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The Correlation between Cytokines Imbalance and Vitamin D level in Patients with Early Chronic Obstructive Pulmonary Disease (COPD) and the role of Vitamin D in Pulmonary Disease

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A B S T R A C T

Background: Vitamin D, recognized for its dual role as an immune regulator and essential nutrient crucial for human well-being, exerts a multifaceted impact on the intricate immune processes found in individuals suffering from Chronic Obstructive Pulmonary Disease (COPD). In addition to its established functions in maintaining phosphate-calcium balance and bone mineralization, it exerts both direct and indirect effects on cellular elements within the COPD environment, thereby influencing the levels of inflammatory factors in the airways.

Objective: The study sought to ascertain the role of sunlight and vitamin D in lung disease, as well as the relationship between vitamin D levels and cytokine discord in patients in early stage chronic obstructive pulmonary disease (COPD).

Methodology: A prospective study was conducted at Ponch Medical College, Rawalakot between January 2020 to December 2020 and comprised of 120 patients. COPD pulmonary function (LF) I & COPD LF II patients were divided into two groups (n = 60 each). An immunosorbent assay that uses enzymes was used to measure the blood levels inflammatory TNF-α, IFN-γ, interleukin 4 (IL-4), and IL-6 in order to identify the Th1/Th2 marker. A proportion of IFN-γ to IL-4 was also a part of the treatment.

Results: Between the LF I alongside LF II groups, there was a significant difference ($p < 0.04$) in FEV1pred%, FEV1 or IFN-γ, IL-4, F, IFN-γ/IL-4, and IL-6. In patients with early-stage COPD, cytokines (Th1/Th2) were significant correlated with forced volume percent (FEV1pred%) ($r < 0.485$, $p < 0.002$) and compelled exhalation volume/forced lifespan (FEV1/FVC) ($r = 0.254$, $p = 0.015$).

Conclusion: Mostly cases with early-stage COPD had a vitamin D deficiency. Together with the FEV1/FVC LF parameters, it showed a positive correlation. This research offers experimental evidence in favour of vitamin D's role in COPD prevention and treatment, as well as possible anti-inflammatory action mechanisms.

Keywords: COPD; Vitamin D; Cytokines; Th1/Th2

Introduction

Chronic obstructive pulmonary disease, also known as COPD is a progressive illness characterized by irreversible airway obstruction and impaired respiratory function. It is currently the third leading cause of death worldwide.[1-3] Furthermore, it is stated that men are more likely than women to have COPD.[4,5] The world may eventually have to deal with COPD as a global health, social, and economic burden due to its increased incidence over the last ten years.[6] The majority of COPD deaths and morbidities are caused by acute exacerbations, which are marked by a worsening of symptoms and a change in the state of the individual (cough, sputum production, dyspnea, as well as airflow limitation).[7]

As an important immune regulatory factor and essential nutrient for human health, vitamin D (vit D) can impact how associated immune factors are expressed in COPD patients. Apart from controlling the balance of phosphate, which in turn controls calcium, and the mineralization of bone, 25 [OH]D acts in either a direct or indirect way on cells in COPD, affecting the quantity of factors that inflame the airways.[8] A vitamin D deficiency (VDD) was identified as a 25 (OH)D blood level less than 20 ng/mL (50 nmol/L). Research conducted recently has found a connection between VDD and COPD. According to these investigations, pulmonary function and vitamin D levels in COPD patients were positively connected. Lung function deteriorates with decreasing vitamin D levels, suggesting that vitamin D levels can, to some extent, predict the degree underlying lung function impairment.[9]

The early stages of COPD include grade I and II, based according to the Worldwide Initiative for Persistent Obstructive Pulmonary Disease. There are a remarkably large number of patients overall who have early COPD. Lung function (LF) can rapidly deteriorate in COPD cases, and those with the condition are rarely diagnosed in time for treatment. Consequently, early identification and timely management of treatment are still crucial for COPD prevention and control.[10]

In the lung, vitamin D reduces inflammation. Vitamin D has been shown in several studies to suppress inflammation by strengthening the connection between VDR and the NF-κB p65 subunit. Therefore, it is particularly intriguing to investigate if a vitamin D deficiency raised the frequency of COPD and the degree of irritation in COPD patients.[11,12]

The medical department of the present authors launched the public well-being initiative "The testing and early identification of COPD" based on the previously mentioned justifications. The results of the COPD testing were utilized to guide a correlational study that looked at the relation with vitamin D levels and the balance of Th1/Th2 cytokines in individuals with early-stage COPD. We examined the relationship between alterations in the Th1/Th2 inequalities, expression of vitamin D, and LF variables. This study provides experimental support for vitamin D's role in managing and preventing COPD and sheds light on potential anti-inflammatory mechanisms at play.

