# Oxi-inflammatory stress and vascular cell adhesion molecule-1 in post-COVID active rheumatoid arthritis patients

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## ABSTRACT

**Background:** Coronavirus disease-19 (COVID-19) has sparked the deterioration of human health at an unprecedented scale globally and affected the patient's musculoskeletal health also. It is conceivable that active rheumatoid arthritis (ARA) patients recovered from COVID-19 after second wave are at enhanced risk of cardiovascular complications. **Aim and Objectives:** In this context, the present study was intended to estimate the soluble vascular cell adhesion molecule-1 (sVCAM-1), serum paraoxonase (PON), and markers of oxi-inflammatory stress in ARA patients diagnosed reverse transcriptase-polymerase chain reaction negative after second wave of COVID-19 and to determine their role in predicting cardiovascular disease (CVD) risk. **Materials and Methods:** Sixty ARA patients (30–45 years) of Delhi-NCR region were recruited and categorized into two groups (n = 30 in each group; on the basis of their history of COVID infection). Using standard methods, study group parameters were estimated in ARA patients and statistically compared it with that of 30 healthy controls by using student's t-test. **Results:** Serum sVCAM-1, malondialdehyde (MDA), and C-reactive protein (CRP) levels were significantly high (P < 0.001) in Group II and Group III subjects as compared to healthy controls. Conversely, serum PON activity was found to be significantly low (P < 0.001) in Group III as compared healthy controls. However, PON activity was altered insignificantly (P < 0.1) with respect to Group II subjects. sVCAM-1 levels were positively correlated with MDA, CRP, and atherogenic index; and negatively correlated with PON activity (P < 0.001) in post-COVID ARA patients. **Conclusion:** Thus, enhanced sVCAM-1 and reduced PON activity along with enhanced oxi-inflammatory stress status are more efficient molecular signatures of CVD risk among post-COVID ARA patients. Therefore, the present study emphasizes the dire need of special attention to provide cardiovascular rehabilitation strategy among post-COVID ARA patients along with reduction of oxi-inflammatory stress to reduce the CVD mortality in ARA population.

**Key words:** Vascular cell adhesion molecule-1; Interleukin-6; Malondialdehyde; Paraoxonase; Inflammation; Free radicals

## INTRODUCTION

Disaster, caused by coronavirus disease-19 (COVID-19) pandemic, is a curse to humanity which affects the human health in a multidimensional way. Despite numerous scientific efforts have been done to achieve successful and healthy life after second wave of COVID-19 in India, the long-term of COVID-19 is unrevealed and it is characterized by ingress of systematic deterioration of health accompanied with an envelope of various health related complications.[1]

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Interestingly, immune hyperactivation and cytokine involvement in alveolar structures have been identified as the key contributors to produce severe lung disease in COVID-19 patients. Although coronavirus is largely a respiratory disease, one of the complication of COVID-19 infection is arthritis, and it is present in 14.9% of cases.[2]

Active rheumatoid arthritis (ARA), a chronic autoimmune disease, is the most common arthritic condition characterized by synovial inflammation, hyperactivation of T cells, and oxidative stress.[3] Moreover, chronic disability in arthritic population and systemic inflammation due to occurrence of several pro-inflammatory cytokines in COVID-19 patients and ARA as well act as contributing factors in developing cardiovascular complications. In this context, C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), well known marker of systemic inflammation, have been reported to be associated with disease severity and mortality, in separate studies pertaining to COVID-19, ARA, and cardiovascular disease (CVD) patients. Recently, the soluble vascular cell adhesion molecule-1 (sVCAM-1) is one of established plasma markers of inflammation and endothelial activation, that is, a hallmark of atherosclerotic complication, and received much attention in COVID-19 and ARA patients.[4]

Oxidative stress mediated by free radicals can evade or overwhelm the antioxidant protective mechanism of cells and may cause cell membrane and cartilage destruction, lipid peroxidation, DNA strand breakage, rises in intracellular free $Ca^{2+}$, damage to membrane ion transporters, and other specific proteins leading to cell death followed by disease development.[5] Free radicals production is efficiently controlled by antioxidant defense system which includes antioxidant enzymes and non-enzymic antioxidants. In this context, assessment of paraoxonase (PON), a HDL-associated enzyme carried on apolipoprotein A-1 (apoA-1) that protects lipoproteins against oxidative modification, has received much attention. The previous studies have shown that PON level alters in various complications such as COVID-19, cardiovascular diseases, musculoskeletal, and neurological disorders.[6,7] However, alteration in PON activity in post COVID ARA patients and in determining future risk of CVD complications is still in obscure and has received much attention to explore hidden facts related to commencement of secondary complications in post-COVID ARA.

