversion of vitamin D to its biologically active form. These genetic variations may exert discernible impacts on the metabolic fate of vitamin D, thereby modulating the bioavailability of its active metabolites. Furthermore, SNPs in the VDR gene (rs2228570, rs731236, rs7975232) were also found introducing variations in the vitamin D receptor, a pivotal mediator of the biological actions of vitamin D. The allelic diversity within the VDR gene may precipitate heterogeneous responses to vitamin D supplementation, influencing intricate physiological processes including calcium homeostasis and immune modulation.

Turning attention to B vitamin metabolism, the allelic variants in the MTHFR gene (rs1801133, rs1801131) present noteworthy implications for folate metabolism. These polymorphisms may exert regulatory control over the conversion of folate to its bioactive form, thereby exerting nuanced effects on the methylation processes critical for DNA synthesis and repair. Additionally, the MTR gene SNP (rs1805087) inserts genetic variability into the methionine synthesis pathway, thereby influencing the metabolism of homocysteine. This intricate genomic landscape underscores the necessity of personalized supplementation strategies.

Genes:	CYP27B1	VDR	MTHFR	MTR
SNP	rs10877012 rs4646536	rs2228570 (FokI) rs731236 (TaqI) rs7975232 (ApaI)	rs1801133 (C677T) rs1801131 (A1298C)	rs1805087 (A2756G)
Function	Involved in Vitamin D activation from 25OHD3 (calcifediol) to 1,25OHD3 (calcitriol).	Vitamin D Receptor responsible for responding to vitamin D levels.	Methylenetetrahydro- folate Reductase enzyme responsible for converting 5,10- methylenetetrahydro-folate to 5- methyltetrahydrofolate	Methionine Synthase Reductase enzyme responsible for converting homocysteine to methionine
Health Implication	May influence vitamin D metabolism conversion and overall vitamin D status of active vitamin D3 (Calcitriol).	Impact on Calcium absorption, bone health, and immune system function.	Associated with altered folate metabolism, potentially affecting methylation processes.	Involved in homocysteine and methionine metabolism, impacting DNA methylation and protein synthesis.

TABLE 2: SNPs found during genetic testing with impact on vitamin D metabolism and methylation cycle.

First GI Map - Microbiome Test Analysis

The inital GI Map Microbioime test results obtained on 05/31/2019 (Figure 6) delineate a dysbiotic profile marked by profound alterations in the composition and abundance of pivotal bacterial species. Elevated levels of Bacteroides fragilis, Escherichia spp., coupled with reduced Bifidobacterium, Lactobacillus, and the absence of Akkermansia muciniphila, underscore a compromised microbial balance and reduced diversity. The absence of Akkermansia muciniphila particularly indicates potential disruption in the mucin layer, intensifying the interaction between the microbiome and the immune system. The abnormal Firmicutes to Bacteroidetes ratio further accentuates the perturbed microbial ecology, indicative of potential metabolic and immunological repercussions.

The overgrowth potential of opportunistic pathogens such as Staphylococcus and Klebsiella signifies the potential for pathogenic dominance within the gastrointestinal milieu. Notably high levels of Candida spp., including Candida Albicans, suggest a substantial fungal overgrowth potentially (SIFO), further contributing to the dysbiosis. These intricate microbial imbalances are implicated in fostering intestinal inflammation, as evidenced by heightened Calprotectin levels. While intestinal health markers, such as normal Steatocrit and Elastase levels, suggest competent fat digestion, the elevated Calprotectin levels denote ongoing inflammation within the gastrointestinal tract. Additionally, the heightened Zonulin levels indicate increased gut permeability, potentially facilitating the translocation of microbial antigens (Figure 7). The intricate interplay between dysbiosis, inflammation, and compromised gut barrier integrity underscores the multifaceted nature of the gastrointestinal pathology unveiled in the GI Map results.

