Egyptian Journal of Medical Microbiology Volume 28 / No.2 / April 2019 1-10 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Correlation between Serum Level of Interleukin-6 and Osteoarthritis Disease Activity and Disability in Beni-Suef University Hospital

[1]Abeer A. Abdelmonem*, [1]Doaa M. Ahmed, [1]Doaa Abdel Rahman Abdel Fattah*, [2]Shaimaa A. K. Rady

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Beni-Suef University,
[2]Department of Rheumatology, Faculty of Medicine, Beni-Suef University

Key words:
Interleukin-6, Osteoarthritis, WOMAC, M.HAQ, K-L score

Corresponding Author:
Abeer Ahmed Abdelmonem
Department of Medical Microbiology & Immunology,
Faculty of Medicine,
Beni-Suef University
Tel.: 01099200769
d.abeer@hotmail.com

ABSTRACT

Background: *Osteoarthritis (OA) is considered one of the most common musculoskeletal disorders and the leading causes of disability nowadays. IL-6 plays a key role in local and systemic manifestation of OA.* ***Objectives:*** *The aims of this work were to evaluate the level of serum IL-6 and its correlation with the activity, severity, disability, early development of osteoarthritis and early structural bone damage in OA patients using Western Ontario and McMaster Universities Osteoarthritis (WOMAC), Modified Health Assessment Questionnaire disability (M.HAQ) and Kellgren and Lawrence (K-L) scores.* ***Methodology:*** *This case-control study had been conducted on 40 patients with OA attending the Rheumatology and Rehabilitation outpatient clinic, Beni-Suef University Hospital from December 2017 until April 2018. The study included 20 healthy individuals as well. The following parameters were investigated: IL-6 serum level, BMI, ESR, CRP, CBCs, Kidney function tests (blood urea and serum creatinine) and liver function tests (AST and ALT). Radiological assessment was done by plain X-ray to the affected joint. Drug history was taken stressing on steroid therapy. Data were processed and analyzed using computer-based program.* ***Results:*** *The results of the study revealed higher levels of IL-6 in serum of OA patients which was significantly correlated with WOMAC, M.HAQ, K-L scores, ESR and CRP.* ***Conclusion:*** *IL-6 may play a synergistic role in OA pathogenesis and the degree of severity and disability of the disease, so it can be used as a biomarker for disease diagnosis and predictor of disease progression.*

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage breakdown, the formation of osteophytes at joint margins and generalized aches and pains [1]. Detailed information on pathogenesis of OA is not available yet. It is traditionally considered that aging, obesity, wear and tear, genetic factors and other systemic diseases might be related to its pathogenesis [2,3].

More than one study revealed increasing activity of the inflammatory factor IL-6 produced by inflammatory synovial tissues, activated chondrocytes [4]. IL-6 is mainly secreted by monocytes /macrophages, vessel endothelial cells, and fibroblasts [5].

In OA, IL-6 can stimulate chondrocytes and synovial cells to produce prostaglandin, collagenase, and metalloproteinase, which induces cartilage degradation[6]. IL-6 activates bone resorption pathways; it is a major activator of the hepatic acute-phase response [7].

IL-6 may indirectly encourage osteoclastogenesis by increasing the release of Receptor Activator of Nuclear factor κB ligand (RANK-L) by synovial cells [8].

The effects of IL-6 on osteoblasts may vary with the cell differentiation stage [9].

The progress in preventive strategies and early-stage interventions for OA is likely to depend on identification of the biologic mechanisms and biomarkers that lies behind progressive deterioration of joint structure and function.

The current study aimed to measure serum level of IL-6 in patients of OA and to correlate it with clinical parameters, disease activity and disability. This study will compare the serum concentrations of IL-6 in patients with OA to those in normal controls and then investigate the correlation between serum levels of IL-6 and the clinical parameters that indicate the disease activity and disability of OA.

METHODOLOGY

The present case - control study was conducted on 60 selected outpatient clinic of Rheumatology and Rehabilitation department, Beni-Suef university hospital; cases: 6 males and 34 females and controls: 9 females and 11 males. They are divided into 2 groups as follows:

- ***Group 1:*** Comprising 40 patients having OA and fulfilling the American College of Rheumatology (ACR) criteria and mirrored by the Osteoarthritis Research Society International (OARSI) include the presence of joint pain, osteophytes or bone spurs on X-ray and one or more associated symptoms for the diagnosis of disease for each joint separately (10). Exclusion criteria are: (a) Symptoms suggestive of any other chronic inflammatory disease; (b) History of corticosteroid treatment; (c) Any other form of arthritis as rheumatoid arthritis (RA) (d) Cancer (11). Patients were 6 male and 34 female with age range from 37 to 86 years old.
- ***Group 2:*** the control group included 20 healthy individuals of matched age, not suffering from any autoimmune disease or on interfering medications.

