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RESEARCH ARTICLE

Interplay of interleukins (IL6, IL10) and 25 hydroxycholecalciferol in asthmatic subjects with chronic post-COVID condition (PCC)

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ABSTRACT

The study aimed to compare and correlate serum levels of IL-6, 10, and 25-hydroxycholecalciferol in individuals with asthma with and without post-COVID condition (PCC). The study was designed to investigate the inflammatory response and serum 25-hydroxycholecalciferol status in asthmatics with and without PCC. A cross-sectional study of 252 subjects (128 asthmatics and 124 non-asthmatic subjects) was carried out. Interleukins and 25-hydroxycholecalciferol levels were estimated on ELISA. The principle findings were that IL-6 and 25-hydroxycholecalciferol levels were significantly increased ($p<0.001$), while IL-10 levels were non-significant in asthmatics with PCC compared to those without PCC. However, 25-hydroxycholecalciferol levels were significantly increased, but no significant change was observed in IL-6, and IL-10 levels in non-asthmatics with and without chronic PCC. A significant positive correlation ($r = 0.258$) was found between 25-hydroxycholecalciferol and IL-6 but a significant negative correlation ($r = -0.227$) with IL-10 in asthmatics with PCC. Similarly, a significant negative correlation ($r = -0.285$) was found between 25-hydroxycholecalciferol and IL-10 but was non-significant with IL-6 in asthmatics without PCC. The correlation of 25-hydroxycholecalciferol with IL-10 was significant (0.683), but IL-6 was non-significant in non-asthmatics with PCC. Multiple regression analysis showed that age, IL-6, gender, and PCC were significantly related in adjusted values to 25-hydroxycholecalciferol. This study sheds light on the complex liaison between 25-hydroxycholecalciferol levels and inflammatory responses in asthmatics, especially those with PCC. The findings suggest that although asthmatics with PCC maintain sufficient levels of 25-hydroxycholecalciferol, they show a substantial increase in the pro-inflammatory response. This suggests that PCC exacerbates the pro-inflammatory response in asthma. Moreover, the study reveals that asthmatics, whether with or without PCC, display a negative correlation between 25-hydroxycholecalciferol and the anti-inflammatory response. This emphasizes the main influence of asthma on the overall inflammatory response. These findings reveal a complex interplay between vitamin D levels and inflammatory mediators in asthmatic individuals with and without PCC.

Keywords: Asthma; post-COVID condition (PCC); interleukin 6; interleukin 10; 25-hydroxycholecalciferol.

INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, has resulted in mortality and morbidity across the world since it was identified in December 2019 as a pandemic. It involved multiple organ systems and those who recovered from the illness reported various symptoms that persist even after the PCR report became negative. "Post-COVID condition (PCC) is the persistence of symptoms beyond 4 weeks after the diagnosis of acute COVID-19 infection". It is classified as sub-acute if there is persistence of symptoms up to 12 weeks after an acute attack, and chronic refers to persistence of symptoms beyond 12 weeks (Nalbandian *et al*., 2021) [Figure 1]. The most common symptoms reported by the people were headaches, fluctuations in blood pressure, weakness, fatigue, bone pains, and breathlessness (Dryden *et al*., 2022; Pazukhina *et al*., 2022).

Asthma has been a global concern, impacting 300 million people across the world, and it is anticipated to see a further rise of 100 million cases by the year 2025 (Moradi-Lakeh *et al*., 2015; Maciag & Phipatanakul, 2020). The prevalence of asthma is 0.4% to 11.9% in Asia, as reported by Song *et al*. (2014). The prevalence of asthma in Pakistan is 4.3%, out of which 11.3% is in the urban population (Gupta *et al*., 2015; Razzaq *et al*., 2018). Asthma is an allergic disease of complex gene-environment interactions. Two studies show that over 70% of the variation in asthmatic tendency is explained by genetic factors, and several contributing genes have been identified (Sacco & Milner, 2019; Morales & Duffy, 2019). However, individual genes have been ineffective in altering the expression of asthma, indicating the necessity of environmental factors.