Objective

The study sought to ascertain the role of sunlight and vitamin D in lung disease, as well as the relationship between vitamin D levels and cytokine discord in patients in early stage chronic obstructive pulmonary disease (COPD).

Methodology

The present study was conducted at Ponch Medical College, Rawalakot, from January 2020 to December 2020 and encompassing a sample size of 120 patients. The inclusion criteria stipulated that participants must be over 20 years of age and diagnosed with high-risk Chronic Obstructive Pulmonary Disease (COPD). Employing a prospective assigned controlled methodology, eligible patients were randomly assigned to two distinct groups denominated as COPD LF I and COPD LF II. Notably, the randomization process was based on an ordered appearance order, ensuring a balanced allocation of patients.

It is noteworthy that all participants in both groups exhibited normal pulmonary function test results and X-ray findings, thus indicating an absence of prior COPD history and symptoms. Additionally, there were no discernible differences in terms of age, gender, body mass index (BMI), or smoking history among the three groups. Moreover, liver and kidney functions of each participant were within the normal range, and none of them were taking medications that might interfere with the metabolism or absorption of vitamin D.

All data were collected through special designed proforma and entered into SPSS for further analysis. After entering complete data, statistical analysis was carried out to discern differences among the groups. Between-group paired contrasts, as well as t-tests or rank-sum tests, were employed for this purpose. A statistically significant threshold was set at $p < 0.05$, meaning that differences were considered significant if the p-value fell below this threshold.

Furthermore, the study explored the relationships between blood concentrations of Interferon-gamma (IFN-γ), Interleukin-4 (IL-4), Interleukin-6 (IL-6), and LF variables. This was achieved through the calculation of Pearson's or Spearman's correlation coefficients. It is noteworthy that a correlation was deemed statistically significant if the calculated p-value was less than 0.05.

Results

Within the present study cases, a clear gender distribution

Table 1. Baseline information of the study cases

Variables	Frequency (120)	Percentage
Gender		
Male	84	70
Female	36	30
mean age (years)	51.1 ± 12.74	
Mena BMI (kg/m2)	24.7 ± 5.43	
Smokers		
Yes	58	48.3
No	62	51.7
Family History of COPD		
Yes	46	37.5
No	74	62.5

was observed, with the majority consisting of 84 individuals (70%) being males, while the remaining 36 individuals (30%) were females. The study population exhibited a mean age of 51.14 years with a standard deviation of 12.74, indicating a relatively diverse age range. Additionally, the participants displayed an average Body Mass Index (BMI) of 24.7 kg/m2, reflecting a moderate level of variation in body composition. Furthermore, among the study cases, 58 individuals (48.3%) were identified as smokers, highlighting a substantial proportion of individuals with a history of tobacco use. Additionally, 46 individuals (37.5%) had a family history of COPD, underscoring the relevance of genetic predisposition in the context of this study (Table 1). These demographic and clinical characteristics provide a comprehensive overview of the study population and its diverse attributes, which are essential for interpreting the study's findings and implications.

Significant differences were detected between the COPD groups LF I and LF II in several key parameters, including FEV1pred% (Forced Exhalation Volume in one second as a percentage of the expected value), FEV1/FVC (Forced Exhalation Volume in one second to Forced Vital Capacity ratio), as well as the cytokines IL-4, IFN-γ, IL-6, and the IFN-γ/IL-4 ratio, with p-values below 0.04. Notably, Th1/Th2 cytokine ratios were positively correlated with lung function measures, specifically FEV1pred% and FEV1/FVC, in patients with early-stage COPD. The correlation coefficients (r) for these relationships were 0.485 ($p < 0.002$) and 0.254 ($p = 0.015$), respectively, as shown in Table 2. These findings indicate that the interplay between Th1 and Th2 cytokines may have a meaningful impact on the pulmonary function of individuals in the early stages of COPD, shedding light on potential mechanisms and avenues for further investigation in the field of COPD research.