The study was enrolled from December 2017 to April 2018.

All subjects signed an informed consent form prior to the initiation of the study, which was approved by the Ethics Committee of the Beni-Suef Hospital University.

Methods:

All patients of this study were subjected to:

Personal history:

Name, age, sex, residence (rural – urban), special habits (smoking, coffee intake, etc….).

Full history:

The patients were asked about: 1) duration of pain, 2) Crepitus during knee movement, 3) Morning stiffness (more or less than 30 min), 4) Swelling,5) Tenderness, 6) body weight and height to calculate BMI.

BMI is the ratio between person`s weight in kg and his height in meter squared (BMI= kg/m2) Underweight: < 18.5, normal weight: 18.5 - 24.9, overweight: 25 - 29.9, obese: ≥ 30 (12).

Patients were also asked for the duration of the disease, joints pain duration, drugs used, history of diabetes, cancer, previous bone fracture, chronic inflammatory diseases and operation.

Musculoskeletal Examination:

As regards presence of swelling, effusion, hemarthrosis, scars of previous injury or surgery.

Cases were also inspected for any gait abnormality

Clinical Evaluation:

The disease activity was evaluated by using the ***a-WOMAC score*** [13]

Patients' disease activity was categorized by the WOMAC score to the following categories

1. Severe disease activity (score > 5.1)
2. Moderate disease activity (score 3.2–5.1)
3. Low disease activity (score 2.6–3.2)
4. Remission if score <2.6).

b- The extent of the patients' functional ability: was evaluated the by using M.HAQ. Scoring of the M.HAQ is patterned after the American Rheumatism Association, American College of Rheumatology as functional classes[14].

For each item, there is a four-level difficulty scale that is scored from 0 to 3 representing:

score 0: normal, no difficulty

score 1: some difficulty,

score 2: much difficulty,

and score 3: unable to do (completely disabled)[15].

c- Radiological assessments:

Plain x-ray film to the affected joint was also required to classify the severity of the disease in case of knee and hip OA according to the K-L score as follow:

- Grade 0: no radiographic features of OA are present.
- Score1: doubtful joint space narrowing (JSN) and possible osteophytic lipping.
- Score 2: definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph.
- Score 3: multiple osteophytes, definite JSN, sclerosis, possible bony deformity.
- Score 4: large osteophytes, marked JSN, severe sclerosis and definite bony deformity.

Laboratory assessments:

The laboratory tests done for each patient/ control were: CBC, Blood urea, serum creatinine, Hepatic enzymes (AST, ALT), (ESR), (CRP).

Measurement of IL-6 by ELISA:

A volume of 5 ml peripheral venous blood was withdrawn from the 40 patients as well as the 20 healthy volunteers on tubes by clean venipuncture under complete aseptic condition. Each tube was labeled by the patient's name and date of collection. Serum was separated and stored at – 80 C till the start of the assay.

Serum level of IL-6 were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (IL-6 Human ELISA Kit, Koma biotechnic, Korea)

Principle of the assay:

This assay employs a quantitative EIA technique. A monoclonal antibody specific for IL-6 has been pre-coated onto a microplate. The absorbance of specimens was read with a spectrophotometer at 450 nm by ELISA reader.

According the manufacturer instructions, a standard curve was generated by plotting the OD at (450nm) obtained for each of the standard concentrations (500, 250,125, 62.5, 31.25, pg/ml).

From this standard curve the concentration of IL-6 in the tested sample can be determined.

Statistical analysis:

Mean and standard deviation or median and range when appropriate were used to describe IL-6 concentrations and other numerical data like laboratory values.

Qualitative data were expressed as frequency and percentage. All the statistical calculations were performed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL).

The associations between baseline clinical or laboratory characteristics and serum IL-6 levels were

analyzed with Mann–Whitney U test for categorical variables and Spearman's correlation coefficient for continuous variables.

Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). A *P-value* <0.05 was considered significant.

RESULTS

Cases' and controls' mean age ±SD was 54.1±7.5 years and 55.2±7.5 years which non-significant (*P-value=0.660*).

There was no statistically significant difference between cases and controls regarding residence and associated chronic diseases *(P-value >0.05)* but, the distribution of sex was significantly unequal distributed among cases and controls *(P-value=0.001)* Table (1).