Recent studies demonstrate the role of cytokines and tumour necrosis factor-α in asthmatics as well as in those who suffered from COVID-19 infection. The study by Queiroz *et al*. (2022) demonstrates increased serum IL-6 levels in broncho-alveolar lavage of acute COVID-19 subjects. However, post-COVID-19 subjects also reported higher serum levels of IL-6 along with other cytokines (Masiá *et al*., 2022; Schultheiß *et al*., 2022; Plocque *et al*., 2023). Tumour necrosis factor-α, which is expressed in mast cells, has been studied as a pro-

inflammatory cytokine whose concentration is shown to increase in the bronchial fluids of asthmatic subjects (Porsbjerg *et al*., 2022). Low levels of IL-10 were found in asthmatic subjects compared with controls (Maffeis *et al*., 2022). IL-6 is produced from lung epithelial cells in response to stimuli like allergens, UV radiation, microbial products, and respiratory viruses that produce cell stress. It is an important regulator of effector CD-T cell fate (Rayavara *et al*., 2022; Albano *et al*., 2022). These studies strengthen the role of IL-6 as pro-inflammatory and IL-10 as anti-inflammatory markers.

The COVID-19 pandemic started in Pakistan in February 2020, and frequent waves were observed. (Jawed, 2020). The subjects with co-morbidities, especially previous lung involvement with COPD, asthma, or emphysema, showed severe mortality and morbidity (Bektas *et al*., 2020). People who recovered from the illness still had varied symptoms for a long time, which include loss of sense of smell and taste, breathlessness on climbing stairs or on slight exertion, as well as variations in blood pressure for a long time. Some also died of myocarditis after a complete recovery from COVID-19 (Bektas *et al*., 2020). Those who suffered from COVID-19 and/or asthma were prescribed 25-hydroxycholecalciferol as it was thought to help in speedy recovery (Zaazouee *et al*., 2023).

People in developing countries like Pakistan live in different environments and are exposed to different climatic conditions and pollutants with unique socioeconomic impacts and dietary habits. Moreover, the genetic makeup of the people living in this part of the world may be different from the other continents, as shown by the response of people in Pakistan to the COVID-19 pandemic (Jaleel, 2020). There is a growing concern about 25-hydroxycholecalciferol deficiency among the general population. General practitioners prescribe 25-hydroxycholecalciferol to asthma patients without conducting a laboratory test, believing it can help prevent asthma exacerbations. Moreover, the onset of COVID-19 and access to information through the internet lead to self-medication of 25-hydroxycholecalciferol, especially in people who suffered from COVID-19. A study conducted at Agha Khan University in Pakistan reveals that patients were commonly prescribed vitamin D supplements for diverse reasons, and there was a notable rise in self-medication as well (Muneer *et al*., 2022).

The study was designed to correlate serum cytokine levels, specifically IL-6 and IL-10, with 25-hydroxycholecalciferol in individuals with asthma who had previously recovered from COVID-19 at least three months ago. These individuals had negative PCR reports for the SARS-CoV-2 virus but were still exhibiting symptoms.

The objectives of the study were to measure serum levels of IL-6, IL-10, and 25-hydroxycholecalciferol in asthmatic subjects and non-asthmatic controls with and without chronic PCC. To compare and correlate serum IL-6, IL-10, and 25-hydroxycholecalciferol levels in asthmatic subjects and non-asthmatic controls with and without chronic PCC.

MATERIAL AND METHODS

A cross-sectional study of 252 subjects, including 128 asthmatics and 124 non-asthmatics, was carried out. The study was approved by the Institutional Review Board of Shalamar Medical and Dental College (SMDC), and informed consent was signed by the participants. The study was carried out from June 2022 to January 2023 at the pulmonology and biochemistry departments of Shalamar Medical and Dental College.

Included were (i) subjects with a diagnosis of asthma for 6 months based on GINA guidelines. (ii) Subjects with chronic PCC with symptoms of breathlessness, weakness, loss of taste and smell, fluctuation in blood pressure, or bone pain (PCR report negative for SARS-CoV-2 virus at least three months ago). Chronic PCC refers to the persistence of symptoms beyond 12 weeks after the diagnosis of an acute COVID-19 infection. (iii) Subjects of both genders with an age between 18 and 75 years visiting the pulmonology department at SMDC (iv) Asthmatics with presentation of cough, tightness in chest, wheezing, early morning, or night exacerbations; (v) Volunteers who were non-asthmatic with no other comorbidity. These also included subjects with chronic PCC (PCR reports were negative for SARS-CoV-2). These individuals were previously diagnosed with COVID-19 based on a positive PCR report, and currently, their last PCR report has been negative for SARS-CoV-2 virus for at least three months.

