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ORIGINAL ARTICLE

STUDY OF IMMUNOLOGICAL MARKERS IN KNEE OSTEOARTHRITIS PATIENTS

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ABSTRACT

Introduction: The role of inflammation in osteoarthritis (OA) pathogenesis is unclear. Proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) play important roles in immune responses and bone metabolism. TNF-α and IL-6 enhance macrophage activation and antigen presentation and regulate immunity through different mechanisms. Hence, we aimed to study the immunological markers in knee osteoarthritis patients.

Methodology: In the present case-control study, 100 KOA cases and 100 healthy were enrolled as per inclusion-exclusion criteria. Patient data, including age, sex, Kellgren–Lawrence (KL) score, and level of immunological markers (IL-6 and TNF-alpha), were recorded and compared.

Results: The mean age in both cases [56.78±9.61] and the control group [55.37±8.42] were comparable. Males outnumbered females in both groups. The majority of patients were of OA Grade II (56%). IL-6 was higher in the case group [3.62±1.00] compared to the control group [1.66±0.67]. So was the level of TNF-alpha. At a cut-off of 2.900, the sensitivity of IL-6 was recorded as 95.00%, and the specificity was 73.00%. On the other hand, at a cut-off of 4.90, the sensitivity was recorded as 100.00%, and the specificity was 98.00%, which was more than IL-6. A significant positive correlation was noted between OA grade vs IL-6 [$p<0.0001$*; $r=0.7091$] and OA grade vs. TNF-α [$p<0.0001$*; $r=0.8127$]

Conclusion: Immunological markers will help in the early detection of osteoarthritis, thus providing early treatment and a better prognosis.

KEYWORDS: Knee Osteoarthritis, Immunological Marker, KL Grading, Interleukin 6, Tumor Necrosis Factor-alpha

INTRODUCTION

Knee osteoarthritis (KOA) is one of the leading causes of pain and disability among the elderly. [1] It is a severe joint disorder that primarily affects the knee's articular cartilage. Joint space constriction, the development of osteophytes, and sclerosis are the most frequent clinical manifestations of KOA. It is characterised by progressive degeneration, loss of articular cartilage, and anatomical and functional changes in the synovium, meniscus, periarticular ligaments, and subchondral bone of the joint. [2] KOA is more prevalent in the elderly, rotund, and sedentary. In its advanced stages, it causes intense discomfort and frequently results in total joint replacement. Even though age is the primary risk factor for osteoarthritis (OA), there are numerous other contributing factors, including genetics, heritable metabolic disorders, muscle weakness and other underlying anatomical and orthopaedic disorders (e.g., congenital hip dislocation), crystal accumulation (e.g., gout), previous rheumatoid

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arthritis (RA), and multiple disorders of bone turnover and blood clotting. [3] Therefore, biomarkers have been recommended as essential tools not only for early diagnosis and prognosis but also for medication formulation, intervention monitoring, and the development of individualised, evidence-based implementation plans [4,5]. Biomarkers of joint tissue turnover can reflect disease-relevant biological activity and provide beneficial diagnostic and therapeutic data, enabling a more rational and individualised approach to healthcare administration. Utilizing biochemical indicators in clinical settings is a relatively new phenomenon, and the optimal techniques for implementing this application are presently the subject of medical and technological development research. (i.e., the development of reliable detection methods). As prospective biomarkers for the assessment of disease burden, prognosis, and diagnosis, biochemical indicators of proinflammatory cytokines are being investigated. These investigations are ongoing. CRP, IL-1, and TNF-alpha are among the most significant proinflammatory cytokines associated with OA. Moreover, IL-6 is essential to the pro-inflammatory process of OA. Previous research has also documented alterations in the ratios of lymphocytes, monocytes, neutrophils, platelets, and uric acid during the progression of OA. Combinations of biochemical markers can improve the diagnostic specificity and sensitivity of individual biochemical markers for a given pathological condition. (e.g., cartilage loss or bone remodelling). [6] In addition, by integrating particular markers, the predictive relationship between biochemical indicators and OA can be strengthened. In this manner, our research will aid in identifying the immunological markers required for diagnosing knee osteoarthritis.