The socio-demographic characteristics of the studied groups are shown in table (1)

The mean age of cases were 54.1± 7.5 years and the control group was 55.2±7.5 years with no statistical significant difference (*P-value=0.660*).

There was no statistically significant difference between cases and controls regarding residence and associated chronic diseases *(P-value >0.05)* but, the distribution of sex was significantly unequal distributed among cases and controls *(P-value=0.001)*.

Table 1: Socio-demographic characteristics of cases and controls

Characteristics	Groups		Chi-value	*P-value*
	Cases 40(100%)	Controls 20(100%)		
Sex				
Male	6(15)	11(55)	10.5	**0.001***
Female	34(85)	9(45)		
Residence				
Urban	31(77.5)	15(75)	0.047	0.892
Rural	9(22.5)	5(25)		
Special habits				
No	35(87.5)	15(75)	1.5	0.221
Smoking	5(12.5)	5(25)		
Chronic diseases				
No	27(67.5)	19(95)	6.1	0.108
Diabetes	5(12.5)	1(5)		
Hypertension	5(12.5)	0(0)		
Liver disease	3(7.5)	0(0)		

Data presented as number and percent P-value is significant at <0.05

There were statistical significant positive moderate correlation between IL-6 and both CRP and ESR *(P-value <0.05)*, but here was no significant correlation between Il-6 and age or between Il-6 and BMI *(P-value >0.05)* Table (2).

Table 2: Correlation between Il-6 and age, BMI, ESR and CRP:

		Age	BMI	ESR	CRP
IL-6	Pearson Correlation (r)	0.124	0.204	0.275	0.379
	P-value	0.344	0.119	**0.035***	**0.016***

** Correlation is significant at the 0.05 level*

Cases had significant higher levels of IL-6 levels than controls *(P-value<0.001)*, which confirms the contribution of IL-6 in the pathogenesis of osteoarthritis (Table 3).

Table 3: Comparison between cases and controls regarding IL-6 levels

Characteristics		Mean±SD	*P-value*	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
IL-6 pg/ml	**Cases**	72.4±50.9	**<0.001***	56.2	88.7	3.1	270.2
	Controls	16±14.2		9.4	22.7	1.4	52.6

Data presented as mean ±SD **P-value is significant at <0.05* ***P-value is highly significant at ≤0.001*

There was no statistical significant difference between cases and controls regarding the CRP level but; cases had significant higher levels of ESR levels than controls *(P-value<0.001)* (Table 4).

Table 4: Comparison between cases and controls regarding the ESR, CRP levels

Characteristics		Mean±SD	*P-value*	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
ESR Mm/hr	**Cases**	46.9±21.1	**<0.001***	40.2	53.7	8	95
	Controls	13.9±2.7		12.6	15.2	8	19
CRP Mg/l	**Cases**	6.4±5.7	0.169	4.6	8.2	0.2	23.1
	Controls	4.4±3.6		2.7	6.1	1.1	17.6

Data presented as mean ±SD **P-value is significant at <0.05* ***P-value is highly significant at ≤0.001*

Frequency distribution of the clinical and radiological assessment scores of disease severity:

As regards radiological assessment scores (K-L scores), most of cases 24 (60%) had moderate score, 15 (37.5%) had sever score and only one case (2.5%) had mild score.

Most of cases 29 (72.5%) had severe disease activity (WOMAC score > 5.1) and 11 (17.5%) had moderate disease activity (score 3.2–5.1)

M-HAQ scores (for disease disability) was almost equally distributed between moderate 19(47.5%) and severe 21(52.5%) Figure (1).