Exclusion criteria are (i) asthmatics with allergic conditions or lung and cardiac diseases other than asthma; (ii) those on medications affecting asthma other than the asthma treatment regimen; (iii) smokers and alcoholics, as active smoking interacts to cause severe symptoms, a decline in lung functions, and an impaired response to corticosteroids and additives in alcohol drinks that cause bronchoconstriction.

Study Protocol

Group A consists of 128 asthmatic subjects already diagnosed by the pulmonologist and on a standard regimen of asthma management (oral corticosteroids + β-agonists according to GINA guidelines for asthma management) with 600 to 800 IU oral vitamin D supplements advised for three months. Group B consists of 124 non-asthmatic volunteers with no comorbidities. They were recruited after obtaining informed consent.

Venous blood of 3 ml was extracted from subjects in both groups by aseptic technique by a phlebotomist. It was centrifuged at 3000 rpm for 20 minutes. The separated serum was placed in three separate tubes and stored at -80°C. The levels were estimated by ELISA. Serum concentrations of 25-hydroxycholecalciferol were assessed by ELISA (CALBIOTECH catalogueueue No. VD220B 25-hydroxycholecalciferol (96 tests); Human Interleukin-10 by ELISA (BIoVendor Research and Diagnostic Products catalogueueue No. RD194572200R); and Interleukin-6 by ELISA (BioVendor Research and Diagnostic Products catalogueueue No. RD194015200R) Multiple Analyte Profiling) assay. It has a sensitivity of 91.7% and a specificity of 95.7%.

Statistical Analysis

All statistical analysis was performed using SPSS-23. The data were expressed as mean ± standard deviation. Comparisons between two groups were performed using an independent student's t-test. Correlations were found using Pearson's r coefficient. Non-parametric alternatives were employed for non-normal data. Regression analysis was used to analyse associations between 25-hydroxycholecalciferol levels and IL-6 and IL-10 levels and several other variables.

RESULTS

Out of a total of 252, 128 subjects with asthma (mean age 45.70 ± 16.81 years) and 124 non-asthmatics (mean age 38.21 ± 10.10) were included in the study. Males were 58.3%, while females were 41.66%. Chronic post-COVID condition (PCC) was present in 60.93% of asthmatics and 33.87% of non-asthmatics. Asthmatic subjects had persistent asthma and were receiving oral corticosteroids and β-agonists according to GINA guidelines for asthma management. 25-hydroxycholecalciferol level was deficient (<15ng/ml) in 2 (1.56%), sufficient (>30ng/ml) in 120 (93.75%), toxic levels (>100 ng/ml) in 6 (4.68%) asthmatic subjects, and deficient in 2 (1.61%) and sufficient in 122 (98.38%) non-asthmatic subjects. Serum IL-6 and IL-10 levels were non-significant, while serum 25-hydroxycholecalciferol levels were significantly increased in asthmatics compared to non-asthmatic subjects (Table 1).

Table 2 shows that serum IL-6 and 25-hydroxycholecalciferol levels were significantly increased ($p<0.001$) in asthmatic subjects with chronic PCC compared to asthmatics without chronic PCC. However, 25-hydroxycholecalciferol levels were significantly

Table 1. Demographics and biochemical parameters of asthmatics and healthy controls

Study variables	Total (252)	Asthmatic subjects (N=128)	Non-asthmatic subjects (N=124)	p-value
Mean age (years)	42.02±14.41	45.70±16.81	38.21±10.10	0.000*
Gender				
Male	147 (58.33%)	60 (46.87%)	87(70.16%)	0.000*
Female	105 (41.66%)	68 (53.12%)	37 (29.83%)	
PCC				
Present	120 (47.61%)	78(60.93%)	42(33.87%)	0.000*
Absent	132 (52.38%)	50(39.06%)	82(66.12%)	
25-hydroxycholecalciferol				
Deficient	4(1.58%)	2(1.56%)	2(1.61%)	0.019*
Sufficient	242 (96.03%)	120(93.75%)	122(98.38%)	
Toxic	6 (2.38%)	6 (4.68%)	Nil	
Serum IL-6 (pg/ml)	6.61±4.66	7.12±5.36	6.09±3.74	0.079
Serum IL-10 (pg/ml)	12.34±5.03	11.94±4.65	12.76±5.37	0.197
Serum 25-hydroxycholecalciferol (ng/ml)	59.78±9.73	62.78±10.08	56.68±8.31	<0.001*
Total	**252 (100.0%)**	**128 (50.79%)**	**124 (49.20%)**	_