GI MAP Comensal Bacteria/Keystone Bacteria	***Result***	***Normal Reference Range***
Bacteroides fragilis:	3.42e11 **High**	1.6e9 to 2.5e11
Bifidumbacterium:	5.28e7 **Low**	> 6.0e7
Escherichia spp:	1.54e10 **High**	3.7e6 to 3.8e8
Lactobacillus:	9.08e2 **Low**	8.6e5 to 6.2e8
Akkermansia muciniphila:	**Undetectable**	1.01e3 to 8.2e6
Roseburia spp	1.56e8	5.0e7 to 2.0e10
Bacterial Phyla	***Result***	***Normal Reference Range***
Bacteroidetes:	7.18e12 **High**	8.6e11 to 3.3e12
Firmicutes:	2.09e10 **Low**	5.7e10 to 3.0e11
Firmicutes:Bacteroidetes Ratio	0.00	< 1.0
Opportunistic/Overgrowth Microbes - Dysbiotic & Overgrowth Bacteria	***Results***	***Normal Reference Range***
Staphylococcus spp:	1.11e4 **High**	< 1.00e4
Inflammatory & Autoimmune-Related Bacteria:	Result	Normal Reference Range
Klebsiella spp:	7.23e4 **High**	< 5.00e3
Commensal Inflammatory & Autoimmune-Related Bacteria:	***Result***	***Normal Reference Range***
Escherichia spp:	4.23e9 **High**	< 3.80e9
Fungi/Yeast:	***Result***	***Normal Reference Range***
Candida spp.:	1.98e6 **High**	< 5.00e3
Candida Albicans:	1.83e5 **High**	< 5.00e2

TABLE 3: First GI Map prior to starting the LGS Protocol

INTESTINAL HEALTH MARKERS

DIGESTION	Result	Reference
Steatocrit	< dL	< 15 %
Elastase-1	>750	> 200 ug/g
GI MARKERS		
β-Glucuronidase	639	< 2486 U/mL
Occult Blood - FIT	3	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	<210 L	510 - 2010 ug/g
Anti-gliadin IgA	21	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	0.40	< 2.34 ug/g
INFLAMMATION		
Calprotectin	429 H	< 173 ug/g
ADD-ON TESTS		
Zonulin	358 H	< 175 ng/g

FIGURE 6: Intestinal Health Markers before starting LGS Protocol

Second GI Map - Microbiome Test Analysis

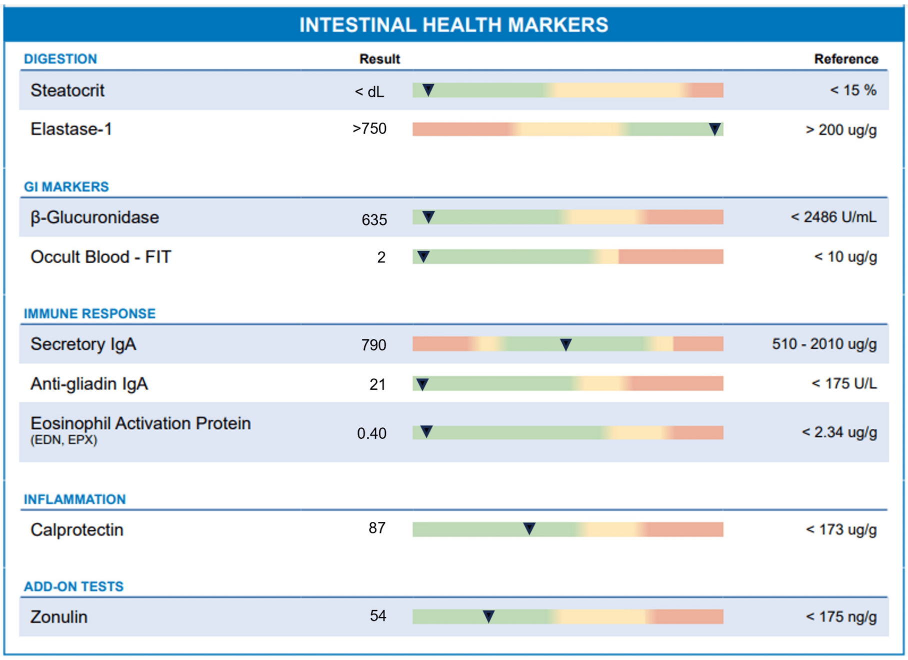
The second GI Map Microbioime test results obtained on 10/27/2019 (Table 4) reveal significant improvement in the microbial profile. Bacteroides fragilis was found within the normal reference range, and beneficial bacteria like Bifidobacterium and Lactobacillus show increased levels. Akkermansia muciniphila levels are within the reference range, signifying a positive change in fostering a healthier mucin layer, crucial for providing a protective barrier in the gastrointestinal tract. Opportunistic pathogens, such as Staphylococcus and Klebsiella, exhibit significant reductions, and fungal overgrowth markers are undetectable. The Firmicutes to Bacteroidetes ratio has increased and remains within an acceptable range.