In addition, the previous studies have documented that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection that caused COVID-19 can attack musculoskeletal systems through oxidative stress and immune-inflammation-dependent mechanisms, which may develop inflammatory arthritis during the infective or post-infective stage.[8] However, little is known about the CVD manifestations or worsening of ARA by this infection.

It is conceivable that there is a close link between long-term effect of COVID-19 and ARA pathophysiology with the future CVD risk due to augmented oxi-inflammatory stress along with traditional CVD risk factor such as dyslipidemia.

### Aims and objectives

To enhance our understanding on post-COVID ARA etiopathophysiology, the present study was intended to evaluate the extent of oxi-inflammatory stress, VCAM-1 level along with atherogenic index in post-COVID ARA patients and to determine their role in prediction of CVD risk as a long-term effect of COVID-19 in post-COVID ARA patients.

## MATERIALS AND METHODS

Sixty patients of either sex with ARA belonged to age group 30–45 years and who were residents of Delhi-NCR region were included in the study. ARA patients were divided into two groups on the basis of their history of COVID-19 infection. Thirty ARA patients who were not affected with COVID during COVID pandemic were included in Group II. In Group III, Post-COVID-19 patients diagnosed reverse transcriptase-polymerase chain reaction (RT-PCR) negative after second wave of COVID and belonging to age 30–45 years of either sex. Thirty age-matched healthy individuals were recruited from hospital staffs, friends, and relatives of patients as Group I (control group). A general information or pre-experimental questionnaire regarding demographic information, family history, and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

### Inclusion criteria

Written informed consent was obtained from all the subjects included in the study. Subjects who do not under any medical treatment or taking antioxidant supplement for at least 1 month before blood collection were included in the study. Criteria recommended by the American Rheumatism Association were used for the diagnosis of ARA.[9] All patients had active RA, defined as the presence of at least three of the following criteria: Six or more tender joints; three or more swollen joints; ≥30 min of morning stiffness; an erythrocyte sedimentation rate (ESR) of ≥28 mm/h. The number of swollen and tender joints (28 joint count) and patient's assessment of pain on visual analog score (VAS) was registered. Disease activity score-28 (DAS-28) was calculated using ESR.[10]

Height was measured using wall mounted scale whereas weight was measured with subject barefoot and lightly dressed using digital weighing machine. The body mass index (BMI) was calculated as (BMI=Weight [kg]/height [metre²]). Blood pressure was measured by mercury sphygmomanometer using auscultatory method. To diminish any confounders developed by other arthritic complications, patients with positive rheumatoid factor were recruited and their disease duration was recorded. However, RA patients with family history of arthritis and hypertension were not excluded from the study. In addition, ARA patients who had previously under any medical treatment including supplementation of antioxidants or non-steroidal anti-inflammatory drugs were not excluded from the study if the subject agreed that no supplements or analgesic drug would be taken in the 7 days before entry into the study. However, there was no restriction or withdrawal on the conventional anti-rheumatoid drugs treatment.

### Exclusion criteria

None of the patients and control subjects had family history of concomitant diseases, such as diabetes mellitus, hepatitis, renal failure, and neurological disorder. In addition, patients with established cardiovascular complications, pregnancy, lactation, obesity (BMI>30), Stage I and Stage II hypertension (BP>129/89 mmHg), smoking habit, renal failure, liver disease, hypothyroidism or who did not follow study instructions were also excluded from the study.

Fasting blood samples were collected in plain vial from anticubital veins avoiding venostasis from each patients and healthy controls. Blood samples destined for the measurement of study group parameters were centrifuged at 3500 rpm for 10 min within 1 h of collection and serum was stored at −80°C until analysis. The serum concentrations of sVCAM-1, IL-6, TNF-α, and CRP were measured with commercially available enzyme-linked immunosorbant assay kits (R&D Systems, USA) according to the manufacturer's instructions.