p-value ≤0.05 is considered significant and Independent-t test was applied for significance.

Table 2. Comparison of biomarkers between asthmatic and non-asthmatic subjects with and without PCC

Biochemical parameters	Asthmatic subjects N=(128)			Non-asthmatic subjects N=(124)		
	Chronic PCC present (PCC) n=(78)	Chronic PCC absent n=(50)	p-value	Chronic PCC present n=(42)	Chronic PCC absent n=(82)	p-value
Interleukin-6 (IL-6) (pg/ml)	8.55±5.89	4.88±3.43	<0.001*	6.99±4.78	5.66±3.09	0.053
Interleukin-10 (IL-10) (pg/ml)	11.84±5.04	12.10±4.03	0.752	12.23±5.40	13.18±5.43	0.438
25-hydroxycholecalciferol (ng/ml)	66.53±8.70	56.94±9.35	<0.001*	61.68±6.66	54.12±7.93	0.000

*p-value ≤ 0.001: Highly Significant compared to the groups.

Table 3. Correlation of 25-hydroxycholecalciferol with Interleukin-6 and 10 in asthmatic and non-asthmatic subjects with and without PCC

Groups		Asthmatics		Non-asthmatics	
		Interleukin-6 (IL-6) pg/ml	Interleukin-10 (IL-10) pg/ml	Interleukin-6 (IL-6) pg/ml	Interleukin-10 (IL-10) pg/ml
PCC present	25-hydroxycholecalciferol	0.258*	-0.227*	0.220	0.683*
	Interleukin-6	—	-0.219	—	0.154
PCC absent	25-hydroxycholecalciferol	0.042	-0.285*	0.110	-0.173
	Interleukin-6	—	-0.010	—	-0.031

Pearson's correlation was applied to determine r value.

increased, but no significant change was observed in IL-6 and IL-10 levels in non-asthmatic subjects with and without chronic PCC (Table 2).

Table 3 shows significant positive correlation (r = 0.258) between 25-hydroxycholecalciferol and IL-6, but a significant negative correlation (r = -0.227) of 25-hydroxycholecalciferol with IL-10 in asthmatic subjects with PCC .

Similarly, a significant negative correlation (r = -0.285) was found between 25-hydroxycholecalciferol and IL-10 in asthmatic subjects without PCC. However, the correlation of 25-hydroxycholecalciferol with IL-6 and between IL-6 and IL-10 was non-significant in this group.

The correlation of 25-hydroxycholecalciferol with IL-10 was significant (0.683) in non-asthmatic subjects with PCC but was non-

significant with IL-6. The correlation between IL-6 and IL-10 was also non-significant in this group. There was no significant correlation found between any parameters in non-asthmatics without PCC.

Table 4 showed that age, IL-6, gender, and PCC were significantly related in adjusted values to 25-hydroxycholecalciferol.

Figure 1 shows the definition, signs, and symptoms of the COVID-19 transient phase and PCC sub-acute and chronic conditions.

DISCUSSION

This cross-sectional observational study was designed to assess the pro-inflammatory and anti-inflammatory cytokine status of asthma subjects with and without chronic PCC. The asthmatic subjects with PCC exhibited significantly higher levels of IL-6 and 25-hydroxycholecalciferol. Similarly, non-asthmatic subjects with PCC also showed significantly higher levels of 25-hydroxycholecalciferol. Serum levels of IL-10 did not show any significant difference between asthmatic subjects with and without chronic PCC. In asthmatic subjects with PCC, there was a significant positive correlation between 25-hydroxycholecalciferol and IL-6 and a significant negative correlation between 25-hydroxycholecalciferol and IL-10. Non-asthmatic subjects with PCC, on the other hand, exhibited a significant positive correlation between 25-hydroxycholecalciferol and IL-10. Asthmatic subjects without PCC demonstrated a significant negative correlation with IL-10. However, IL-6 shows a non-significant correlation with 25-hydroxycholecalciferol (r = 0.042) in asthmatic subjects without PCC.

