Endothelial activation in Post COVID knee osteoarthritis patients: A deteriorative clinical incident

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Abstract:

Background: Musculoskeletal health has also affected during COVID 19 pandemic at an unprecedented scale globally and characterized by marked deterioration of human health multidimensionally. It is conceivable that knee osteoarthritis (KOA) patients recovered from COVID-19 after second wave are at enhanced risk of Cardiovascular complications. **Aim:** The present study was intended to estimate the soluble vascular cell adhesion molecule (sVCAM-1), serum Paroxonase (PON) and markers of oxi-inflammatory stress in KOA patients diagnosed RT-PCR negative after second wave of COVID-19 and to determine their role in predicting Cardiovascular complications. **Methodology:** 80 KOA patients (35-50 years) of Delhi-NCR region were recruited and categorized into two groups (n=40 in each group; on the basis of their history of COVID infection). By using standard methods, study group parameters were estimated in KOA patients and statistically compared it with that of 40 healthy controls by using student's t-test. **Result:** Serum sVCAM-1, MDA and IL-6 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, IL-6 and Atherogenic index; and negatively correlated with PON activity ($P<0.001$) in post COVID KOA patients. **Conclusion:** Endothelial activation characterized by enhanced sVCAM-1 and reduced PON activity along with enhanced oxi-inflammatory stress status are more efficient molecular signatures of cardiovascular complications among Post COVID KOA patients. Therefore, Cardiovascular rehabilitation strategy along with reduction of oxi-inflammatory stress can be an effective approach in order to reduce the burden of CVD mortality among Post COVID KOA patients.

Keywords: VCAM-1, IL-6, Malondialdehyde, PON, inflammation, free radicals.

Introduction:

Knee osteoarthritis (KOA), a multifactorial process of joint degeneration, is the most common arthritic condition generally affects the knee, hip, metatarsophalangeal joints, distal interphalangeal, proximal interphalangeal, cervical and lumbar spine [(1)]. Although coronavirus is largely a respiratory disease, one of the complications of COVID-19 infection is arthritis, and it is present in 14·9% of cases [(2)]. Moreover, physical disability and systemic inflammation due to occurrence of several pro-inflammatory cytokines in arthritis and COVID 19 patients as well, act as contributing factors in developing Cardiovascular complications in arthritic population [(3)].

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, KOA and cardiovascular disease (CVD) patients. Recently, the soluble vascular cell adhesion molecule (sVCAM-1) is one of established plasma markers of inflammation and endothelial activation i.e., a hallmark of atherosclerotic complication, and received much attention in COVID 19 and KOA 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 apo A-I that protects lipoproteins against oxidative modification, has received much attention. 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 KOA patients and in determining future risk of CVD complications is still in obscure, and has received much attention in order to explore hidden facts related to commencement of secondary complications in Post COVID KOA.

In addition, 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 KOA by this infection.

It is conceivable that there is a close link between long term effect of COVID 19 and KOA pathophysiology with future CVD risk due to augmented oxi-inflammatory stress along with traditional CVD risk factor such as dyslipidemia. In order to enhance our understanding on Post COVID KOA etio-pathophysiology, the present study was intended to evaluate the extent of endothelial activation and oxi-

inflammatory stress along with atherogenic index in Post COVID KOA patients and to determine their role in prediction of CVD risk as a long-term effect of COVID19 in post COVID KOA patients.

Material and Methods:

80 patients of either sex with knee osteoarthritis (KOA) belonged to age group 35-50 years and who were residents of Delhi-NCR region were included in the study. KOA patients were divided into two groups on the basis of their history of COVID 19 infection. 40 KOA patients who were not affected with COVID during COVID pandemic were included in Group II. In Group III, Post COVID 19 patients diagnosed RT-PCR negative after second wave of COVID and belonging to age 35 – 50 years of either sex. 40 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 prior to blood collection were included. Criteria recommended by the American Rheumatism Association Clinical diagnostic criteria were used for the diagnosis of KOA [(9)]. In addition, patients had radiological evidence of grade 3 knee OA in at least one or both of the knees (as per KL grading scale) were included. Assessment of pain on Visual Analog Score (VAS) were registered [(10)].