The study also unveiled a noteworthy positive correlation between the levels of 25(OH)D (25-hydroxyvitamin D) and FEV1pred% (Forced Exhalation Volume in one second as a percentage of the expected value), with a correlation coefficient (r) of 0.571 and a p-value less than 0.001. This finding underscores the potential influence of vitamin D status on pulmonary function, suggesting that higher 25(OH)D levels may be associated with improved lung function in individuals with early-stage COPD. Additionally, the study revealed a positive correlation between Th1/Th2 cytokines and vitamin D levels, with a correlation coefficient (r) of 0.21 and a p-value of 0.02. This correlation indicates that vitamin D levels may be linked to the balance of Th1 and Th2 immune responses in COPD patients. These correlations are visually depicted in Figure 1, providing a clear graphical representation of these relationships.

The observed positive correlation between 25(OH)D levels and FEV1pred% is of particular significance, as it suggests that maintaining adequate vitamin D status may

Table 2. Comparing the two groups' Th1/Th2 ratios, cytokines, 25(OH)D levels, and lung function-related indices

Variables	Group I	Group II	P value
FEV1/FVC	64.42 ± 25.58	63.28 ± 16.80	0.015
FEV1pred%	82.63 ± 42.75	61.21 ± 46.88	0.002
25(OH)D (ng/mL)	18.5 ± 11.22	14.6 ± 8.67	0.002
IFN-γ/IL-4	51.14 ± 12.74	51.14 ± 12.74	0.004
IL-6 (pg/mL)	1.14 ± 3.42	20.14 ± 10.17	0.004
IL-4 (pg/mL)	1.13 ± 4.11	1.98 ± 1.25	0.004
IFN-γ (pg/mL)	1.46 ± 1.72	1.91 ± 3.29	0.004
TNF-α(pg/ml)	1.30 ± 16.71	1.83 ± 5.31	0.004

play a role in preserving lung function among individuals with early-stage COPD. Vitamin D has known immunomodulatory properties and can influence inflammatory pathways, which could impact the progression of COPD. The positive correlation between Th1/Th2 cytokines and vitamin D levels hints at the potential immunomodulatory effects of vitamin D in the context of COPD. However, further research is needed to elucidate the mechanistic aspects of these associations and their clinical implications. These findings contribute valuable insights into the complex interplay between vitamin D, immune responses, and lung function in COPD patients, highlighting avenues for future research and potential therapeutic interventions.

Discussion

In the context of Chronic Obstructive Pulmonary Disease (COPD), several intricate pathogenic factors and mechanisms come into play, ultimately contributing to the progression of this chronic lung condition. Epithelial apoptosis, oxidative stress, imbalances in protease/antiprotease activities, and chronic inflammatory responses stand as prominent pillars in the understanding of COPD pathogenesis. These factors collectively lead to the progressive deterioration of lung function observed in individuals afflicted with COPD. Moreover, systemic and pulmonary inflammations represent additional significant pathogenic causes, further exacerbating the disease's severity.

One of the pivotal factors contributing to COPD's onset and progression is the disruption of the immune system, particularly the role played by T cells. Research has established a strong correlation between the development and course of COPD and immune dysregulation driven by T cells.[13,14] Notably, there is evidence suggesting that patients with initial COPD exhibit elevated levels of Th-1 cells and Interferon-gamma (IFN-γ) when compared to healthy individuals. IFN-γ, apart from inhibiting Th2 cell function, also activates neutrophils and stimulates macrophages, thus intensifying airway inflammation in COPD. Furthermore, IFN-γ induces the production of interferon-inducible protein 10 and monocyte-associated factors, aggravating inflammation and contributing to tissue damage, particularly through the production of matrix metalloproteinase-12 (MMP-12) by lung macrophages.[15]

Imbalances in multiple immune modulations play a pivotal role in the onset and progression of chronic lung conditions, including COPD. Notably, within the COPD LF II group, studies have shown higher levels of key immune mediators such as Tumor Necrosis Factor-alpha (TNF-α), Interferon-gamma (IFN-γ), Interleukin-4 (IL-4), and Interleukin-6 (IL-6) compared to the COPD LF I group. Importantly, the data reveal a lower IFN-γ/IL-4 ratio, signifying an imbalance in immune responses that may contribute to the disease's severity. These immune imbalances further underscore the intricate nature of COPD pathogenesis and its association with immune dysregulation.[15,16]