MATERIAL AND METHODS

This case-control study was conducted at the Department of Biochemistry, LNCT Medical College, Bhopal, after obtaining approval from the Institutional Ethical Committee and informed consent. A total of 200 patients aged between 40-65 years with newly diagnosed osteoarthritis were included. However, patients with a history of gout in the knee, Periarticular fracture, Joint infection, Total knee replacement, or Diabetes mellitus were excluded. Patients were randomly divided into two groups as follows: Cases: Knee osteoarthritis patients and Controls: Healthy participants. Details regarding age, gender and OA grading were noted, and a further examination of the patients was carried out, including haematological examination, level of IL-6 and TNF-alpha, which were recorded and compared. Under aseptic conditions, 5 ml of 10-12 hrs fasting venous blood was collected; 2 ml was collected in an EDTA vial, and 3 ml was collected in a plain vial. Immunological markers were analysed using an ELISA kit as per the manufacturer's protocol.

Statistical Analysis:

Data collected were entered in MS Excel and analyzed using SPSS, version 22.0, licensed to Dr RMLIMS. A descriptive summary, such as mean, standard deviation, median, IQR, range etc., was calculated for various blood parameters for LID. The dichotomous variables were presented in number/frequency and were analyzed using the Chi-square test. Statistical difference in blood parameters was determined using the Unpaired T-test and Spearman Correlation at 95% confidence interval. ROC analysis was done to determine the test's validity and appropriate cut-off levels.

RESULTS

Both cases (42.0%) and controls (48.0%) consisted primarily of males aged between 56 and 65 years. The preponderance of cases (n=56) had grade II OA, which was statistically significant. [Table-1] Cases had higher mean values for WBC, neutrophils, and monocytes than controls. Additionally, controls had higher levels of haemoglobin, platelets, and lymphocytes than cases. All of these haematological parameters showed substantial differences. [Table-2] Further evaluation of the immunological markers revealed that the level of IL-6 was substantially higher in the case group than in the control group [3.62±1.00 vs 1.66±0.67]. TNF-alpha was also considerably elevated in the case group [9.695±3.311] compared to the control group [2.482±0.3039]. [Table-3; Figure-1 and 2] Upon performing receiver operating characteristic (ROC) analysis on IL-6 and TNF-alpha, we noticed that, at a cut-off of 2.900, the sensitivity of IL-6 was 95.0% and the specificity was 73.0%, whereas at a cut-off of 4.90, the sensitivity of TNF-alpha was 100.0% and the specificity was 98.0%. Higher sensitivity and specificity indicated that these are better KOA prognostic markers. [Table-4; Figure-3 and 4] In addition, correlation analysis revealed a significant positive correlation between OA grade and IL-6 [$p<0.0001$*; $r=0.7091$]

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and between OA grade and TNF-alpha [p<0.0001*; r=0.8127]. [Table-5] Consequently, as the level of these markers increases, so does the OA grade.

