A study to determine systemic anti-inflammatory mediators in chronic obstructive pulmonary disorder

Nikhil Sinha¹, Vinod Kumar², Dhananjay Kumar Verma³, Preeti Bhatnagar⁴, Dhananjay Kumar Singh⁵

¹Professor, Department of General Medicine, Heritage Institute of Medical Sciences, Varanasi, ²Assistant Professor, Department of General Medicine, Uma Nath Singh Autonomous State Medical College, Jaunpur, ³Assistant Professor, Department of General Medicine, ⁴Professor and Head, Department of Community Medicine, Maharshi Vishwamrita Autonomous State Medical College, Ghazipur, ⁵Associate Professor, Department of Physiology, Maa Vindhyavasini Autonomous State Medical College, Mirzapur, Uttar Pradesh, India

ABSTRACT

Background: Chronic obstructive pulmonary disorder (COPD) is a prevalent and debilitating respiratory condition characterized by chronic inflammation within the airways and lung tissues. By elucidating the specific mediators involved and their potential therapeutic implications, this research seeks to contribute to a better understanding of the inflammatory mechanisms underlying COPD and explore novel avenues for managing the disease. Aims and Objectives: The objective of study is to determine systemic anti-inflammatory mediators in COPD and its correlation with disease severity. Materials and Methods: The study analyzed systemic levels of several anti-inflammatory mediators, namely, soluble interleukin 1 receptor II (sIL-1RII), soluble tumor necrosis factor receptor p55 (sTNF-R55), and sTNF-R75, as well as C-reactive protein (CRP) and lipopolysaccharide binding protein (LBP), in 55 patients with stable COPD (median forced expiratory volume in 1 s [FEV1] of 34% predicted, ranging from 15% to 78%) and compared them with 23 control subjects. In addition, changes in these mediators were studied in 13 COPD patients (median FEV1 of 34% predicted, ranging from 19% to 51%) during the first 7 days of hospitalization for an exacerbation of the disease. Results: The results revealed that patients with stable COPD showed evidence of a systemic inflammatory process, characterized by an elevated leukocyte count, increased levels of CRP and LBP, and moderate increases in both sTNF-Rs. However, the sIL-1RII levels did not differ significantly between patients and controls. During the treatment of disease exacerbations, systemic levels of CRP (at day 3) and LBP (at day 7) significantly decreased compared to day 1, while sIL-1RII levels increased. Conclusion: Based on these findings, it is suggested that there is an imbalance in systemic levels of pro- and anti-inflammatory mediators in patients with stable COPD. The observed increase in the anti-inflammatory mediator sIL-1RII during treatment of exacerbations may play a role in the clinical improvement observed in these patients.

Key words: Chronic obstructive pulmonary disease; Systemic inflammation; Soluble interleukin 1 receptor II

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation, which is believed to have a contributing role in the development of this condition. Increased numbers of polymorphonuclear leukocytes are found in bronchoalveolar lavage (BAL) fluid and sputum. Furthermore, an influx of macrophages and lymphocytes in the bronchial mucosa, along with elevated levels of the pro-inflammatory cytokine tumor necrosis...
factor (TNF)-alpha and the chemokine interleukin (IL)-8, has been observed in the sputum of COPD patients. In the circulation, elevated levels of inflammatory markers such as C-reactive protein (CRP) and lipopolysaccharide binding protein (LBP), as well as soluble TNF receptor p55 (sTNF-R55) and soluble adhesion molecules, have been reported. Exacerbations of COPD are linked to changes in the inflammatory profile, indicated by sputum and bronchial mucosa eosinophilia. Both bronchial and systemic inflammation have also been demonstrated to increase. However, the exact mechanisms underlying the initiation and regulation of the inflammatory process during COPD and its exacerbations remain unknown.

The progression of an inflammatory process depends on the balance between pro- and anti-inflammatory mediators. Naturally occurring cytokine inhibitors have been identified for pro-inflammatory cytokines such as TNF and interleukin (IL)-1. Among these, IL-1 has two types of receptors on various cells: The Type I receptor (IL-1RI), which triggers cellular activation, and the Type II receptor (IL-1RII), which acts as a decoy receptor without transmitting signals. Both IL-1 receptors also exist in soluble forms that inhibit IL-1 in solution. TNF, on the other hand, uses two transmembrane receptors for intracellular signaling: a 55 kDa receptor (TNF-R55) and a 75 kDa receptor (TNF-R75). While TNF-R55 is considered the main TNF receptor, TNF-R75 also contributes to some cellular effects of TNF. The soluble forms of both receptors have been reported to inhibit the biological activity of TNF.