It is important to note that the patient followed our anti-inflammatory diet and underwent treatment with 4 cycles of Oregano Oil, Curcumin Longa, along with probiotic supplementation (Bifidumbacterium, Lactobacilus and Akkermansia Munciphila) with and increased healthy fiber intake (inulin).

GI MAP Comensal Bacteria/Keystone Bacteria	***Result***	***Normal Reference Range***
Bacteroides fragilis:	2.2e11	1.6e9 to 2.5e11
Bifidumbacterium:	8.2e7	> 6.0e7
Escherichia spp:	3.5e9	3.7e6 to 3.8e8
Lactobacillus:	9.2e5	8.6e5 to 6.2e8
Akkermansia muciniphila:	7.9e6	1.01e3 to 8.2e6
Roseburia spp	7.8e8	5.0e7 to 2.0e10
Bacterial Phyla	***Result***	***Normal Reference Range***
Bacteroidetes:	9.7e11	8.6e11 to 3.3e12
Firmicutes:	2.7e11	5.7e10 to 3.0e11
Firmicutes:Bacteroidetes Ratio	0.27	< 1.0
Opportunistic/Overgrowth Microbes - Dysbiotic & Overgrowth Bacteria	***Results***	***Normal Reference Range***
Staphylococcus spp:	Undetectable	< 1.00e4
Staphylococcus aureus:	Undetectable	< 5.00e2
Inflammatory & Autoimmune-Related Bacteria:	**Result**	**Normal Reference Range**
Klebsiella spp:	4.23e3	< 5.00e3
Commensal Inflammatory & Autoimmune-Related Bacteria:	***Result***	***Normal Reference Range***
Escherichia spp:	3.05e9	< 3.80e9
Fungi/Yeast:	***Result***	***Normal Reference Range***
Candida spp.:	Undetectable	< 5.00e3
Candida Albicans:	Undetectable	< 5.00e2

TABLE 4: Second GI Map after 5 months on the LGS Protocol

Intestinal health markers (Figure 7), digestion, GI markers, immune response, and inflammation markers all fall within the normal range after treatment. This suggests a positive impact on the patient's gut health, with reduced inflammation, improved immune defense, and a more balanced microbial ecosystem. The normalization of gut permeability (Zonulin) further supports the efficacy of the integrated therapeutic approach. Overall, the simulated analysis indicates a successful transformation from a dysbiotic state to a healthier gut microbiome following the comprehensive treatment plan.



INTESTINAL HEALTH MARKERS	Result	Reference
DIGESTION		
Steatocrit	< dL	< 15 %
Elastase-1	>750	> 200 ug/g
GI MARKERS		
β-Glucuronidase	635	< 2486 U/mL
Occult Blood - FIT	2	< 10 ug/g
IMMUNE RESPONSE		
Secretory IgA	790	510 - 2010 ug/g
Anti-gliadin IgA	21	< 175 U/L
Eosinophil Activation Protein (EDN, EPX)	0.40	< 2.34 ug/g
INFLAMMATION		
Calprotectin	87	< 173 ug/g
ADD-ON TESTS		
Zonulin	54	< 175 ng/g

FIGURE 7: Intestinal Health Markers After 5 months on the LGS Protocol

Correlating Clinical Improvements with VDR immunoflouresence

The first picture depicted the inflamed state of the skin, showcasing the typical red, thickened, and scaly patches associated with psoriasis vulgaris. A skin biopsy was subjected to VDR (Vitamin D Receptor) immunofluorescence analysis. The results revealed little expression of VDRs, suggesting a potential role of vitamin D dysregulation in the pathogenesis of psoriasis and implicating the immune-modulatory functions associated with VDRs in skin health.

Following successful treatment and the achievement of complete remission, a second picture documented the transformed state of the patient's skin. In correlation with this improvement, a second skin biopsy was performed, revealing a significant shift in the expression of VDRs. The immunofluorescence analysis displayed a notable increase, indicating a restored or enhanced function of VDRs. This correlation between clinical improvement and increased VDR expression supports the notion that VDRs may play a crucial role in regulating immune responses and cellular processes involved in psoriasis vulgaris. The comparison between the initial and remission states, along with VDR immunofluorescence findings, provides valuable insights into the potential therapeutic implications of modulating vitamin D pathways in the management of psoriasis vulgaris.