DISCUSSION

Despite the fact that ageing is the leading cause of KOA, there are numerous other risk factors, such as genetics, heritable metabolic disorders, muscle weakness and other underlying anatomical and orthopaedic disorders, crystal accumulation, prior rheumatoid arthritis, and multiple disorders of bone turnover and blood clotting. [3] Recent estimates indicate that approximately 250 million people suffer from knee osteoarthritis worldwide. KOA is anticipated to become the fourth leading cause of disability among women and the eighth leading cause of disability among men. [4] The present case-control study enrolled 100 KOA cases and 100 healthy participants. In both groups, males outnumbered females. The mean age in both cases [56.78±9.61] and the control group [55.37±8.42] were comparable. **Gao K et al.** [7] recruited 239 patients (119 cases and 120 controls) with a mean age of both controls [54.96 ± 8.28] and cases [55.47 ± 9.23]. Both groups have the highest proportion of female participants. **Sargin et al.** [8] reported 38 patients (8 males, 30 females, mean age 56.8 ± 11.8 years) diagnosed with RA and 30 healthy controls were included in the study. As far as age and sex distribution were concerned, they did not find a significant difference between the two groups in our study either. **Du et al.** [9] examined 66 RA patients, 163 osteoarthritis (OA) patients, and 131 healthy controls (HC). In our study, the mean interleukin-6 was higher in the case group [3.62±1.00] compared to the control group [1.66±0.67]. Statistically, a significant difference was observed among groups [p<0.0001*]. At a cut-off of 2.900, the sensitivity was recorded 95.00%, and the specificity was 73.00%. **Stannus et al.** [10] determined that blood levels of interleukin-6 and tumour necrosis factor-alpha are linked with knee cartilage degradation in older individuals, indicating that low-level inflammation plays a role in the pathogenesis of knee osteoarthritis. In the Hartley guinea pig model of osteoarthritis, independent of age and weight, blood levels of interleukin-6 were observed to correlate favourably with the total histological score of osteoarthritis (assessing cartilage structural anomalies and proteoglycan loss). [11] The results of clinical investigations are considerably less persuasive. In 41 patients with knee osteoarthritis, interleukin-6 expression in the synovial membrane was unrelated to Kellgren-Lawrence radiological score and pain [12], while in 29 patients with knee or hip osteoarthritis, interleukin-6 in serum and urine was unrelated to clinical measures including soft tissue swelling. [13] Both of these investigations are restricted by their modest sample sizes. **Penninx et al.** [14] observed that the blood concentration of interleukin-6 was not connected with pain, stiffness, function, or ROA in 272 individuals with knee osteoarthritis but was correlated with slower walking speed. **Toncheva et al.** [15] reported that serum levels of interleukin-6 were significantly elevated in patients with active osteoarthritis (exhibiting swelling, local hyperthermia, and a high erythrocyte sedimentation rate) but not in those with inactive osteoarthritis (lacking the aforementioned symptoms) when compared to healthy controls; however, the association with JSN or cartilage loss was not reported. **Livshits et al.** [16] revealed that circulating interleukin-6 predicted the occurrence of KOA measured by the Kellgrene-Lawrence grading system in a female population. In addition, **Sakao et al.** [17] reported that interleukin-6 expression was significantly higher in subchondral bone osteoblasts with knee osteoarthritis than in subchondral bone osteoblasts without knee osteoarthritis and that interleukin-6 expression was higher in patients with severe cartilage damage than in those with mild cartilage damage. **Stannus et al.** [10] discovered that blood levels of interleukin-6 were linked with JSN in the medial tibiofemoral and entire compartment. Significantly, independent of tumour necrosis factor-alpha, baseline interleukin-6 levels were related to loss of tibial cartilage volume, and change in interleukin-6 was similarly associated with the change in tibial cartilage volume over about 3 years. These relationships were irrespective of potential confounding variables such as age, gender, BMI, tibia bone area, smoking, physical activity and another illness status. These findings imply that interleukin-6 may have a role in early-stage knee osteoarthritis cartilage loss. This may have clinical significance; for instance, if rates of loss are constant over time, it can be estimated that patients with low-level interleukin-6 (<2 pg/ml, 0.6% per annum loss in this sample) will never lose the amount of medial tibial cartilage needed to reach end-stage osteoarthritis [18] (when 60% of cartilage is lost) because it will take 100 years, whereas it will take only 15 years for patients with high interleukin-6 levels (>4.0 pg/ml, 4.1% per annum loss). This result would have significant clinical significance. In addition, **Stannus et al.** [10] discovered that the occurrence of osteophytes was larger in participants with high blood interleukin-6 levels (median) than in those with low interleukin-6 levels. This is consistent with a recent report which demonstrated that interleukin-6 production in osteoblasts isolated from osteoarthritis osteophytes was

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significantly higher than that of osteoblasts isolated from the subchondral bone without osteoarthritis [19], and suggests that interleukin-6 may play a role in osteophyte formation in osteoarthritis. However, longitudinal studies are required to confirm this. IL-6 plays a significant role in osteoarthritis cartilage matrix breakdown and bone resorption. It can stimulate the production of other cytokines, matrix metalloproteinases, and prostaglandins [20] while inhibiting the development of proteoglycans and type II collagen. [21] In the present study, the mean tumour necrosis factor-alpha was higher in the case group [9.695±3.311] compared to the control group [2.482±0.3039]. Statistically, a significant difference was observed among groups [p<0.0001*] At a cut-off of 4.90, the sensitivity was 100.00%, and the specificity was 98.00%. In osteoarthritis-affected cartilage, tumour necrosis factor-alpha mRNA levels were elevated relative to normal cartilage. [22] **Penninx et al.** [14] reported that in patients with KOA, serum levels of tumour necrosis factor-alpha were not associated with knee pain, stiffness, or radiographic scores; however, higher serum levels of the soluble receptors, tumour necrosis factor-sR1 and tumour necrosis factor-sR2 were significantly associated with greater knee pain and stiffness and tended to be associated with poorer radiographic scores. A Dutch group reported that high innate ex vivo production of tumour necrosis factor-alpha in whole-blood assay upon stimulation with lipopolysaccharide was not associated with an increased risk of osteoarthritis [23]; however, longitudinally, patients in the highest quartile of tumour necrosis factor-alpha production had a 6-fold increased risk of JSN progression over 2 years20 compared to those in the lowest quartile of tumour necrosis factor-alpha production. **Stannus et al.** [10] discovered in this investigation that serum levels of tumour necrosis factor-alpha were substantially linked with JSN in tibiofemoral compartments. **Stannus et al.** [10] findings connecting tumour necrosis factor-alpha with widespread JSN do not match the negative findings of **Livshits et al.** [16], research, possibly due to their distinct KOA measurements. Although, baseline blood levels of tumour necrosis factor-alpha were not associated with cartilage volume loss, changes in tumour necrosis factor-alpha levels were associated with changes in cartilage volume over about three years. The causal link between tumour necrosis factor-alpha and cartilage loss in early osteoarthritis deserves more investigation.