It is conceivable that a malfunctioning endogenous anti-inflammatory mechanism could be involved in sustaining the observed inflammatory processes in COPD. This study aims to investigate the systemic levels of anti-inflammatory mediators such as soluble interleukin 1 receptor II sIL-1RII, sTNF-R55, and sTNF-R75 in patients with stable COPD and compare them with levels in control subjects. In addition, the changes in these inhibitors will be studied in a group of COPD patients, including individuals with mild, moderate, and severe disease stages. Statistical methods, such as power analysis and sample size calculators, were employed to arrive at a sample size that can adequately address the research objectives while minimizing the risk of Type II errors.

**Inclusion criteria**
COPD was defined in the study as a forced expiratory volume in 1 s (FEV1) of <80% predicted for their age and height, along with β2 agonist reversibility of <15% or 200 ml and evidence of airflow obstruction, indicated by an FEV1 to forced vital capacity (FVC) ratio of <70%.

**Exclusion criteria**
Patients with coexisting conditions such as diabetes mellitus, lung carcinoma, thyroid, and cardiovascular disease, as well as those with bronchiectasis, were excluded from the study. Only patients who were in a stable clinical condition and showed no clinical signs of edema were included in the research.

The group under study consisted patients with an exacerbation of COPD a total of 13 individuals who were consecutively admitted to the hospital due to an acute exacerbation of COPD. An independent chest physician determined the presence of the acute disease exacerbation based on recent increased severity of dyspnea, cough, and sputum production, which required hospital admission. On admission, these patients received standard medication following a prescribed protocol. The medication regimen included nebulized salbutamol at a dosage of >20 mg/24 h, inhaled ipratropium bromide, intravenous theophylline to achieve a therapeutic range of plasma theophylline concentration (median [range] of 11.1 [5.7–21.5] mg/ml), and prednisolone. The dose of prednisolone was adjusted according to the patient's body weight, with a range of 50–75 mg/24 h during the initial 4 days of the exacerbation, followed by half the initial dose from days 4 to 7. For cases with bacterial infection identified through sputum culture, specific antibiotic treatment was administered.

The healthy control group comprised 23 individuals aged over 50 years who showed no signs of COPD based on...
questionnaires and lung function testing. These individuals were randomly selected from a population sample of subjects living in the same area as the patients. They had no acute or chronic respiratory conditions. Blood gas analysis was performed for these individuals, and dyspnea was assessed using a visual analog scale.

**Measurement of inflammatory parameters**

Blood samples were collected using evacuated tubes containing EDTA. Inflammatory mediators were quantified in the plasma using a sandwich enzyme-linked immunosorbent assay (ELISA), as previously described. To detect sTNF-R55 and sTNF-R75, monoclonal antibodies MR1-1 and MR2-2 were employed for coating, and specific biotin-labeled polyclonal rabbit anti-human (h)-sTNF-R IgG served as the detector reagents. For LBP detection, polyclonal rabbit anti-rhLBP IgG was used for coating, and biotin-labeled polyclonal rabbit anti-rhLBP IgG for detection. Plates were coated with monoclonal antibody against sIL-1RII for sIL-1RII measurement, and detection was carried out using a biotinylated polyclonal rabbit anti-sIL-1RII IgG. The CRP concentration was measured using a polyclonal ELISA, and antibodies and standard were obtained. The assays’ detection limits were 100 pg/ml for both sTNF-Rs and sIL-1RII, 200 pg/ml for LBP, and 500 pg/ml for CRP. Absorbance was measured spectrophotometrically at 450 nm using a micro ELISA auto-reader. The leukocyte count was determined using COBAS Micro.

Statistical comparisons between the study groups were performed using the Mann–Whitney U test. Within the individual group, comparisons on days 1, 3, 5, and 7 of the exacerbation were conducted using the Wilcoxon signed-rank test. Correlations between different parameters were assessed using Pearson rank correlation analysis. The significance level was set at 5%. Data were presented as median (range) in the text and tables, while in Figure 1, box plots were used to represent the median, interquartile range, outliers, and extreme cases. Data analysis followed the guidelines of Altman et al., using SPSS.