The research has shown that TNF-α and interleukins (IL) like IL-1β and IL-6 are classical pro-inflammatory cytokines made by various inflammatory cells and by prime lung epithelial cells in response to a diverse set of stimuli such as allergens, airway viruses, and physical activity (Jaén *et al*., 2022). The increased levels of IL-6 in plasma, bronchoalveolar lavage fluid, and sputum of asthmatic subjects have been documented (Mettelman *et al*.,

Table 4. Final model explaining variation in 25-hydroxycholecalciferol due to study variables

Coefficients	Crude Value					Adjusted Value				
	B	Standard error	Significance	95% confidence interval for B		B	Standard error	Significance	95% confidence interval for B	
				Lower Bound	Upper Bound				Lower Bound	Upper Bound
Age	0.244	0.040	0.000	0.165	0.322	0.110	0.037	0.004*	0.037	0.184
IL 6	0.613	0.126	0.000	0.364	0.862	0.353	0.109	0.001*	0.138	0.569
IL 10	-0.227	0.122	0.063	-0.466	0.12	-0.161	0.097	0.097	-0.352	0.029
Gender	5.622	1.195	0.000	3.269	7.974	5.588	1.013	0.000*	3.59	7.584
Asthma	6.104	1.167	0.000	3.806	8.402	1.623	1.051	0.124	-0.448	3.694
Covid-19	9.646	1.068	0.000	7.542	11.750	6.858	1.101	0.000*	4.689	9.026

Univariate Linear regression was applied.