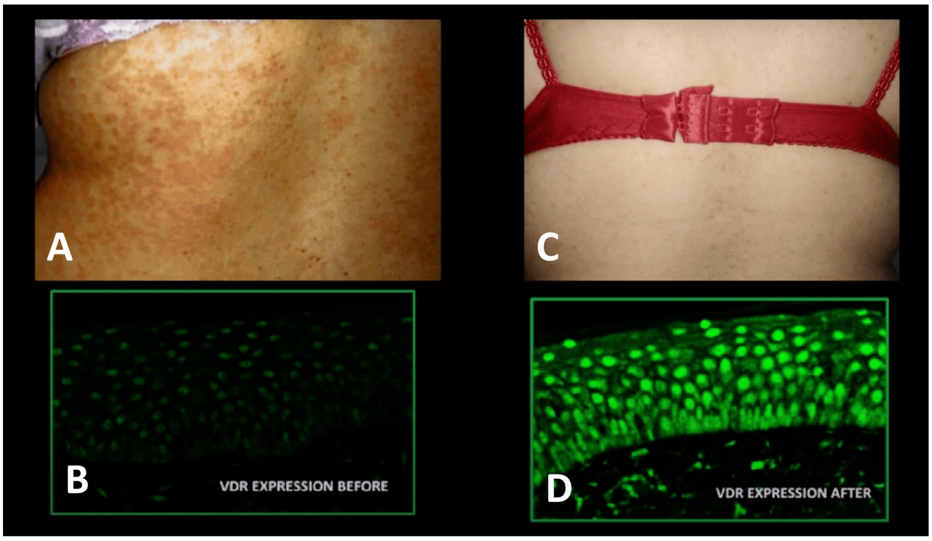


FIGURE 8: Clinical Improvements with VDR immunoflouresence

Image B displays the VDR immunofluorescence obtained from the initial skin biopsy, revealing reduced VDR expression on the epidermis. This biopsy aligns with Image A, illustrating the patient's skin with characteristic psoriatic plaques, erythema, and desquamation.

In contrast, Image D showcases the VDR immunofluorescence from the second skin biopsy, indicating heightened VDR expression on the epidermis. This biopsy corresponds to Image C, portraying the patient in complete remission.

Discussion

This case report meticulously delineates the patient's clinical trajectory, incorporating rigorous diagnostic methodologies, including the Gastrointestinal Microbial Assay Plus (GI Map) and genetic testing targeting Single Nucleotide Polymorphisms (SNPs) in genes intricately linked with vitamin D metabolism and methylation cycle genes. The scope of laboratory monitoring extended to the nuanced evaluation of vitamin D metabolites (25OHD3 and 1,25OHD3) and its antagonist parathyroid hormone (PTH). Simultaneously, serum and ionized calcium levels were diligently scrutinized for potential hypercalcemia, concomitant with a comprehensive assessment of kidney and liver function. This vigilance was necessitated by the patient's adherence to a High Dose Vitamin D protocol and an anti-inflammatory diet rooted in the principles of the LGS Protocol developed by Dr. Eduardo Beltran [7].

The overarching objective of this holistic approach was to unravel the intricate nexus between the gut microbiome, the immune system, and genetic determinants influencing the genesis of psoriatic pathology. The findings illuminate the detrimental impact of lipopolysaccharides (LPSs), endotoxins, and mycotoxins on the expression of Vitamin D Receptors (VDRs). Furthermore, the study elucidates how dietary interventions, coupled with the administration of herbal antimicrobials such as oregano oil and turmeric, contribute to mitigating intestinal hyperpermeability and enhancing VDR expression, as evidenced by findings from skin biopsy and immunofluorescence analyses.

The ensuing discourse meticulously interprets and analyzes the accrued results, establishing intricate connections with the underlying research question and delving into the far-reaching implications thereof.

Mechanism of Oregano Oil's Antimicrobial Properties:

The mechanisms underlying the antimicrobial properties of oregano oil is attributed to a complex chemical composition consisting of bioactive compounds, including phenols, terpenes, and flavonoids, known for their antimicrobial, antioxidant, and anti-inflammatory attributes.

The bioactive constituents of oregano oil are highlighted, with phenols like carvacrol and thymol playing a crucial role in its therapeutic potential. Carvacrol, a major phenolic compound, exhibits potent antimicrobial activity, while thymol complements these actions. Terpenes, such as beta-caryophyllene and thymol, contribute to the oil's anti-inflammatory and antioxidant effects, while flavonoids like apigenin and luteolin enhance its antioxidant capabilities [8].