Serum malondialdehyde (MDA) levels were estimated by thiobarbituric acid (TBA) reaction.[11] Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (pH 2–3) and boiled with TBA which reacts with malondialdehye, forming a MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured using a spectrophotometer at 530 nm.

Serum PON activity was estimated by Gan et al., method using p-nitrophenyl acetate (5.5 mM/L) as a substrate.[12] The increase in the absorbance of p-nitrophenol formed at 412 nm was measured spectrophtometrically. The activity of PON was measured in tris buffer (20 mM/L; pH=8.0) containing 1 mM $CaCl_2$. The generated product p-nitrophenol was calculated using molar extinction coefficient of 17000/mole/cm at pH=8.0. Results are expressed as Units/mL (1 nmol p-nitrophenol formed per minute).

### Statistical analysis

After estimating study group parameters, data were entered manually in Microsoft Excel sheet of windows 2007 and result was processed using online Graphpad software. Values were expressed as Mean±SD and Student's t-test was used to compare the significance of mean difference between study group subjects. Pearson correlation coefficient was used to determine the relationship among the markers, $P<0.05$ and $<0.001$ were considered as significant and highly significant, respectively.

## RESULTS

Demographic indices and clinical profile including mean age and blood pressure of the study group subjects are depicted in Table 1. BMI and VAS of pain measurement revealed significant and continuous elevation in Group II and III ARA patients. Out of 30 post-COVID ARA patients, 12 patients (40%) were overweight and 18 patients (60%) of post-COVID ARA patients were pre-hypertensive as per JNC 7th guidelines which reflect the detrimental effect of COVID infection among ARA patients. However, they were not taking any antihypertensive drug and were being managed by diet and exercise. The ESR level of ARA patients was significantly high ($P<0.001$; 38% high in Group II and 49% high in Group III) as compared to healthy controls. In addition, both category of ARA patient population had a moderate disease activity with a mean DAS-28-ESR of 3.84±0.23 and 4.28±0.25, respectively.

**Table 1: Demographic and clinical profile of study group subjects (mean±SD)**

| S. No. | Particulars | Group I (n=30) | Group II (n=30) | Group III (n=30) |
|---|---|---|---|---|
| 1. | Age (years) | 37.3±5.0 | 40.4±4.2 | 39.3±3.9 |
| 2. | M: F ratio | 16:14 | 17:13 | 19:11 |
| 3. | BMI (Kg/m²) | 24.8±1.4 | 25.7±1.1* | 28.2±0.97** |
| 4. | Systolic blood pressure (mmHg) | 105.5±3.4 | 116.4±3.2 | 124±3.0 |
| 5. | Diastolic blood pressure (mmHg) | 75.0±2.4 | 78.3±2.0. | 84.0±2.5 |
| 6. | VAS pain (mm) | 0.0 | 33.5±4.6** | 37.65±5.1** |
| 7. | ESR (mm/h) | 16.2±2.21 | 28.4±3.40** | 34.5±3.52** |
| 8. | DAS-28 | 0.0 | 3.84±0.23 | 4.28±0.25** |

*P<0.1: Non-significant, **P<0.05: Significant, ***P<0.001: Significant. BMI: Body mass index, VAS: Visual analog score, ESR: Erythrocyte sedimentation rate, DAS-28: Disease activity score-28, M: Male, F: Female, SD: Standard deviation

Marked occurrence of atherogenic profile along with abnormalities in lipid profile contents was observed in post-COVID active RA patients as compared to healthy controls (Table 2). Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very LDL-C (VLDL-C) levels were found to be increased significantly in Group II ($P<0.05$ i.e., 18.37%, 13.84%, 25.83%, and 12.56% high) and in Group III ARA subjects ($P<0.001$ i.e., 34.59%, 24.10%, 54.17%, and 25.58% high), respectively, as compared to healthy controls. On the other hand, serum HDL-C levels were decreased significantly in Group II and Group III patients ($P<0.05$; 18.16% and $P<0.001$; 29.19% low, respectively). However, these levels were altered insignificantly ($P<0.1$) in Group III as compared to Group II subjects. Moreover, statistically significant ($P<0.05$) and high atherogenic index (TC/HDL-C ratio was higher than 5) were observed in post-COVID ARA patients which revealed the increased risk of atherosclerotic complication during post-COVID infection.