A restriction in airflow is a defining feature of the illness known as chronic pulmonary obstructive condition (COPD). According to the current study's findings, FEV1/FVC and Th1/Th2 cytokines may positively correlate in the early stages of COPD. An explanation for this observation could be that the discharge of lung inflammatory mediators from COPD-related inflammation sites results in alterations to the cells lining the airways, a

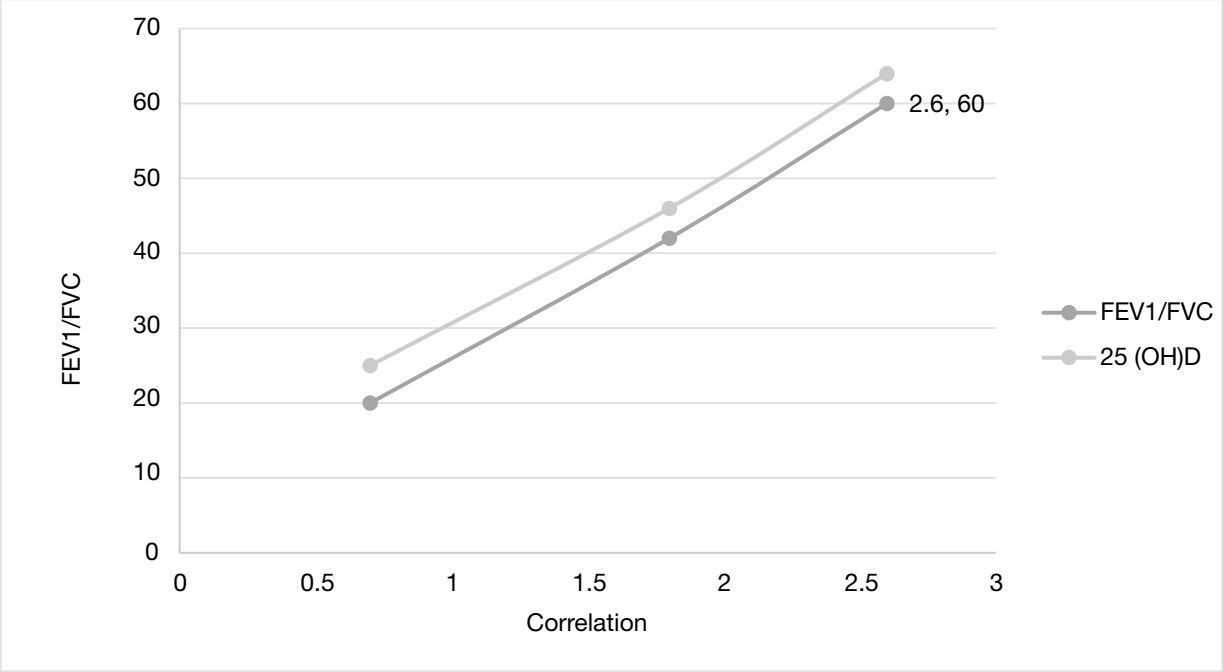

Figure 1. In early COPD, there is a significant relation between Th1/Th2 cytokines and FEV1/FVC.

rise in the settling of airway collagen fibres, rebuilding of the airways, and a decline in lung function.[17] According to certain research, IFN-γ may stimulate cytotoxic T-cells to release porin and granzyme B, which is linked to bronchial epithelial cell apoptosis. This causes the extracellular matrix to degrade and rebuild, breaking down pulmonary fundamental cells and obstructing small airways. Airway fibrosis has been associated with IL-4 because of its ability to stimulate eosinophil transforming growth factor beta secretion.[18]

The main outcome of the study indicates that individuals in both the LF I and LF II groups exhibited lower levels of 25-hydroxyvitamin D (25(OH)D) compared to the healthy control group. This observation underscores the prevalence of Vitamin D deficiency (VDD) in the early stages of Chronic Obstructive Pulmonary Disease (COPD). The study's findings resonate with previous research that has reported a frequent occurrence of VDD in COPD patients, particularly in the initial phases of the disease. However, the impact of vitamin D levels on the development and progression of COPD remains uncertain and warrants further investigation.

Several studies utilizing observational data have shed light on the potential role of vitamin D in regulating and bolstering the immune system's defense mechanisms. However, despite these insights, definitive conclusions regarding the intricate interactions between vitamin D, specific clinical inflammatory markers, and pulmonary ventilation performance remain elusive. It is important to acknowledge that while observational studies can reveal associations, they cannot establish causation.