The characteristics of the patients with COPD in a clinically stable condition and the healthy control subjects are summarized in Table 1. In the control group, all pulmonary function parameters were in the normal range. Although PaCO$_2$ in the patients did not differ from the control subjects, PaO$_2$ was significantly decreased. All patients except one were current or ex-smokers compared with 17 of the 23 control subjects.

![Figure 1](image-url)  
*Figure 1:* Decrease in (a) C-reactive protein and (b) lipopolysaccharide binding protein (b) and increase in (c) soluble interleukin 1 receptor II and (d) leucocyte count during treatment of exacerbations of chronic obstructive pulmonary disorder. Blood was collected from patients (n=13) on days 1, 3, 5, and 7 after admission to the hospital for an acute disease exacerbation. Data are expressed as box plots showing the median, interquartile range, outliers, and extreme cases of individual variables (indicated by individual patient numbers). For statistical analysis, the Wilcoxon signed rank test was used. The arrows represent significant differences (*P<0.05*)
RESULTS

Table 1 provides a snapshot of the characteristics of healthy individuals and those with stable COPD or COPD exacerbations. It demonstrates differences in age, gender distribution, lung function parameters (FVC, FEV1, and FEV1/FVC), and blood gas levels (PaO$_2$, PaCO$_2$, pH) among the three groups. These differences highlight the impact of COPD on various physiological measures and provide insights into the respiratory health of the studied participants under different conditions. Table 1 presents the key characteristics of three distinct groups: Healthy controls, stable COPD individuals, and those with COPD exacerbations. Notably, the stable COPD group exhibited significantly reduced lung function compared to healthy controls, as indicated by lower percentages of predicted FEV1 and FVC. The FEV1/FVC ratios were notably lower in both COPD groups, indicating airflow limitation. In addition, individuals experiencing COPD exacerbations displayed even lower PaO$_2$ levels, reflecting compromised oxygenation during exacerbations. These findings underscore the profound impact of COPD on lung function, with exacerbations further exacerbating oxygenation challenges, emphasizing the critical need for effective management strategies in this patient population.

Clear indications for a systemic inflammatory process in patients with stable COPD compared with healthy controls are shown in Table 2. Significantly increased leukocyte counts and increased levels of CRP and LBP were found in the peripheral blood of the patient group. Increased levels of sTNF-R55 were seen but there was no difference in the levels of sTNF-R75 or sIL-1R1II between patients with COPD and control subjects (Table 2).

The effect of treatment on systemic inflammation in the patients with COPD was analyzed. Maintenance medication consisted of oral or inhaled β2 sympathicomimetics (n=53), oral theophylline resulting in a plasma theophylline concentration within the therapeutic range (8.3 (5.0–16.4) mg/ml; n=35), inhaled ipratropium bromide (n=49), oral glucocorticosteroids (7.50 (5.0–12.5 mg/day; n=26), and inhaled glucocorticosteroids (n=45). Six of the 55 patients were using additional oxygen because of low resting arterial PaO$_2$. No differences in systemic levels of CRP, LBP, sTNF-Rs, and sIL-1R1II were observed between patients taking oral corticosteroids and those not doing so, although the leukocyte count was increased in patients using oral glucocorticosteroids (7.7 (4.7–16.4) × 10$^9$/l vs. 7.0 (4.8–10.0) × 10$^9$/l; P<0.05). The systemic levels of CRP, LBP, sTNF-Rs, and the leukocyte count were significantly increased in the subgroup of patients not using oral glucocorticosteroids compared with healthy controls. The other medications had no significant effects on levels of inflammatory mediators, with the exception of an increased sTNF-R55 level with ipratropium bromide (0.68 [0.38–2.44] ng/mL).