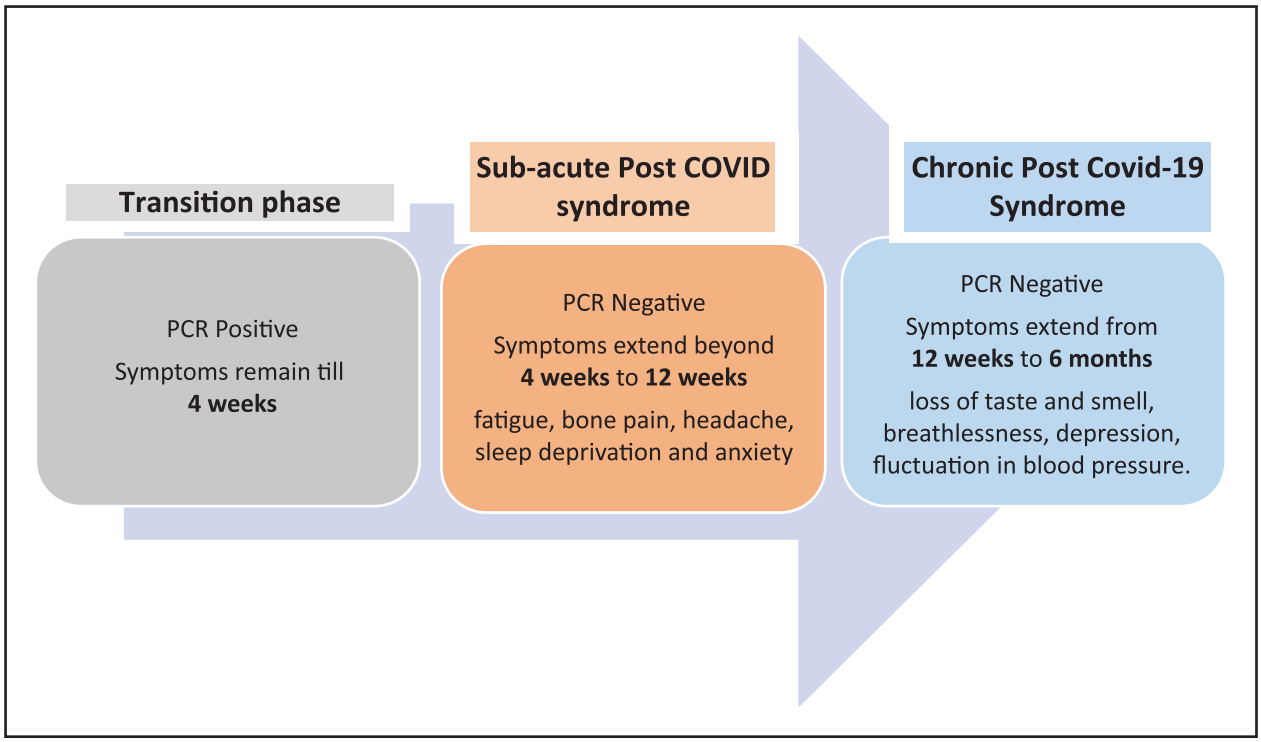

Figure 1. Subacute and chronic post COVID-19 syndrome.

2022). IL-6 exerts its pro-inflammatory effect in asthma by promoting the differentiation of Th2 and Th17 cells (Li *et al*., 2022). It has a suppressive effect on Treg cells that may lead to an upsurge of the immune response and thus potentiate the inflammation detected in the lungs of asthmatic subjects (Jaén *et al*., 2022). It is also found in increased amounts in the serum, exhaled breath, and sputum of COPD subjects, predominantly during disease severity (Li *et al*., 2022). Raised IL-6 levels were also observed in our study in asthmatic subjects with PCC.

High IL-6 and other pro-inflammatory cytokines have been demonstrated in moderate to severe cases of active COVID-19, while the level of anti-inflammatory cytokines was low, correlating IL-6 to the severity of COVID-19. However, no significant difference was reported between controls and recovered COVID-19 subjects (Han *et al*., 2020; Pandolfi *et al*., 2020). Following SARS-CoV-2 infection, the virus is recognised by the TLRs (toll-like receptors), which trigger the production of pro-inflammatory cytokines such as IL-6 and TNF-α, which have been associated with hyper-inflammatory status (Costela-Ruiz *et al.*, 2020; Bae *et al*., 2022). High IL-6 concentrations were detected in the bronchoalveolar lavage fluid of the COVID-19 subjects (Proal & VanElzakker, 2021). In a Brazilian cohort study conducted in 2022, advanced age, comorbidities, and elevated serum IL-6 levels were associated with severe acute COVID-19. However, post-COVID-19 subjects who had recovered from COVID-19 without PCC reported higher levels of IL-6 along with other cytokines (Queiroz *et al*., 2022). A recent post-COVID-19 digital epidemiology study, including biomarker analysis, reports PCC continuing for up to two years in more than half of subjects after mild COVID-19. Serum cytokine analysis in these cases revealed raised IL-1β, IL-6, and TNF levels possibly released from hyperactive monocytes and/or macrophages (Schulthei *et al*., 2022). This may be due to ongoing immune responses against persisting viral antigens, the virus itself, or chronic immune cell responses (Salmanpour *et al*., 2022). Our analysis aligns with the findings of these studies, as we also observed elevated serum expression of IL-6 in asthmatic subjects with PCC.

However, in the present study, the levels of 25-hydroxycholecalciferol were also significantly higher in asthmatic subjects with PCC compared to asthmatics without PCC. Moreover, a significant positive correlation of 25-hydroxycholecalciferol with IL-6 and a significant negative correlation with IL-10 was observed in asthmatic patients with PCC. A significant negative correlation of 25-hydroxycholecalciferol with IL-10 was also observed in asthmatic patients without PCC. The subjects were taking oral corticosteroids and β-agonists according to GINA guidelines for asthma management. In non-asthmatic subjects with PCC, a significant positive correlation of 25-hydroxycholecalciferol with IL-10 was observed.