In the context of the current investigation, a noteworthy correlation was identified between vitamin D levels and the Th1/Th2 ratio in early COPD ($r = 0.27$, $p = 0.02$). This suggests a potential link between vitamin D status and the balance of T-helper cell subsets involved in immune responses. Additionally, a significant association was established between forced expiratory volume in one second as a percentage of predicted (FEV1pred%) and 25(OH)D levels in early COPD ($r = 0.695$, $p < 0.002$). This finding corroborates the favorable relationship previously observed between Th1/Th2 ratios and lung function (FEV1pred%) in the early stages of COPD.

Comparing these findings with other studies in the literature, it becomes evident that the relationship between vitamin D and COPD is a topic of ongoing research. While some studies have reported associations between low vitamin D levels and COPD severity, others have yielded mixed or inconclusive results. Variability in study designs, populations, and methodologies may account for these discrepancies. Therefore, further well-designed studies, including randomized controlled trials, are necessary to elucidate the potential therapeutic implications of vitamin D supplementation in COPD management and to clarify its role in disease progression. The multifaceted nature of COPD and its interplay with vitamin D status underscores the complexity of this area of research.[17]

The primary outcome of the present research highlights a consistent finding that 25-hydroxyvitamin D (25(OH)D)

levels were significantly lower in both the LF I and LF II groups when compared to the healthy control group. This observation underscores the prevalence of Vitamin D deficiency (VDD) in the early stages of Chronic Obstructive Pulmonary Disease (COPD), with nearly all participants in the early-stage COPD group exhibiting VDD. This finding aligns with numerous prior studies that have also reported a negative correlation between VDD and the severity of COPD.

It is well-documented that vitamin D plays a multifaceted role in COPD pathogenesis. Research has consistently shown that low vitamin D levels are associated with worse lung function and more severe COPD symptoms. This correlation is particularly significant in the context of airway remodeling, immunological regulation, and anti-inflammatory effects attributed to vitamin D. These mechanisms suggest that vitamin D may have a protective role in COPD by modulating the immune response, reducing inflammation, and preventing lung tissue damage.[18]

Furthermore, research has delved into the molecular aspects of vitamin D's impact on COPD. One study, for instance, has highlighted that 1,25-dihydroxyvitamin D3, an active form of vitamin D, controls inflammation in macrophages or airway epithelial cells through various signal transduction pathways. By doing so, it can potentially prevent lung tissue damage and slow down the progression of COPD.

Comparing these findings with other studies in the literature, it becomes evident that there is a consistent trend of associating lower vitamin D levels with more severe COPD outcomes. This aligns with the broader body of research indicating the potential benefits of vitamin D supplementation in COPD management. However, it's important to note that while these associations are compelling, causation has not been definitively established, and the specific mechanisms through which vitamin D affects COPD pathogenesis require further exploration.[19,20]

Variability in study populations, methodologies, and geographic locations may account for some differences in study outcomes. Nevertheless, the accumulating evidence underscores the importance of vitamin D in COPD and the potential for targeted interventions to improve outcomes in individuals with this chronic lung condition. Further research, including clinical trials, is warranted to elucidate the precise role of vitamin D in COPD and to guide clinical practice effectively.

Conclusion

In conclusion, the findings from the research highlight a significant prevalence of vitamin D deficiency (VDD) in patients with early-stage Chronic Obstructive Pulmonary Disease (COPD). Moreover, the study reveals a positive correlation between vitamin D levels and the FEV1/FVC lung function parameters in these individuals. These results offer valuable experimental evidence supporting the potential role of vitamin D in both the prevention and treatment of COPD. Furthermore, the study hints at possible anti-inflammatory mechanisms associated with vitamin D. The association between VDD and early-stage COPD emphasizes the need for further investigation into the precise mechanisms through which vitamin D influences the disease. It also suggests that assessing and addressing vitamin D levels in COPD patients, particularly those in the early stages, may have clinical significance in managing the condition and potentially slowing its progression. While the study underscores the potential benefits of vitamin D, it is important to recognize that further research, including randomized controlled trials, is required to establish causation and determine the most effective strategies for incorporating vitamin D supplementation into COPD management. Nonetheless, these findings provide a promising avenue for future research and clinical practice, offering hope for improved outcomes and enhanced understanding of the role of vitamin D in COPD.

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