Serum sVCAM-1 levels were found to be increased significantly only in post-COVID ARA ($P<0.001$) patients, that is, 32.7% high as compared to healthy controls whereas in Group II subjects serum sVCAM-1 levels were increased ($P<0.1$) insignificantly, as depicted in Figure 1. Similarly, marked alteration in the levels of oxi-inflammatory stress was observed in study group subjects, as represented in Figures 2 and 3, respectively. Serum MDA, IL-6, TNF-alpha, and CRP levels were also found to be significantly high in Group III ($P<0.001$) ARA patients, that is, 53.8%, 39.6%, 40.1%, and 36.4% high as compared to Group I. These levels revealed continuous elevation in post-COVID ARA patient as compared to ARA patients who did not infected with COVID-19 which reflect the deteriorative effect of COVID-19 infection in ARA patients. However,
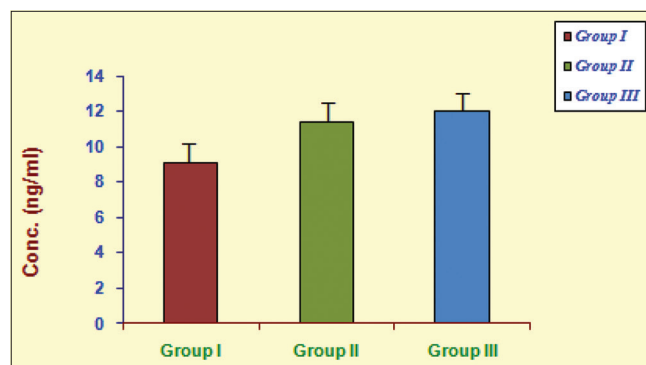
statistically, these values were altered insignificantly on comparing with each other.

Interestingly, correlation studies revealed that sVCAM-1 was significantly correlated with the disease severity, that is, with VAS ($r=0.604$, $P=0.002$), ESR ($r=0.618$, $P=0.001$), DAS-28 score ($r=0.563$, $P=0.082$), and atherogenic index ($r=0.512$, $P=0.04$), as presented in Figure 4. Remarkably, we observed a negative correlation between sVCAM-1 with PON activity ($r=-0.448$, $P=0.05$), whereas marker of lipid peroxidation and inflammation such as MDA, CRP, TNF-$\alpha$, and IL-6 levels was positively correlated
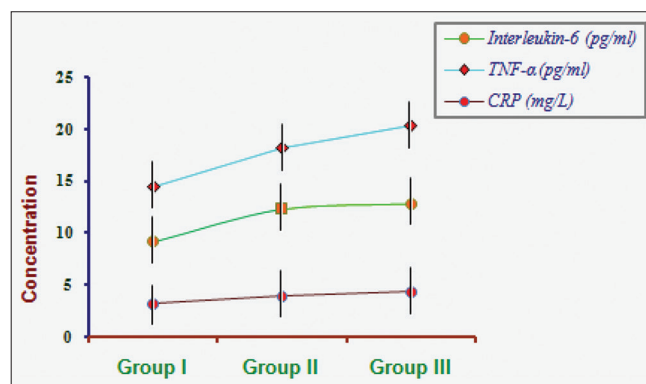