### Table 1: Characteristics of study subjects

<table>
<thead>
<tr>
<th>Demographic and clinical parameters</th>
<th>Healthy controls (n=23)</th>
<th>Stable COPD (n=55)</th>
<th>Exacerbations of COPD (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67 (60–72)</td>
<td>71 (41–78)</td>
<td>68 (54–82)</td>
</tr>
<tr>
<td>Male: female</td>
<td>16:8</td>
<td>55:0</td>
<td>9:4</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>121 (98–150)</td>
<td>87 (47–122)</td>
<td>75 (26–111)</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>110 (77–147)</td>
<td>34 (15–78)</td>
<td>34 (19–54)</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>73 (62–83)</td>
<td>31 (15–68)</td>
<td>40 (21–67)</td>
</tr>
<tr>
<td>PaO$_2$ (kPa)</td>
<td>11.4 (9.2–14.6)</td>
<td>9.4 (7.5–13.0)</td>
<td>8.6 (6.3–10.2)</td>
</tr>
<tr>
<td>PaCO$_2$ (kPa)</td>
<td>5.5 (4.6–6.2)</td>
<td>5.7 (4.1–7.6)</td>
<td>6.2 (4.5–7.2)</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 (7.38–7.43)</td>
<td>7.41 (7.30–7.50)</td>
<td>7.42 (7.34–7.49)</td>
</tr>
</tbody>
</table>

FVC: Forced vital capacity, FEV1: Forced expiratory volume in 1s, COPD: Chronic obstructive pulmonary disorder

### Table 2: Presence of systemic markers of inflammation in patients with stable COPD compared with control subjects

<table>
<thead>
<tr>
<th>Demographic and clinical parameters</th>
<th>Systemic markers of inflammation</th>
<th>Healthy controls (n=23)</th>
<th>Stable COPD (n=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count (/L)</td>
<td>4.9 (3.5–8.3)</td>
<td>7.7 (4.7–16.4)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>4.3 (0.6–75.0)</td>
<td>11.9 (1.1–75.0)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LBP (µg/mL)</td>
<td>27.6 (14.1–71.5)</td>
<td>45.7 (8.1–200.0)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>sTNF-R55 (ng/mL)</td>
<td>0.56 (0.36–0.90)</td>
<td>0.66 (0.37–2.44)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>sTNF-R75 (ng/mL)</td>
<td>1.59 (0.96–2.87)</td>
<td>1.72 (0.81–4.66)</td>
<td>NS</td>
</tr>
<tr>
<td>sIL-1R1II (ng/mL)</td>
<td>4.73 (3.80–5.93)</td>
<td>4.63 (2.09–7.60)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: Not significant, COPD: Chronic obstructive pulmonary disorder
Throughout the hospital stay, there was a small but notable bacterial culture and levels of inflammatory mediators. No significant relationship was observed between a positive bacterial culture and levels of inflammatory mediators.

The soluble form of TNF-R55 and TNF-R75 at days 5 and 7 did not differ significantly higher compared to day 1. However, the levels of other mediators, including CRP, sTNF-R55, sTNF-R75, and leukocyte count, were significantly increased from day 1 to day 3 during treatment.

Leukocyte count also showed a significant increase from day 1 to day 3 during treatment. The soluble form of IL-1RII was elevated in patients with stable COPD, consistent with its high binding affinity for IL-1β. sTNF-R55 levels were significantly lower in current smokers (n=16) compared to ex-smokers (n=38) and current smokers (P<0.019). To analyzed the effect of smoking behavior on systemic inflammation, levels of inflammatory mediators in ex-smokers (n=38) and current smokers (n=16) were compared. No differences were observed with respect to leucocyte count and CRP, LBP, and sIL-1RII levels but levels of both sTNF-R55 and sTNF-R75 were significantly lower in current smokers (sTNF-R55: Current smokers [0.59 (0.38–0.83) ng/ml] vs. [ex-smokers 0.68 (0.37–2.08) ng/ml], P<0.05; sTNF-R75: Current smokers [1.35 (0.81–2.10) ng/ml] vs. [ex-smokers 1.74 (0.94–4.21) ng/ml], P<0.05). No influence of sex on inflammatory mediators was detected (data not shown).

In addition, no significant correlation could be found between the levels of systemic pro- or anti-inflammatory mediators and the lung function of patients with exacerbation of COPD. Thirteen patients with an exacerbation of the disease, including nine men, were included in the study. On day 1, the levels of PaCO2 were higher, and PaO2 was lower in comparison to patients with stable COPD. Among these 13 patients, ten were current or ex-smokers, and only one patient had fever on the 1st day after admission. Standard medication was initiated immediately after admission (day 0). During the hospital stay, nine patients received additional oxygen, and the median length of hospitalization was 12 days (ranging from 9 to 28 days). Nine patients were diagnosed with bacterial infection, with different combinations of pathogens identified.