Height was measured by using wall mounted scale whereas weight was measured with subject barefoot and lightly dressed by using digital weighing machine. The Body Mass Index (BMI) was calculated as [BMI = *Weight (kg) / Height (metre^2)*]. Blood pressure was measured by mercury sphygmomanometer using auscultatory method. To diminish any confounders developed by other arthritic complications, patients with grade 3 were recruited. However, KOA patients with family history of arthritis and hypertension were not excluded. In addition, KOA 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 seven days before entry into the study. However, there was no restriction or withdrawal on the conventional 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 patient and healthy controls. Blood samples destined for 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 and TNF-α were measured with commercially available enzyme-linked immunosorbant assay (ELISA) kits (R&D Systems, USA) according to the manufacturer's instructions.

Serum 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 thiobarbituric acid which reacts with Malondialdehye, forming MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured by using a spectrophotometer at 530 nm.

Serum paraoxonase 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 1mM $CaCl_2$. The generated product p-nitrophenol was calculated by using molar extinction coefficient of 17000 per 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 were 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 co-relation coefficient was used to determine the relationship among the markers P value <0.05 and <0.001 were considered as significant and highly significant respectively.

Result:

Demographic indices and clinical profile including mean age and blood pressure of the study group subjects, are depicted in Table 1. Body mass index (BMI) and visual analogue scale (VAS) of pain measurement revealed significant and continuous elevation in Group II and III KOA patients. Out of 40 Post COVID KOA patients, 24 patients (60%) were overweight and 20 patients (50%) of Post COVID KOA patients were pre-hypertensive as per JNC 7^{th} guidelines which reflect the detrimental effect of COVID infection among KOA patients. However, they were not taking any antihypertensive drug and were being managed by diet and exercise. Marked occurrence of atherogenic profile along with abnormalities in lipid profile contents were observed in Post COVID KOA patients as compared to healthy controls (Table 2.0). Serum TC, TG, LDL-C and VLDL-C levels were found to be increased significantly in Group II ($p<0.05$ i.e. 20.5%, 14.61%, 31.17% and 7.45% high) and in Group III KOA subjects ($p<0.001$ i.e. i.e. 37.96%, 28.08%, 57.20% and 20.61% 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 ($p<0.05$; 18.09% and $p<0.001$; 26.19% low respectively). However, these levels were altered insignificantly ($P<0.1$) in Group III as compared to Group II subjects. Moreover, statistically significant ($p < 0.05$) high atherogenic index (TC/ HDL-C ratio was higher than five) were observed

in Post COVID KOA patients which revealed the increased risk of atherosclerotic complication during Post COVID infection.

Post COVID KOA patients revealed significantly high ($p<0.001$) serum sVCAM-1 levels i.e. 35.0% 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 were observed in study group subjects, as represented in Figure 2 and 3 respectively. Serum MDA, IL-6 and TNF-alpha levels were also found to be significantly high in Group III ($p<0.001$) KOA patients i.e., 58.62%, 44.34% and 40.54% high as compared to Group I whereas serum PON activity was decreased significantly in Group II and Group III patients ($p<0.001$; 34.04% and 43.61% low respectively). However, these levels were altered insignificantly ($P<0.1$) in Group III as compared to Group II subjects. These levels revealed continuous elevation in Post COVID KOA patient as compared to KOA patients who did not infect with COVID19 which reflect the deteriorative effect of COVID 19 infection in KOA patients. However, statistically these values were altered insignificantly on comparing with each other.

Remarkably, correlation studies revealed that sVCAM-1 was significantly correlated with the disease severity i.e., with VAS ($r = 0.604$, $p = 0.002$) and Atherogenic index ($r = 0.512$, $p = 0.04$), as presented in Table 3.0. Moreover, negative correlation was observed between sVCAM-1 with PON activity ($p = 0.05$), whereas marker of lipid peroxidation and inflammation such as MDA, TNF-α and IL-6 levels were positively correlated with sVCAM-1 (Table 4.0, $p<0.001$) which indicates the association of endothelial activation with oxi-inflammatory stress and elevated disease complexity in terms of pain, clinical symptoms, and CVD risk in Post COVID KOA patients.

Discussion:

In post COVID era, risk of cardiovascular disease (CVD) has been implicated as a curse of COVID to humanity and the patients of musculoskeletal disease are at great risk to develop secondary complication due to lock down mediated limited physical mobility. Both, Knee osteoarthritis (KOA) and COVID 19 patients, share the involvement of oxidative stress and immune-inflammatory dependent etio-pathophysiology so closely that CVD can be considered as an extra articular manifestation of post COVID KOA [8,13]. Amongst 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 towards solving the unanswered question related to the development of future risk of CVD in post COVID KOA patients and, thus, can help to prevent and reduce the CVD burden in KOA and post COVID patients.