The antimicrobial-antifungal mechanisms of oregano oil efficacy against bacteria, fungi, parasites, and viruses work through the disruption of bacterial cell membranes by carvacrol and thymol, leading to structural perturbations and subsequent demise. Carvacrol and thymol, possessing lipophilic properties, effortlessly traverse the bacterial cell membrane. Their structural similarity to fatty acids within the cell membrane enables intrusion and initiation of transformative interactions. Upon entry, carvacrol and thymol orchestrate a series of structural perturbations, unsettling the arrangement of lipids. This disorganization inflicted upon the lipid bilayer induces destabilization, making the cell membrane permeable. This heightened permeability facilitates the escape of crucial ions and molecules from the bacterial cell, leading to its demise. Notably, these phenolic compounds selectively target key proteins and enzymes, intensifying the disruption and sealing the fate of the bacterial cell [9].

Inhibition of Fungal Enzymes

Additionally, oregano oil's impact extends to fungal enzymes, where carvacrol and thymol denature enzymes critical for metabolic pathways, inhibiting fungal growth. Carvacrol and thymol engage in intricate interactions with fungal enzymes, initiating structural alterations that result in enzyme denaturation [8]. This process modifies the essential structures of enzymes involved in critical functions such as cell wall synthesis and energy production, halting fungal growth and replication [9].

Hindrance of Fungal Spores:

Furthermore, carvacrol and thymol intervene in the germination process of fungal spores, significantly impeding their development. By curtailing the germination stage, oregano oil restricts the spread of fungal infections and impedes the establishment of new colonies [9].

In summary, the multifaceted therapeutic properties of oregano oil, driven by its bioactive components, make it a promising agent with broad-spectrum antimicrobial effects against various pathogens.

Adding Turmeric to the Protocol: VDR upregulation

Moreover, the incorporation of turmeric (Curcumin longa), recognized for its robust anti-inflammatory properties and its capability to elevate VDRs, further amplifies the therapeutic approach.

Anti-inflammatory Effects of Turmeric (Curcumin Longa):

Curcuma longa, a vivid yellow spice extracted from the rhizomes of the Curcuma plant, has garnered considerable attention for its potent anti-inflammatory attributes. At the core of turmeric's therapeutic potential lies its active compound, curcumin, renowned for its versatile mechanisms that address inflammation at multiple levels, offering potential benefits for a diverse range of inflammatory conditions [10].

Inhibition of Inflammatory Pathways:

Curcumin's pivotal anti-inflammatory effects stem from its capacity to inhibit various pro-inflammatory pathways. A notable target is the nuclear factor-kappa B (NF-κB) pathway, a master regulator of inflammatory responses. Curcumin suppresses NF-κB activation, preventing the transcription of pro-inflammatory genes and the subsequent production of cytokines and chemokines [10].

Modulation of Inflammatory Mediators:

Curcumin influences the production of inflammatory mediators, including interleukins (IL-1β, IL-6, and IL-8), TNF-α, and prostaglandins (PGE2). By intervening at these crucial points, curcumin limits the amplification of inflammatory signals [10].

Antioxidant Effects:

Given the interconnected nature of inflammation and oxidative stress, curcumin acts as a potent antioxidant, extinguishing free radicals and mitigating oxidative damage. This antioxidant action contributes to restraining the propagation of inflammatory cascades [11].

Regulation of Immune Cells:

Curcumin's impact extends to immune cells, modulating their responses. It can enhance the production of anti-inflammatory cytokines, such as IL-10, countering pro-inflammatory cytokines. Furthermore, curcumin may promote a shift towards regulatory T cells (Tregs), immune cells that foster immune tolerance and suppress excessive inflammation [11].

Epigenetic Regulation: Specifically, curcumin's ability to alter deoxyribonucleic acid (DNA) methylation patterns and modify the structure of histones leads to sustained anti-inflammatory effects, even after its metabolism and clearance from the body. This persistence contributes to a reduction in chronic inflammation related to curcumin's beneficial effects on inflammation-related genes [12].