CONCLUSION

We conclude that elevated or rising levels of IL-6 and TNF- are associated with an increased risk for subsequent progression of knee osteoarthritis. Thus, IL-6 and TNF- may be worthwhile as immunological biomarkers to identify individuals at risk for the progression of knee osteoarthritis and could also be valid treatment targets. Our study had a few limitations, such as results being limited to a single tertiary care hospital that may not be generalized for all settings. Hence, it cannot be incorporated into the larger population. So multicentric analysis with high precision and accuracy may be advised for a more reliable interpretation of the data.

CONFLICT OF INTEREST- All authors declare no conflict of interest.

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REFERENCES

1. Cooper C. Epidemiology of osteoarthritis. In: Klippel JH, Dieppe PA, editors. Rheumatology. 2nd ed. London: Mosby; 1998:1–20.
2. Martin JA, Buckwalter JA. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology. 2002 Sep;3:257-64.
3. Mobasheri A. Osteoarthritis year 2012 in review: biomarkers. Osteoarthritis and Cartilage. 2012 Dec 1;20(12):1451-64.
4. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The lancet. 2012 Dec 15;380(9859):2197-223.
5. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. The lancet. 1997 May 3;349(9061):1269-76.
6. Emery CA, Whittaker JL, Mahmoudian A, Lohmander LS, Roos EM, Bennell KL, Toomey CM, Reimer RA, Thompson D, Ronsky JL, Kuntze G. Establishing outcome measures in early knee osteoarthritis. Nature Reviews Rheumatology. 2019 Jul;15(7):438-48.
7. Gao K, Zhu W, Liu W, Ma D, Li H, Yu W, et al. Diagnostic value of the blood monocyte–lymphocyte ratio in knee osteoarthritis. Journal of International Medical Research. 2019 Sep;47(9):4413-21.