The present study group subjects revealed a traditional CVD risk factor i.e., an abnormal lipid profile, characterized by an increase of serum total cholesterol, triglycerides and LDL-C levels, and a reduction in HDL-C levels which enhances the CVD risk in post COVID KOA patients. It could be explained as a long-term impact of COVID19 on KOA 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 KOA 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]. Recently, in consistent with the findings of present study, marked alteration in lipid profile content in Post COVID 19 patients was also observed [16].

Interestingly, integrity and functionality of endothelial cells are critical to maintaining hemostasis 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 [17]. 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; platelet activation, thrombosis and inflammation [18].

In the present study, serum sVCAM-1 levels were increased significantly ($p<0.05$; Figure 1) and positively co-related with atherogenic index along with the markers of inflammation such as IL-6 and TNF-α (Table 3 & 4) in Post COVID KOA patients which reflect the persistence of systemic inflammation mediated endothelial activation in post COVID state and thus, making the post COVID KOA patients more susceptible to develop the CVD risk. Recently, Wong et al. also reported that pro-inflammatory cytokines activate 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 [19]. However, conversely, recovery from severe COVID-19 was associated with reductions in serum CRP, IL-18, TNF-α and sVCAM-1 levels have been well documented [18].

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 KOA 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 via lipid peroxidation which in turn play a crucial role in the development and progression of vascular complications in arthritic patients [20]. 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 KOA patients which clarify the role of oxi-inflammatory stress mediated endothelial activation in shaping the KOA patients more susceptible to develop CVD risk in post COVID state. Interestingly, increased levels of MDA were also reported in arthritis and COVID 19 patients [3]. Moreover, 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 arthritis patients, has also been well documented [21].

In order 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 KOA patients and negatively correlated with sVCAM-1 levels (Table 4) which reflects toward its utilization in preventing ROS mediated lipid peroxidation and its inactivation due to interaction of oxidized lipids with the PON free sulfhydryl group. Similar findings have been documented by Rodriguez-Tomas et al. in COVID19 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 [22].

Conclusion:

Thus, monitoring of sVCAM-1 level may be an effective "treat to target" approach from a lens of therapeutic intervention strategy in treating KOA and its associated complications. Moreover, regular assessment of markers of oxidative stress such as serum PON activity and MDA are additional approach to provide clear clinical picture with advancing of KOA. Thus, based on these observations, our study concludes that COVID19 deteriorates the human health and assessment of more sVCAM-1 and oxi-inflammatory markers incorporation along with conventional lipid profile parameters can be included to the battery of routine analysis of CVD risk determination in knee osteoarthritis patients, even in the era Post COVID 19. Interestingly, this is the first study which emphasizes the plausible connecting link between endothelial activation and atherogenic index along with marker of and oxi-inflammatory stress at a single platform in post COVID era in KOA patients. In addition, inclusion of antioxidant rich food products in diet along with regular aerobic exercise and counseling for boosting immunity and stamina by adopting healthy life style modifications are needed at regular and continuous pace for KOA population in order to reduce the burden of CVD risk.

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Table 1: Anthropometric and clinical profile of study group subjects (Mean ± SD)

S No	Particulars	Group I (n=40)	Group II (n=40)	Group III (n=40)
1)	Age (years)	38.5 ± 4.5	42.8 ± 5.0	41.2 ± 4.8
2)	M:F ratio	21 : 19	12 : 18	24 : 16
3)	BMI (Kg/m^2)	24.6 ± 1.5	25.4 ± 1.2*	28.5 ± 0.98**
4)	Systolic blood pressure (mmHg)	106.4 ± 3.5	115.0 ± 3.4	125.2 ± 3.2
5)	Diastolic blood pressure (mmHg)	76.0 ± 2.6	78.0 ± 2.2.	86.0 ± 2.4
6)	VAS pain (mm)	0.0	38.2 ± 4.8**	39.5 ± 5.0**