The role of 25-hydroxycholecalciferol in both asthma and COVID-19 is still not clear. Some studies have suggested a potential connection between asthma and 25-hydroxycholecalciferol. 25-hydroxycholecalciferol has a regulatory effect on body innate and adaptive immunity, which may partially explain its association with inflammatory epithelial changes observed in asthma. Bae *et al*., 2022). Low serum 25-hydroxycholecalciferol levels have been linked to poor asthma control, and supplementation with this compound has been shown to improve asthma control in individuals with deficiency (Nitzan *et al*., 2022; Wang *et al*., 2022). Low levels in children have been associated with increased asthma frequency, along with deteriorating lung functions and airway hyperresponsiveness (Salmanpour *et al*., 2022; Mullin & Milne, 2022). Clinical trials among asthmatic subjects have shown the shielding effect of 25-hydroxycholecalciferol supplementation (Luo *et al*., 2015; Alkhatatbeh *et al*., 2021; Mullin & Milne, 2022; Nitzan *et al*., 2022). Additionally, the protective effect of 25-hydroxycholecalciferol supplementation during pregnancy against asthma has been observed in both children and adults (Chu *et al*., 2022). A study reveals an association between COVID-19 inflammation and suboptimal levels of serum vitamin D (Bae *et al*., 2022). Patients with COVID-19 with low serum vitamin D levels revealed an increased inflammatory response, including IL-6 levels, thereby suggesting a role of vitamin D in inflammation prevention (Jain *et al*., 2020).

Lung epithelial cells express 1α-hydroxylase, which converts 25-hydroxycholecalciferol to its active form that interacts with immune cells via vitamin D receptors (VDR) and controls the expression of various inflammatory and immunomodulatory genes. Vitamin D suppresses pro-inflammatory gene expression and promotes the Treg-dependent secretion of anti-inflammatory cytokines by directly acting on CD4+ T cells. Moreover, it enhances glucocorticoid receptiveness in asthmatic subjects, controlling its severity (Luo *et al*., 2015). A meta-analysis revealed that polymorphisms in the VDR gene were associated with a predisposition to some enveloped viral infections (Laplana *et al*., 2018).

However, despite adequate 25-hydroxycholecalciferol levels, unlike the above studies, we observed a positive correlation of IL-6 with 25-hydroxycholecalciferol in asthmatics with PCC and a negative correlation of IL-10 with 25-hydroxycholecalciferol in asthmatics with and without PCC. A set of studies proposed that 25-hydroxycholecalciferol supplements are responsible for asthma persistence. A meta-analysis by Luo *et al.* (2015), which included seven randomised controlled trials from different countries, investigated the effect of 25-hydroxycholecalciferol supplements along with asthma treatment but found no improvement in asthma exacerbation, lung functions, or disease symptoms despite adequate 25-hydroxycholecalciferol levels (Luo *et al*., 2015; Salmanpour *et al*., 2022). A randomised placebo control trial by Berlanga-Taylor *et al*. (2018) reported no effect of a year-long supplementation of 25-hydroxycholecalciferol on the serum levels of cytokines, including IL-6 and IL-10. A recently conducted randomised clinical trial using a single high dose of 25-hydroxycholecalciferol did not show any improvement in the cytokine and chemokine status in hospitalised moderate to severe COVID-19 subjects (Fernandes *et al*., 2022). Yegorov *et al*. (2019) reported increased IL-6 levels in 25-hydroxycholecalciferol-supplemented Mongolian children. IL-6 up-regulation has also been reported in the peripheral blood mononuclear cells of 25-hydroxycholecalciferol-supplemented multiple sclerosis patients (Gargari *et al*., 2015).

The IL-10, on the other hand, is an effective anti-inflammatory cytokine that prevents the expression of several inflammatory genes, including cytokines and chemokines that are up-regulated in asthma and COPD (Gargari *et al*., 2015). A reduction in the expression of IL-10 has been reported at transcription and translation levels in macrophages in asthmatic and COPD subjects, and it may be a physiological feedback effect (Borish *et al*., 1996; Albano *et al*., 2022). The decreased levels of anti-inflammatory cytokines in our study may be associated with the unimpeded secretion of pro-inflammatory IL-6 in asthmatic subjects with PCC. Elevated circulatory IL-6 levels were associated with subjects who had recovered from COVID-19, while low IL-10 and increased IL-6 levels were observed in PCC cases (Queiroz *et al*., 2022; Schulthei *et al*., 2022).

The outcomes of these studies support our results that, despite adequate levels of 25-hydroxycholecalciferol in asthmatic subjects with PCC, IL-6 levels were significantly raised and showed a positive correlation with 25-hydroxycholecalciferol, while IL-10 showed a significant negative correlation with 25-hydroxycholecalciferol in asthmatic subjects with and without PCC (Bader *et al*., 2023).