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8. Sargin G, Senturk T, Yavasoglu I, et al. Relationship between neutrophil-lymphocyte, platelet-lymphocyte ratio and disease activity in rheumatoid arthritis treated with ituximab. Int J Rheum Dis 2018; 21(12): 2122–2127.
9. Du J, Chen S, Shi J, et al. The association between the lymphocyte-monocyte ratio and disease activity in rheumatoid arthritis. Clin Rheumatol 2017; 36(12): 2689–2695.
10. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis and cartilage. 2010 Nov 1;18(11):1441-7.
11. Huebner JL, Seifer DR, Kraus VB. A longitudinal analysis of serum cytokines in the Hartley guinea pig model of osteoarthritis. Osteoarthritis and cartilage. 2007 Mar 1;15(3):354-6.
12. Brenner SS, Klotz U, Alscher DM, Mais A, Lauer G, Schweer H, et al. Osteoarthritis of the knee–clinical assessments and inflammatory markers. Osteoarthritis and cartilage. 2004 Jun 1;12(6):469-75.
13. Otterness IG, Weiner E, Swindell AC, Zimmerer RO, Ionescu M, Poole AR. An analysis of 14 molecular markers for monitoring osteoarthritis. Relationship of the markers to clinical end-points. Osteoarthritis and cartilage. 2001 Apr 1;9(3):224-31.
14. Penninx BW, Abbas H, Ambrosius W, Nicklas BJ, Davis C, Messier SP, Pahor M. Inflammatory markers and physical function among older adults with knee osteoarthritis. The Journal of rheumatology. 2004 Oct 1;31(10):2027-31.
15. Toncheva A, Remichkova M, Ikonomova K, Dimitrova P, Ivanovska N. Inflammatory response in patients with active and inactive osteoarthritis. Rheumatology international. 2009 Aug;29:1197-203.
16. Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FM, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford study. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2009 Jul;60(7):2037-45.
17. 102 Sakao K, Takahashi KA, Mazda O, Arai Y, Tonomura H, Inoue A, et al. Enhanced expression of interleukin-6, matrix metalloproteinase-13, and receptor activator of NF-κB ligand in cells derived from osteoarthritic subchondral bone. Journal of Orthopaedic Science. 2008 May 1;13(3):202-10.
18. Cicuttini FM, Jones G, Forbes A, Wluka AE. Rate of cartilage loss at two years predicts subsequent total knee arthroplasty: a prospective study. Annals of the rheumatic diseases. 2004 Sep 1;63(9):1124-7.
19. Sakao K, Takahashi KA, Arai Y, Saito M, Honjo K, Hiraoka N, et al. Osteoblasts derived from osteophytes produce interleukin-6, interleukin-8, and matrix metalloproteinase-13 in osteoarthritis. Journal of bone and mineral metabolism. 2009 Jul;27:412-23.
20. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2001 Jun;44(6):1237-47.
21. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clinical Orthopaedics and Related Research (1976-2007). 2004 Oct 1;427:S27-36.
22. Amin AR. Regulation of tumor necrosis factor-α and tumor necrosis factor converting enzyme in human osteoarthritis. Osteoarthritis and Cartilage. 1999 Jul 1;7(4):392-4.
23. Riyazi N, Slagboom E, de Craen AJ, Meulenbelt I, Houwing-Duistermaat JJ, Kroon HM, et al. Association of the risk of osteoarthritis with high innate production of interleukin-1β and low innate production of interleukin-10 ex vivo, upon lipopolysaccharide stimulation. Arthritis & Rheumatism. 2005 May;52(5):1443-50.

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TABLES AND FIGURES

TABLE-1: Demographic parameters of the enrolled patients among the groups

DEMOGRAPHICS	CONTROL [N=100]		CASES [N=100]		P-VALUE
	N	%	N	%	
AGE DISTRIBUTION					X=2.393 p=0.6640
36-45	9	9.00%	5	5.00%	
46-55	42	42.00%	37	37.00%	
56-65	42	42.00%	48	48.00%	
66-75	5	5.00%	7	7.00%	
76-85	2	2.00%	3	3.00%	
MEAN±SD	55.37±8.42		56.78±9.61		t=1.104 p=0.2711
GENDER					
MALE	53	53.00%	51	51.00%	X=0.08013 p=0.7771
FEMALE	47	47.00%	49	49.00%	
OA GRADING					
GRADE I	-	-	31	31.00%	X=14.70 **p=0.0006***
GRADE II	-	-	56	56.00%	
GRADE III	-	-	13	13.00%	

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TABLE-2: Haematological parameters of the enrolled patients among the groups

HAEMATOLOGICAL PARAMETERS	CONTROL [N=100]	CASES [N=100]	T-VALUE	P-VALUE
	Mean±SD	Mean±SD		
WHITE BLOOD CELLS(WBC)	5.219±0.7423	5.562±1.310	t=2.278	**p=0.0238***
LYMPHOCYTES	3.929±0.5109	2.539±0.3668	t=22.09	**p<0.0001***
NEUTROPHILS	2.419±0.9008	3.881±1.148	t=10.02	**p<0.0001***
MONOCYTES	1.050±0.4184	1.245±0.4253	t=3.271	**p=0.0013***
PLATELETS	245.0±32.41	233.6±36.02	t=2.353	**p=0.0196***
HEMOGLOBIN	138.3±10.78	128.6±8.272	t=7.139	**p<0.0001***
RED BLOOD CELLS	5.614±0.6450	5.314±0.6806	t=3.199	**p=0.0016***

TABLE-3: Level of immunological markers of the enrolled patients among the groups

IMMUNOLOGICAL MARKERS	CONTROL [N=100]	CASES [N=100]	T-VALUE	P-VALUE
	Mean±SD	Mean±SD		
Interleukin-6 (IL-6)	1.66±0.67	3.62±1.00	t=16.2	**p<0.0001***
Tumor Necrosis Factor-alpha (TNF-α)	2.482±0.3039	9.695±3.311	t=21.69	**p<0.0001***