**Figure 1:** Serum soluble vascular cell adhesion molecule-1 levels in study group subjects



**Figure 2:** Markers of inflammation in study group subjects



**Figure 3:** Oxidative stress markers in study group subjects

## Table 2: Dyslipidemia in post-COVID ARA patients (mean±SD)

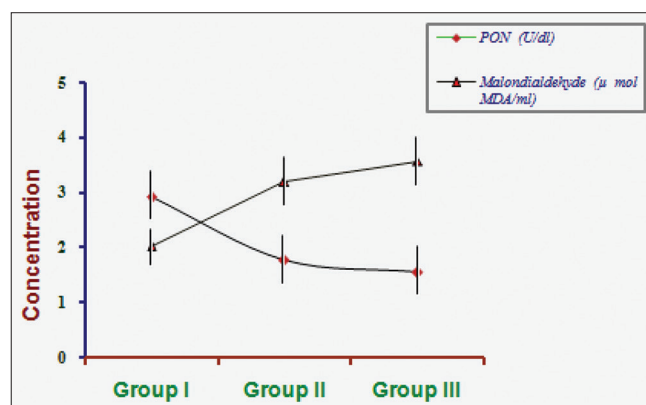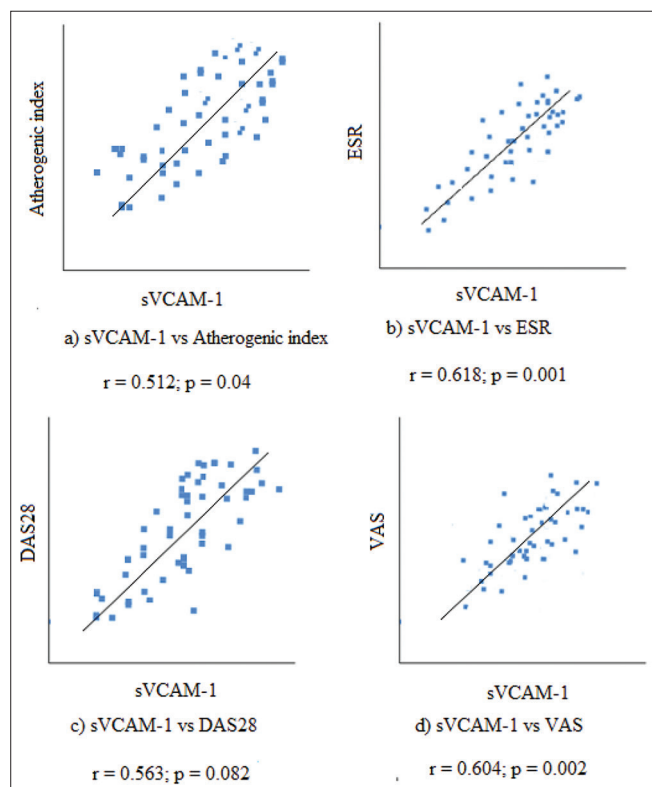| S. No. | Particulars | Group I (n=30) | Group II (n=30) | Group III (n=30) |
|---|---|---|---|---|
| 1. | TC (mg/dl) | 158.4±7.0 | 187.5±9.2** | 213.2±7.3*** |
| 2. | TG (mg/dl) | 112.0±8.1 | 127.5±8.0* | 139.0±7.7*** |
| 3. | HDL cholesterol (mg/dl) | 43.5±5.0 | 35.6±5.2** | 30.8±4.8*** |
| 4. | LDL cholesterol (mg/dl) | 99.5±9.2 | 125.2±10.5** | 153.4±7.8*** |
| 5. | VLDL cholesterol (mg/dl) | 21.5±1.5 | 24.2±1.8* | 27.0±1.6*** |
| 6. | TC/HDL cholesterol ratio | 3.48±0.72 | 4.35±1.32** | 5.38±1.20*** |

*$P<0.1$: Non-significant, **$P<0.05$: Significant, ***$P<0.001$: Highly significant. TC: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very-low-density lipoprotein
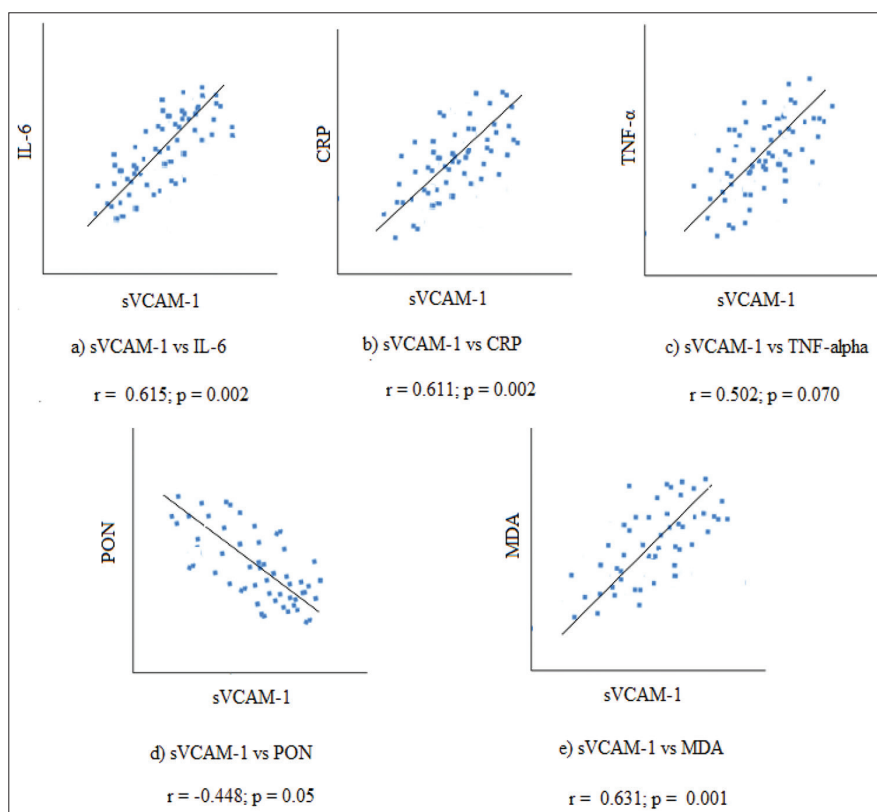
**Figure 4:** Correlation coefficient (r) between soluble vascular cell adhesion molecule-1 and disease activity score-28 along with other variables in post-COVID active rheumatoid arthritis patients