where,
* p<0.1: Non-significant
** p<0.05: Significant
*** p<0.001: Significant

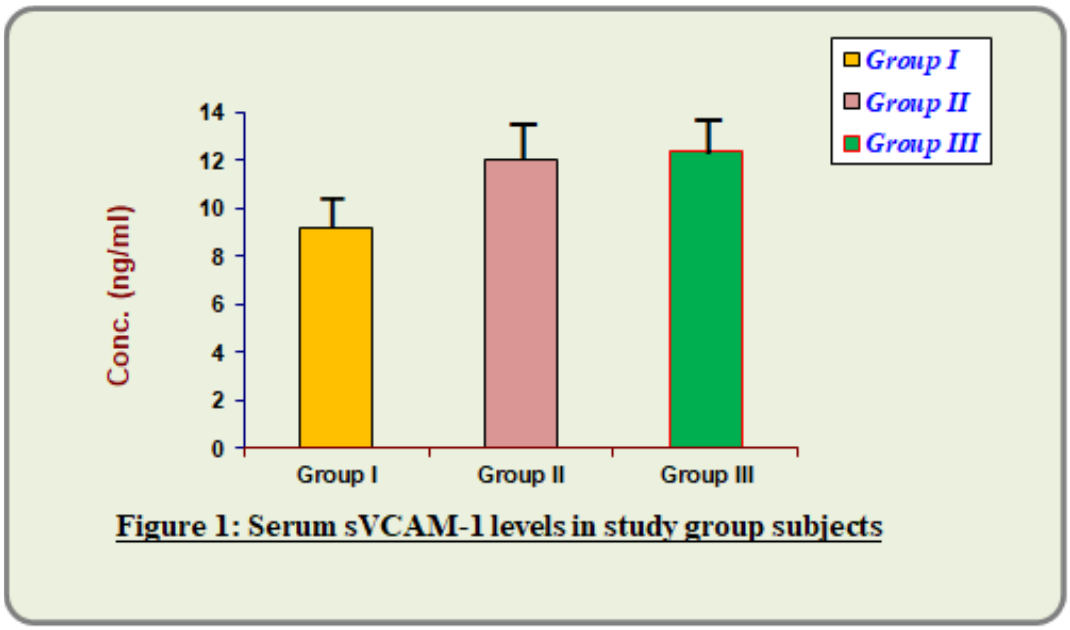

Figure 1: Serum sVCAM-1 levels in study group subjects

Table 2: Dyslipidemia in Post COVID KOA patients. (Mean ± SD)

S.No	Particulars	Group I (n=30)	Group II (n=30)	Group III (n=30)
1.	Total Cholesterol (mg/dl)	156.2 ± 7.2	188.3 ± 9.0**	215.5 ± 7.8***
2.	Triglycerides (mg/dl)	113.6 ± 8.0	130.2 ± 8.2*	145.5 ± 7.9***
3.	HDL cholesterol (mg/dl)	42.0 ± 5.2	34.4 ± 5.0**	31.0 ± 4.7***
4.	LDL cholesterol (mg/dl)	97.2 ± 9.0	127.5 ± 10.2**	152.8 ± 8.2***
5	VLDL cholesterol (mg/dl)	22.8 ± 1.4	24.5 ± 1.9*	27.5 ± 1.9***
6	TC/HDL cholesterol ratio	3.45 ± 0.70	4.48 ± 1.30**	5.42 ± 1.25***

where,

* p<0.1 : Non-significant; ** p<0.05 : Significant;*** p<0.001: Highly significant