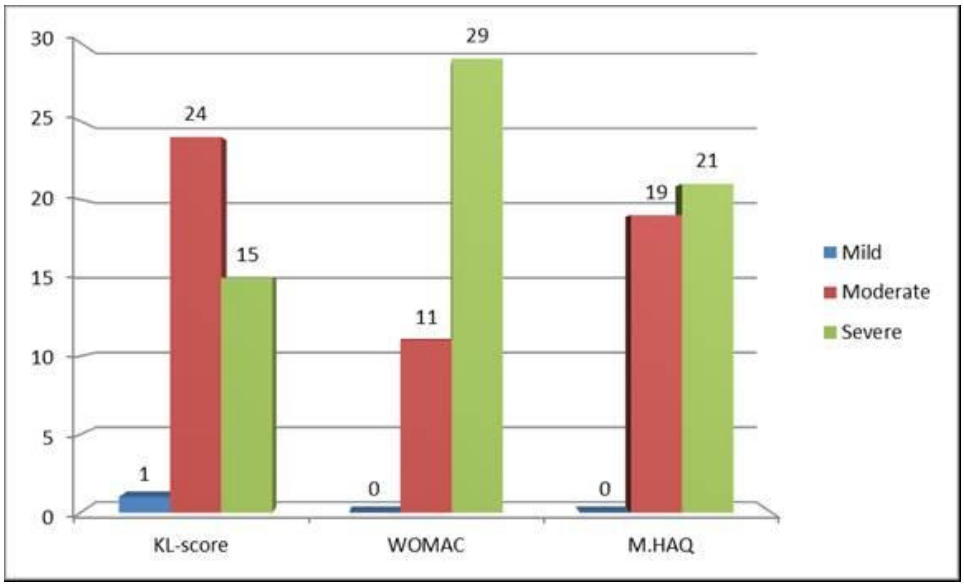

Fig. 1: Frequency distribution of the clinical and radiological assessments.

There was no statistical significant difference between different WOMAC score regarding the ESR, CRP and BMI *(P-value>0.05)* but; cases with severe disease activity had significant higher mean age than who had moderate disease activity *(P-value<0.05).* Also cases with severe disease activity had significant higher mean IL-6 than who had moderate disease activity *(P-value<0.05).* (Table 5).

Table 5: Relation between WOMAC scores and ESR, CRP, Ag , BMI and IL6 level

WOMAC		Mean±SD	*P-value*	95% Confidence Interval for Mean		Mini.	Maxi.
				Lower Bound	Upper Bound		
ESR Mm/hr	**Moderate**	47.6±15.7	0.911	37	58.1	30	80
	Severe	46.7±23.1		37.9	55.5	8	95
CRP Mg/l	**Moderate**	6.5±4.5	0.952	3.4	9.5	0.5	17.7
	Severe	6.4±6.2		4	8.7	0.2	23.1
Age Years	**Moderate**	48.3±6.7	**0.020***	43.9	52.8	38	60
	Severe	56.3±9.9		52.5	60.1	37	86
BMI Kg/m^2	**Moderate**	33.3±5.1	0.575	29.8	36.7	27.5	41.5
	Severe	34.9±9.2		31.4	38.4	25.5	68.4
IL-6 pg/ml	**Moderate**	32.9±16.9	**0.002***	21.5	44.3	3.1	63.1
	Severe	87.4±51.5		67.8	107	10.3	270.2

Data presented as mean ±SD **P-value is significant at <0.05* ***P-value is highly significant at ≤0.001*

ROC curve illustrates the potential of IL-6 as predictors of early stages of osteoarthritis activity (non-severe). At a cut-off value of 38 the sensitivity of IL6 for presence of early stages osteoarthritis was 45.5% and the specificity was 14% (AUC=0.113) Figure (2).

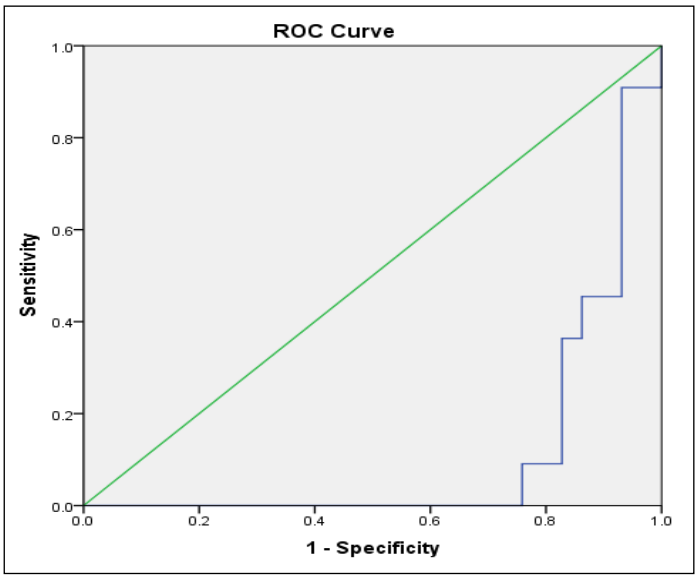

Fig. 2: Receiver operator characteristic curves: prediction of early stages osteoarthritis (non-severe) by IL-6 level (regarding to WOMAQ score).

There was no statistical significant difference between different M.HAQ score grades regarding the ESR, CRP and BMI *(P-value>0.05)* but; cases with severe scores had significant higher mean age than who had moderate score *(P-value<0.05).* Also cases with severe M.HAQ scores had significant higher mean IL-6 than who had moderate score *(P-value<0.05)* Table (6).