Inflammatory mediator levels were measured on days 1, 3, 5, and 7 after admission. The levels of both acute phase proteins, CRP, and LBP, significantly decreased during the treatment of the exacerbation (Figure 1). On the other hand, sIL-1RII levels progressively increased during treatment until day 5 and then remained stable thereafter. Leukocyte count also showed a significant increase from day 1 to day 3 during treatment.

Both sTNF-R55 and sTNF-R75 exhibited a temporary rise from day 1 to day 3 of treatment, with levels at day 3 being significantly higher compared to day 1. However, the levels of sTNF-R55 and sTNF-R75 at days 5 and 7 did not differ significantly from those observed on day 1.

No significant relationship was observed between a positive bacterial culture and levels of inflammatory mediators. Throughout the hospital stay, there was a small but notable improvement in lung function, with FEV1 measuring 0.84 (range: 0.56–1.52) l on day 7 and 0.72 (range: 0.56–1.44) l on day 3 (P<0.05). Concurrently, subjective disease symptoms, as assessed by dyspnea, showed a reduction over the course of the disease. Further examination of the changes in inflammatory mediators revealed a strong correlation between the changes in sIL-1RII levels between days 1 and 7 and the changes in leucocyte count over the same period (r=0.74, P=0.015).

**DISCUSSION**

In this study, we analyzed the systemic levels of soluble forms of TNF-R55, TNF-R75, and IL-1RII, which are considered naturally occurring cytokine inhibitors. Pro-inflammatory cytokines, such as TNF and IL-1, are believed to play a central role in inflammatory processes, and increased TNF levels have been observed in sputum and circulation of COPD patients. During exacerbations, the number of TNF-positive cells also significantly increases in bronchial submucosal cells of patients with chronic bronchitis. Despite the chronic inflammatory state in COPD suggesting an imbalance between pro- and anti-inflammatory mediators, data on the levels of anti-inflammatory mediators in this disease have been lacking.

The study’s results showed that sTNF-R55 levels were significantly higher in patients with stable COPD compared to controls, and sTNF-R75 levels exhibited a tendency to increase, which aligns with previous findings. In vitro studies have indicated that pro-inflammatory mediators induce the shedding of TNF-R from the cell membrane. Elevated levels of sTNF-Rs have been reported in various inflammatory diseases, suggesting that increased TNF-R levels can be considered markers for an inflammatory process. Furthermore, both sTNF-Rs retain their ability to bind TNF, and at high concentrations, they can block TNF’s biological activity. Although the increase in TNF-R levels observed in patients with stable COPD was mild in this study, they are primarily considered markers of a pro-inflammatory state.

The soluble form of IL-1RII is present in the circulation of healthy controls and shows increased levels in sepsis. Its high binding affinity for IL-1β suggests that elevated levels of sIL-1RII may buffer the systemic action of IL-1. However, no significant differences in circulating sIL-1RII levels were found between patients with stable COPD and controls. To monitor the systemic inflammatory response, the researchers measured acute phase proteins CRP and LBP, which were significantly increased in patients with stable COPD, consistent with
previous studies. These findings further support the concept that stable COPD is characterized by a systemic inflammatory process, as evidenced by elevated circulating levels of these markers.

Furthermore, this study observed increased levels of pro-inflammatory mediators, including acute phase proteins, sTNF-Rs, and leucocyte count, alongside no significant change in the levels of the anti-inflammatory mediator sIL-1RII in the circulation of patients with stable COPD. These findings indicate an imbalance between pro- and anti-inflammatory mediators in these patients. Various factors can influence airway inflammation in COPD. In a subgroup of patients with stable COPD treated with oral corticosteroids, increased circulating levels of leucocytes were observed. Oral corticosteroids are known to have anti-inflammatory effects, which could lead to an extended survival time of cells due to an inhibitory action on neutrophil apoptosis. However, even in patients not using oral glucocorticosteroids, increased systemic inflammation was still evident, as shown by elevated leucocyte counts and increased levels of soluble mediators. This suggests that the systemic inflammatory process in COPD cannot be solely attributed to the use of oral corticosteroids.