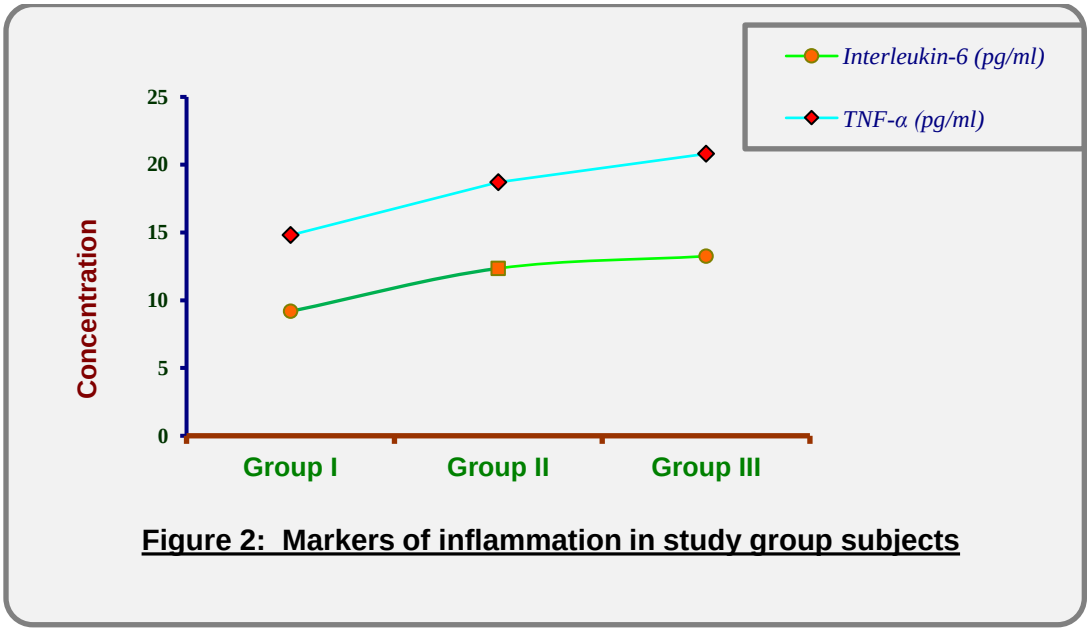

Figure 2: Markers of inflammation in study group subjects

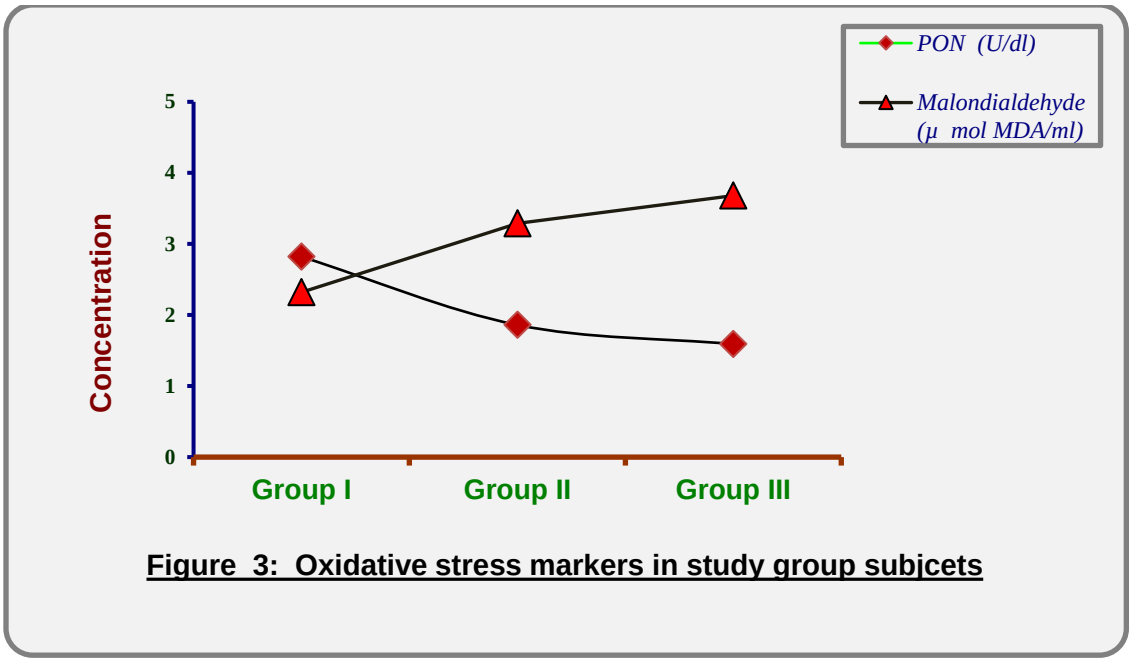

Figure 3: Oxidative stress markers in study group subjcets

Table 3. Correlation coefficient (r) between sVCAM-1 with VAS and Atherogenic index in Post COVID KOA patients.

Particulars	VAS	Atherogenic index
sVCAM-1	0.620 **	0.545 **

Where,
** p < 0.001 : Highly significant

Table 4. Correlation coefficient (r) between sVCAM-1 and oxi-inflammatory stress markers in Post COVID KOA patients.

Particulars	PON activity	MDA	IL-6	TNF-α
sVCAM-1	-0.445 *	0.628 **	0.619 **	0.514 *

Where,
* p < 0.05 : Significant,
** p < 0.001 : Highly significant