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TABLE-4: ROC analysis of the immunological markers of the enrolled patients

ROC ANALYSIS	AUC	Std. Error	95% CI	P value	Cut-off	Sensitivity %	Specificity %	Likelihood ratio
IL-6	0.9417	0.01422	0.9138 to 0.9695	**<0.0001***	2.900	95.00%	73.00%	3.519
TNF-α	0.9998	0.0002804	0.9993 to 1.000	**<0.0001***	4.90	100.00%	98.00%	50.00

TABLE-5: Correlation analysis of OA grade with immunological markers

CORRELATION ANALYSIS	Spearman r	95% confidence interval	P value
OA GRADE vs IL-6	0.7091	0.6302 to 0.7736	**<0.0001***
OA GRADE vs TNF-α	0.8127	0.7579 to 0.8562	**<0.0001***

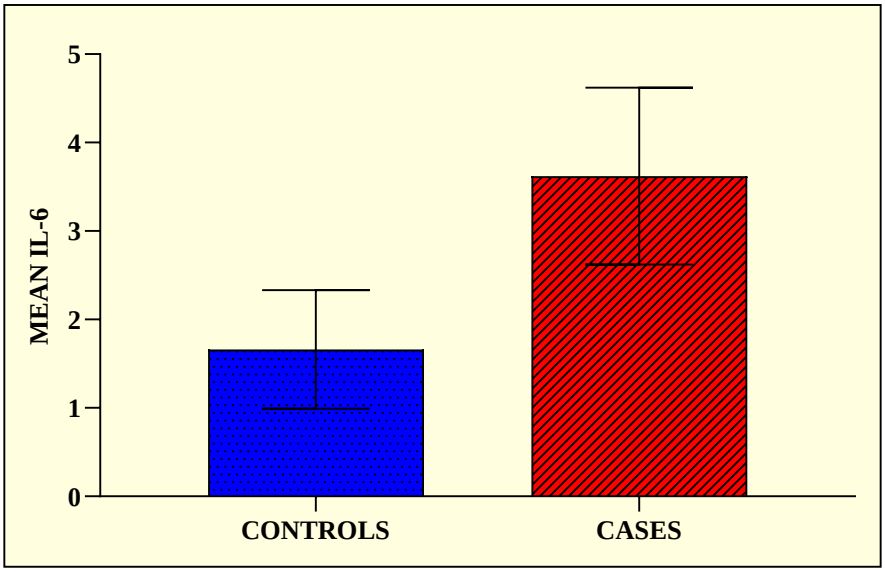

FIGURE-1: Level of IL-6 in enrolled patients among groups

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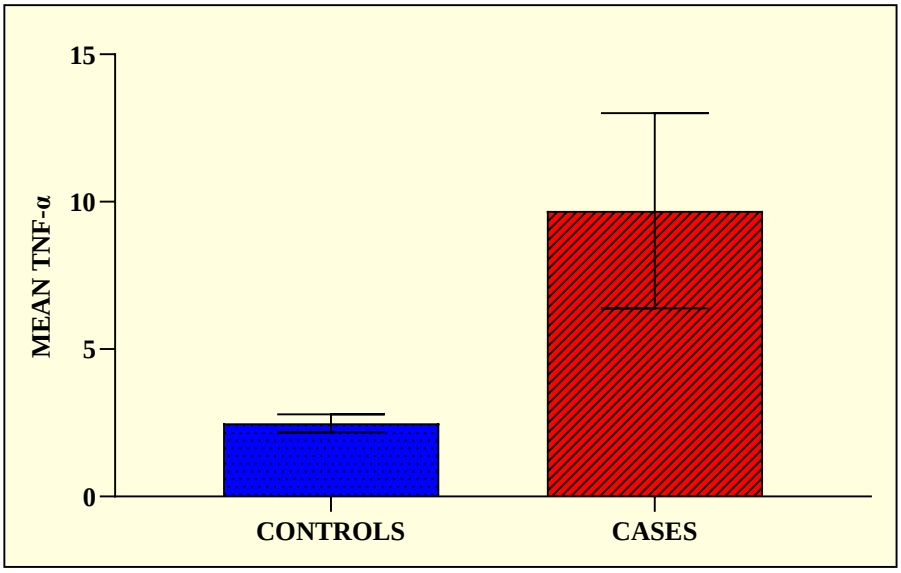