The seemingly contradictory findings in different studies regarding IL-6, IL-10, and 25-hydroxycholecalciferol could be attributed to various confounders and differences among the studies, such as the duration and dosage of 25-hydroxycholecalciferol supplementation, the participants' age, sex, smoking history, BMI,

other environmental factors, genetic background, comorbidities, effects of clinical therapy, vaccination status of the patient, and extent of 25-hydroxycholecalciferol insufficiency. The studies have demonstrated that the severity of COVID-19 was more common in males with increasing age, leading to high mortality rates, while females had mild to moderate symptoms with a higher recovery record. In the present study, a higher percentage of females were suffering from asthma and chronic PCC.

Decreased literacy rates, over-the-counter availability of drugs, and avoidance of hospital visits are common factors associated with self-medication, especially analgesics and anti-inflammatory drugs, in our part of the world. Moreover, poor environmental conditions and a high air pollution index leading to the exacerbation of asthma and other allergic conditions further increase the misuse of anti-inflammatory drugs like aspirin. The asthmatic subjects in our study were on glucocorticoids. Glucocorticoids exert their anti-inflammatory effect by inhibiting pro-inflammatory cytokines and enhancing IL-10 expression by human CD4+ and CD8+ T cells, and glucocorticoid treatment induces the synthesis of IL-10 by lung epithelial cells in asthmatic subjects (Richards *et al*., 2000). However, the synergistic effect of these two anti-inflammatory drugs may suppress the anti-inflammatory response of the body, and this may be the reason behind the low levels of IL-10 in our PCC asthma subjects (Khoo *et al*., 2011).

Reduced sensitivity to the anti-inflammatory effects of corticosteroids is a chief hindrance to effective asthma management. CD4+ T cells from steroid-insensitive asthmatic subjects fail to express IL-10 following stimulation with dexamethasone (Muscari *et al*., 2022). The combined corticosteroid and calcitriol administration induced IL-10-producing T cells, reversing its impaired induction in steroid-insensitive subjects (Xystrakis *et al*., 2006). However, in our subjects, despite adequate 25-hydroxycholecalciferol levels, IL-10 levels were low, indicating that other factors may also play a role.

The positive correlation of 25-hydroxycholecalciferol with IL-10 in non-asthmatic subjects with PCC may possibly be attributed to the vitamin D-dependent up-regulation of anti-inflammatory cytokines (Luo *et al*., 2015; Colotta *et al*., 2017), like IL-10. Considering the crucial function of IL-10 in resolving inflammation, it is conceivable that this cytokine could play a beneficial role in alleviating PCC.

The final regression analysis demonstrated a significant relationship between age, gender, IL-6 levels, and PCC with 25-hydroxycholecalciferol after adjusting for study variables. Further studies are required, especially in PCC subjects, to illustrate the precise mechanism behind this changed cytokine profile despite adequate 25-hydroxycholecalciferol levels.

CONCLUSION

This study sheds light on the complex liaison between 25-hydroxycholecalciferol levels and inflammatory responses in asthmatics, especially those with PCC. The findings suggest that although asthmatics with PCC maintain sufficient levels of 25-hydroxycholecalciferol, they show a substantial increase in the pro-inflammatory response. This suggests that PCC exacerbates the pro-inflammatory response in asthma. Moreover, the study reveals that asthmatics, whether with or without PCC, display a negative correlation between 25-hydroxycholecalciferol and the anti-inflammatory response. This emphasizes the main influence of asthma on the overall inflammatory response. Moreover, a significant relationship between age, gender, IL-6 levels, and PCC was observed with 25-hydroxycholecalciferol after adjusting for study variables. These findings reveal a complex interplay between vitamin D levels and inflammatory mediators in asthmatic individuals with and without PCC. Vitamin D in asthmatics with and without PCC needs to be further explored using well-designed interventional studies. This study has some limitations. The first few participants refused to give a blood sample after giving consent. Secondly, the mean age of the asthmatic patient's group was higher than that of non-asthmatic subjects. Finally, the cross-sectional design of the study was a limitation that did not allow for the assessment of time-sequential associations due to the non-compliance of the participants as exposure and outcome data were collected at one point in time.

Conflict of interest

The authors declare that they have no conflict of interest.

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