Immunologic Mechanisms of Curcumin in Upregulating Vitamin D Receptors (VDRs):

The emerging research area delves into how curcumin enhances the expression and function of Vitamin D receptors (VDRs), holding promising implications for immune regulation. VDRs, located on immune cells, play a pivotal role in modulating immune responses. Various mechanisms elucidate how curcumin contributes to the upregulation of VDRs. [12]

DNA Methylation Patterns and VDR Expression:

Curcumin's epigenetic impact on DNA methylation patterns has the potential to influence the expression of the VDR gene. DNA methylation, a chemical modification of DNA, has the ability to silence gene expression by obstructing the binding of transcription factors. Curcumin's capability to modify DNA methylation patterns may reverse the methylation status of the VDR gene promoter region, facilitating its accessibility to transcription factors. This modification could potentially lead to an increased transcription of the VDR gene, resulting in elevated levels of VDRs within cells [13].

Enhanced Transcriptional Activity of VDRs:

Functioning as a phytoestrogen, curcumin can engage with estrogen receptors in cells. These receptors are linked to the activity of VDRs. The binding of curcumin to estrogen receptors may set off a cascade of events enhancing the transcriptional activity of VDRs. This heightened activity could potentially translate to an increased expression of genes involved in immune modulation and regulation [13].

Interaction with Co-regulators and Amplified VDR Effects:

Curcumin's interactions are not confined to VDRs alone; it can also bind to co-regulators influencing VDR activity. Co-regulators are proteins that assist in fine-tuning gene expression. Curcumin's interaction with these co-regulators could enhance the activity of VDRs, amplifying their immunomodulatory effects. This collaborative interaction might trigger a series of molecular events resulting in robust immune responses mediated by VDRs [13].

Counteracting Inflammation-Induced VDR Suppression:

Inflammatory processes can downregulate VDR expression, diminishing their ability to mediate immune responses. Curcumin's potent anti-inflammatory properties can counteract this suppression. By reducing inflammation, curcumin might create a microenvironment that supports the re-expression of VDRs, restoring their availability for immune regulation [14].

GI MAP & Interpretation

The GI Map test is a powerful tool for assessing the microbiome's impact on immune function and overall health. It enables the identification and quantification of specific pathogens like Candida albicans, informing targeted interventions such as antimicrobial treatments and dietary adjustments to address recurrent fungal infections. The test measures various biomarkers related to intestinal health, offering a comprehensive understanding of the microbial landscape and facilitating personalized approaches to enhance gut health [15].

Biomarkers in GI Map Testing:

The GI Map test evaluates key biomarkers to assess gastrointestinal health. Inflammatory markers like calprotectin indicate conditions such as inflammatory bowel disease (IBD) or infections, providing valuable diagnostic insights. Immunologic markers, including secretory IgA, offer information on mucosal immune defense, with elevated or low levels indicating potential immune responses or dysfunction. Digestive and pancreatic markers like steatocrit and elastase-1 provide crucial information about fat malabsorption and pancreatic function, guiding interventions for gastrointestinal conditions. Additionally, markers like beta-glucuronidase reveal insights into bacterial activity, while occult blood detection aids in identifying potential bleeding and assessing overall gastrointestinal health [15].

Intestinal Permeability and Beyond:

The GI Map test extends its assessment to intestinal permeability through zonulin level measurement. Elevated zonulin indicates increased gut permeability, linked to conditions like leaky gut syndrome. Monitoring zonulin provides valuable insights into the integrity of the intestinal barrier, guiding targeted interventions to support gut health, reduce inflammation, and enhance overall gastrointestinal function [15]. The detection of occult blood in the digestive tract serves as a crucial indicator for various gastrointestinal conditions, including inflammatory bowel diseases and colorectal issues.

GI MAP (Microbiome Test) interpretation of the Clinical Case:

The GI Map unveiled the presence of imprtant bacterial dysbiosis with Small Intestinal Fungal Overgrowth (SIFO), caused by Candida albicans as a significant player in the pathogenesis. The detection of mycotoxins in urine and feces samples further illuminated the intricate interplay between the gut microbiome and psoriatic pathology. After introducing the use of oregano oil and turmeric into the treatment regimen significant clinical skin improvement was observed that correlated with our post secondary microbiome test findings. Degree of dysbiosis improved and SIFO caused by candida was eliminated showing undetectable levels. These finding further support the beneficial antimicrobial and anti-fungal effects of oregano oil combined with (turmeric) curcumin longa.