with sVCAM-1 (Figure 5, P<0.001) which indicates the association of sVCAM-1 with oxi-inflammatory stress and elevated disease complexity in terms of pain, clinical symptoms, and CVD risk in post-COVID active RA patients.

## DISCUSSION

CVD is one of the main causes of mortality and morbidity in patients with ARA and in COVID-19 patients. Both the diseases share the involvement of oxidative stress and immune-inflammatory dependent etiopathophysiology so closely that CVD can be considered as an extra-articular manifestation of post-COVID ARA.[8,13] Among various modifiable risk factors for CVD such as smoking, hypertension, diabetes, and overweight along with dyslipidemia, oxi-inflammatory stress has now been receiving much attention toward solving the unanswered question related to the development of the future risk of CVD in post-COVID ARA patients and, thus, can help to prevent and reduce the CVD burden in ARA and post-COVID patients.

The present study group subjects revealed a traditional CVD risk factor, that is, an abnormal lipid profile, characterized by an increase of serum TC, TG, and LDL-C



**Figure 5:** Correlation coefficient (r) between soluble vascular cell adhesion molecule-1 and oxi-inflammatory stress in post-COVID active rheumatoid arthritis patients

levels, and a reduction in HDL-C levels which enhances the CVD risk in post-COVID ARA patients. It could be explained as a long-term impact of COVID-19 on ARA patients. It has been documented that COVID-19 exert deteriorative effect on human health and responsible for decreased potential and physical activity of patients even after confirmation of RT-PCR report negative.[14] Progressive reduction in HDL cholesterol levels, as observed in post COVID ARA patients, also exposed them to CVD risk because HDL particle is known not only for its ability to facilitate reverse cholesterol transport, but also due to its anti-thrombotic, anti-oxidant, anti-inflammatory, and endothelium-stabilizing properties that may benefit against atherosclerosis.[15,16] Our findings were in consistent with the recent findings of Uyaroglu et al., who also observed marked alteration in lipid profile content in post-COVID-19 patients.[17]

Interestingly, integrity and functionality of endothelial cells are critical to maintaining homeostasis and cardiovascular health. Endothelial cells are an essential component of the coagulation system. Endothelial activation, induced by pro-inflammatory cytokines (IL-6, TNF-α), facilitates the recruitment and attachment of circulating leukocytes to the vessel wall and thereby plays a key role in coagulopathy.[18] Angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2, is expressed by endothelial cells. The interaction of SARS-CoV-2 and ACE2 leads to endothelial activation which may result in loss of vascular integrity; expression of leukocyte adhesion molecules; change in phenotype from antithrombotic to prothrombotic; cytokine production; and platelet activation, thrombosis, and inflammation.[19]

In the present study, serum sVCAM-1 levels were increased significantly ($P<0.05$; Figure 1) and positively correlated with atherogenic index along with the markers of inflammation such as ESR, CRP, IL-6, and TNF-α (Figures 3 and 4) in post-COVID ARA patients which reflect the persistence of systemic inflammation mediated endothelial activation in post-COVID state and thus, making the post-COVID ARA patients more susceptible to develop the CVD risk. Recently, Wong et al., also reported that pro-inflammatory cytokines activates the dysfunctional endothelial cells characterized by elevated levels of endothelial adhesion molecule sVCAM-1 which may contribute to the pathogenesis of thrombosis by altering the expression of pro- and antithrombotic factors.[20] However, conversely, Tong et al., reported that recovery from severe COVID-19 was associated with reductions in serum CRP, IL-18, TNF-α, and sVCAM-1 levels.[19]