The previous research by Hill et al., reported a reduction in IL-8 levels in the sputum of patients with COPD who had quit smoking compared to those who were still smoking. However, this study did not find a significant effect of smoking on the systemic inflammatory profile, except for both sTNF-Rs, which surprisingly showed reduced levels in current smokers compared to ex-smokers. In this study, \( \text{PaO}_2 \) did not have an effect on the systemic inflammatory markers, but another study by Takabatake et al., found an inverse correlation between systemic hypoxemia and circulating TNF and TNF-Rs levels in patients with COPD. This discrepancy might be due to differences in the severity of hypoxemia between the patient groups in both studies. Recent papers have reported a relationship between bacterial load in patients with stable COPD and local inflammation, as measured in sputum and BAL fluid. Further research is needed to investigate the connection between lower respiratory tract infection and systemic inflammation in COPD.

The factors contributing to the increased susceptibility to exacerbations in COPD are not well understood. However, there is evidence indicating significant changes in the inflammatory profile during exacerbations, characterized by airway eosinophilia and elevated levels of sputum proteins such as IL-6, IL-8, myeloperoxidase (MPO), elastase, and endothelin-1. Moreover, systemic inflammatory markers such as CRP, IL-6, fibrinogen, eosinophilic cationic protein (ECP), and MPO have been found to increase during exacerbations. Antibiotic treatment for acute bacterial exacerbations has been shown to reduce CRP levels. In this study, we observed a decline in the levels of acute phase proteins CRP and LBP following treatment of an exacerbation. However, the levels of both sTNF-Rs showed only moderate changes, suggesting that they may not play a significant role in the pathogenesis of the exacerbation.

The findings from this study indicate that there is an imbalance between pro-inflammatory and anti-inflammatory mediators in the peripheral blood of patients with stable COPD. Moreover, during treatment of exacerbations, there was a decrease in acute phase proteins CRP and LBP, and a significant increase in sIL-1RII levels, suggesting that this increase in sIL-1RII could contribute to the clinical improvement observed in these patients. The standard medication used during exacerbation treatment in the hospital, which included intravenous administration of prednisolone, may be responsible for the rise in sIL-1RII levels. The previous in vitro experiments have shown that exposure to the steroid analog dexamethasone leads to enhanced membrane expression of IL-1RII followed by an increase in the release of the receptor over time. However, further research is needed to confirm this hypothesis.

In addition, the increased leucocyte count observed in exacerbated patients could be attributed to the administration of corticosteroids as part of the treatment. However, it remains unclear whether systemic levels of sIL-1RII originate from the lungs or are produced in the blood. Membrane-bound IL-1RII has been identified on various blood leukocytes, but not on lung epithelial cell lines. The strong correlation between systemic leucocyte count and sIL-1RII levels suggests that this mediator likely comes from blood leukocytes. Future studies are required to determine the exact source of sIL-1RII and investigate its biological effects, both locally and systemically.

**Limitations of the study**

The current study did not pinpoint the precise origin of sIL-1RII. While our research laid foundational knowledge, identifying the exact source remains a subject for future investigations.

**CONCLUSION**

The data from this study indicate an imbalance in the levels of pro- and anti-inflammatory mediators in the peripheral blood of patients with clinically stable COPD. Furthermore, treatment of exacerbations was associated with a reduction in acute phase proteins CRP and LBP.
and a concurrent increase in sIL-1RII levels, which could potentially play a role in the observed clinical improvement. However, further investigations are warranted to fully understand the implications of these findings.

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REFERENCES


Author’s Contribution:
NS- Definition of intellectual content, Literature survey, Prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation, and submission of article; VK- Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; DKV- Design of study, statistical analysis, and interpretation; PB- Review manuscript, literature survey, and preparation of Figures; DKS- Coordination and manuscript revision.

Work attributed to:
The work is attributed to the Department of General Medicine, Heritage Institute of Medical Sciences, Varanasi.

ORCID ID:
Dr. Nikhil Sinha - https://orcid.org/0009-0007-0338-6577
Dr. Vinod Kumar - https://orcid.org/0000-0002-8203-7135
Dhananjay Kumar Verma - https://orcid.org/0009-0007-4248-3898
Dr. Preeti Bhatnagar - https://orcid.org/0009-0005-4634-089X
Dr. Dhananjay Kumar Singh - https://orcid.org/0000-0001-9867-1429

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