FIGURE-2: Level of TNF-α in enrolled patients among groups

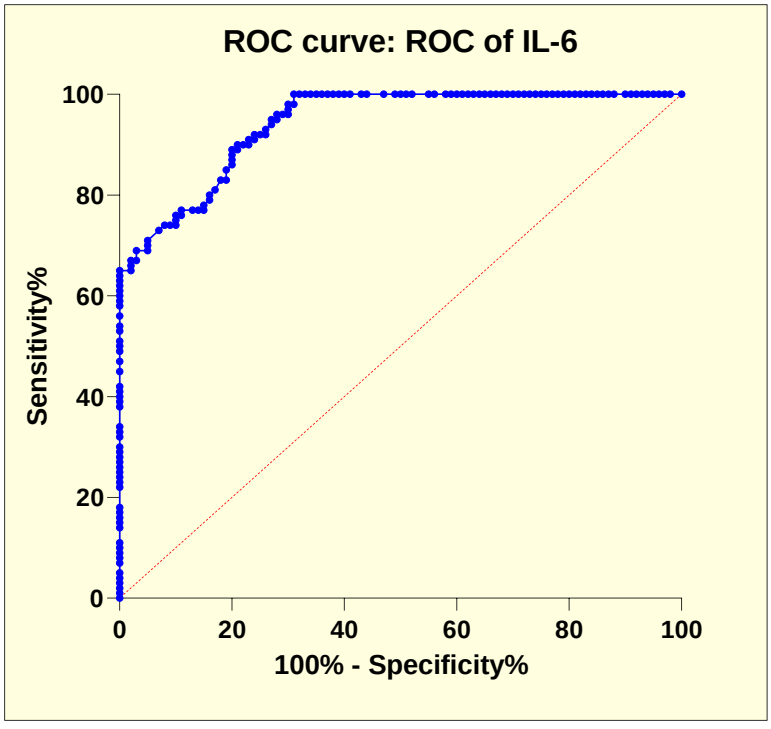

FIGURE-3: ROC curve of IL-6 of the enrolled patients

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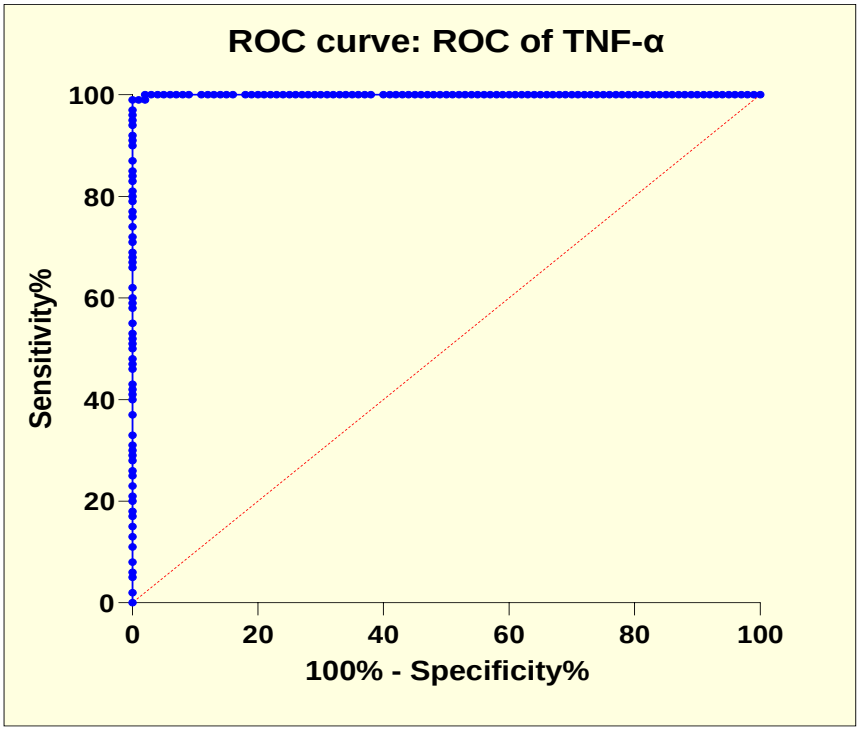

FIGURE-4: ROC curve of TNF-α of the enrolled patients