VDR Renewal through AID confirmed through VDR immunoflouresence:

The initial histopathological analysis of the skin biopsy revealed typical psoriasis features, including hyperplasia, parakeratosis, acanthosis, and Munro microabscesses, consistent with classical psoriatic skin lesions. Immunofluorescence examination further indicated reduced VDR expression in the affected skin, pointing to altered VDR signaling as a contributor to psoriasis pathogenesis. This finding aligns with existing literature linking VDR dysfunction to chronic inflammation and immune dysregulation.

Despite an initial therapeutic regimen involving high-dose vitamin D supplementation with cofactors and an anti-inflammatory diet, the patient did not experience the expected improvement. This noteworthy observation underscores the multifactorial nature of psoriasis, suggesting that modifying VDR signaling alone may not be adequate for achieving significant clinical amelioration.

The subsequent intervention by utilizing oregano oil and turmeric, marked a pivotal moment in the patient's condition. Oregano oil's potent antimicrobial properties against Candida albicans, combined with turmeric's recognized ability to upregulate VDRs and display anti-inflammatory effects, likely contributed to the observed clinical improvement.

The alignment of histopathological findings with VDR immunofluorescence results in both biopsies highlights the intricate relationship between VDR expression and ongoing gut dysbiosis progression. The initial reduction in VDR expression coincided with psoriatic pathogenesis, while the subsequent enhanced VDR expression paralleled clinical improvement, underscoring the potential significance of VDR renewal when gut dybiosis is treated accordingly.

Laboratory Interpretation and Therapeutic Implications:

When analyzing lab results related to vitamin D and PTH levels, it's essential to understand that PTH acts like a "thermometer" to gauge the resolution of vitamin D resistance. In our lab study, we noticed an initial rise in calcitriol levels, even though calcifediol levels remained normal which suggests ongoing active diseases process (autoimmunity)[7]. This was despite the patient taking a daily dose of 50,000 IU of cholecalciferol and following an anti-inflammatory diet. Vitamin D plays a crucial role in preventing zonulin expression, maintaining the integrity of tight junctions of GI barrier. Vitamin D also contributes to the production of antimicrobial peptides (like beta-defensin and cathelicidins), which defend against infections, and supports microbiome diversity [7].

In our specific case, the expected reduction in PTH, signaling the overcoming of vitamin D resistance per the Coimbra Protocol, didn't happen initially. This deviation could be linked to a situation of excessive overgrowth, where High-Dose Vitamin D (HDVD) and an AID alone couldn't effectively counteract the increased burden of LPSs and mycotoxins. Notably, introducing herbal antimicrobials was a turning point, leading to PTH inhibition and at times even require lowering the vitamin D dose. This positive change aligned with improvements in microbiome diversity, coinciding with clinical improvement and a clear rise in Vitamin D Receptor (VDR) expression observed through immunofluorescence. This consistent finding has been evident in some of our patients in our clinical practice that has treated well over 3500 patients so far.

Limitations:

While the interventions showcased remarkable improvements, it's crucial to acknowledge the limitations. This individual case study done with immunoflouresence represents a single patient, and generalizing findings to a broader population requires further research. Additionally, the intricacies of individual responses to interventions may vary, necessitating personalized treatment approaches. Further studies with larger sample sizes and diverse demographics are needed to validate the generalizability of the presented interventions.

Future Research Suggestions:

Looking ahead, future research endeavors could benefit from conducting larger-scale studies to validate the effects observed in the current study. Exploring the impact of herbal antimicrobials, in conjunction with HDVD and cofactors across a spectrum of autoimmune conditions would yield comprehensive insights. Additionally, further investigations into the intricate interplay between the gut microbiome, genetics, and immune responses in autoimmune disorders are imperative for advancing our understanding of these complex relationships.

Conclusions

This comprehensive approach, encapsulated in the LGS Protocol, emphasizes the importance of microbiome modulation, immune regulation, and compensating genetic polymorphisms through tailored supplementation. The study underscores the need for more tailored holistic treatments in autoimmune disorders. The integration of advanced diagnostic tools and a personalized, tailored treatment strategy emerged as a crucial aspect of addressing the multifaceted nature of these conditions. The observed significant increase in VDR expression by immunofluorescence following these targeted interventions presents a promising avenue for not only managing psoriasis but also extending its applicability to a broader spectrum of autoimmune conditions.

Additional Information

Disclosures

Human subjects: Consent for treatment and open access publication was obtained or waived by all participants in this study. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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