In addition to systemic inflammation, oxidative stress due to uncontrolled ROS production plays a crucial role in increasing the chances to develop CVD complications in post-COVID ARA population. ROS produced by endothelial cells and vascular smooth cells not only oxidize low density lipoprotein and initiate atherosclerotic event but also involve in cell membrane damage through lipid peroxidation which, in turn, plays a crucial role in the development and progression of vascular complications in arthritic patients.[21] In the present study, serum malondialdehyde levels (marker of lipid peroxidation) were also found to be significantly high in Group II and Group III subjects ($P<0.001$, Figure 3) and positively correlated sVCAM-1 levels. These findings indicate that excessive ROS generation takes place in post-COVID ARA patients which clarify the role of oxi-inflammatory stress mediated endothelial activation in shaping the ARA patients more susceptible to develop CVD risk in post-COVID state. Interestingly, increased levels of MDA were also reported in rheumatoid arthritis and COVID-19 patients.[3,22] Moreover, Saxena et al., reported that lipid peroxidation mediated electrolyte imbalance and production of protein radical in lipid membranes affects the normal ion transport, and thereby enhances the risk of CVD in RA patients.[23]

To combat with oxidant mediated injury, various sorts of antioxidant enzymes are present in the body. Among them, serum PON contributes to anti-atherogenic and antioxidant activity by regulating oxidation of LDL, by hydrolyzing specific oxidized phospholipids, cholesterol linoleate hydroperoxides, and by neutralizing hydrogen peroxide.[6,7] Alteration in PON activity may have significant effect in inducing CVD risk with disease complexity. In the present study, serum PON activity was found to be decreased significantly in Group II and Group III ARA patients and negatively correlated with sVCAM-1 levels (Figure 5) which reflects toward its utilization in preventing ROS mediated lipid peroxidation and its inactivation due to interaction of oxidized lipids with the PON free sulphydryl group. Similar findings have been documented by Rodriguez-Tomas et al., in COVID-19 patients and implicated the role of reduced PON activity along with marker of systemic inflammation in determining the risk of cardiovascular complications.[6] Gabaldo et al., also emphasized the estimation of PON activity in the diagnosis of COVID-19.[24]

Recently, researchers have focused on the combinational approach of antioxidant therapy such as oral N-acetyl cysteine along with conventional therapy in reducing the ARA complications and CVD risk. In this context, Zeng et al., revealed potential benefits of antioxidant therapy in randomized and controlled trials of RA patients treatment. According to them, antioxidant therapy plays a crucial role in preventing oxidative stress mediated RA pathology by reducing MDA and increasing total antioxidant activity along with glutathione levels in rheumatoid arthritis.[25]

However, to get high quality evidence with respect to antioxidant therapy, large samples and high quality randomized and controlled trials are needed to combat the ARA complexity in post-COVID era.

### Limitation of the study

It is difficult to draw a solid line for developing therapeutic intervention strategy due to inadequacy of sample size of the present study, which is just 60.

## CONCLUSION

On the basis of findings of the present study, it is obvious that induction of endothelial activation, characterized by elevated sVCAM-1 levels, has a role in alteration of vascular homeostasis in post-COVID ARA patients. In toto, it is obvious that cumulative effect of oxi-inflammatory stress is responsible for altered vascular homeostasis and elevated atherogenic index, ensuing post-COVID ARA patients more susceptible to develop future CVD complications. Thus, monitoring of sVCAM-1 level along with markers of oxidative stress such as serum PON activity and MDA may be an effective "treat to target" approach from a lens of therapeutic intervention strategy in treating ARA and its associated complications. Therefore, counseling for boosting immunity and stamina by adopting healthy life style modifications and inclusion of antioxidant rich food products in diet or antioxidant therapy along with regular aerobic exercise are needed at regular and continuous pace for ARA population to reduce not only the burden of CVD risk but also to combat with other secondary complications in rheumatic disorder patients.

## ACKNOWLEDGMENT

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