Table 6: Relation between MHAQ score and ESR, CRP, Age, BMI and IL6 level

M-HAQ		Mean±SD	*P-value*	95% Confidence Interval for Mean		Mini.	Maxi.
				Lower Bound	Upper Bound		
ESR Mm/hr	**Severe**	52.1±22.5	0.143	41.3	62.9	8	95
	Moderate	42.2±19.1		33.5	50.9	15	90
CRP Mg/l	**Severe**	6.5±6.3	0.944	3.4	9.5	0.2	23.1
	Moderate	6.3±5.3		3.9	8.8	0.8	22.1
Age Years	**Severe**	57.6±6.6	**0.030***	54.4	60.8	44	70
	Moderate	50.9±11.1		45.9	56	37	86
BMI Kg/m^2	**Severe**	34.2±10.5	0.870	29.2	39.3	25.5	68.4
	Moderate	34.7±5.9		32	37.3	27.5	49.2
IL-6 pg/ml	**Severe**	96.7±61.3	**0.003***	67.2	126.3	3.1	270.2
	Moderate	50.4±24.3		39.4	61.5	10.3	93.6

Data presented as mean ±SD **P-value is significant at <0.05* ***P-value is highly significant at ≤0.001*

ROC curve illustrates the potential of IL-6 as predictors of early stages of osteoarthritis (non-severe).At a cut-off value of 52.7 the sensitivity of IL-6 for presence of early stages osteoarthritis was 82.8% and the specificity was 99.9% (AUC=0.887) Figure (3).

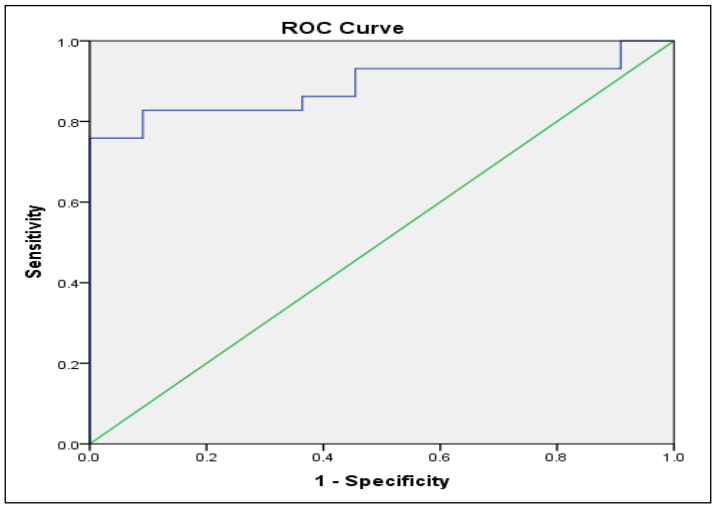

Fig. 3: Receiver operator characteristic curves: prediction of early stages osteoarthritis (non-severe) by IL-6 level (regarding to M.HAQ score)

There was no statistical significant difference between different K-L score regarding the ESR, CRP levels and BMI *(P-value>0.05)* but; cases with severe scores had significant higher mean age than who had moderate score *(P-value<0.05).* Also cases with severe K-L scores had significant higher mean IL-6 than who had mild score *(P-value<0.001)* Table (7).

Table (7) Relation between K-L score and ESR, CRP, Age, BMI and IL6 level

K-L score		Mean±SD	*P-value*	95% Confidence Interval for Mean		Mini.	Maxi.
				Lower Bound	Upper Bound		
ESR Mm/hr	**Mild**	35	0.672			35	35
	Moderate	45.3±20.9		36.48	54.2	15	90
	Severe	50.3±22.3		37.92	62.6	8	95
CRP Mg/l	**Mild**	6.9	0.846			6.9	6.9
	Moderate	5.9±5.4		3.701	8.2	0.5	22.1
	Severe	7.1±6.6		3.425	10.7	0.2	23.1
Age Years	**Mild**	38	**<0.001***			43.1	43.1
	Moderate	50.3±7.2		44.4	75.2	3.1	160.5
	Severe	61.2±9.2		58.9	130.3	16.1	270.2
BMI Kg/m^2	**Mild**	43.1	0.259			43.1	43.1
	Moderate	33±4.7		31	35	27.5	42.2
	Severe	37.1±11.8		30.5	43.6	25	68.4
IL-6 pg/ml	**Mild**	38	**<0.001***			43.1	43.1
	Moderate	50.3±7.2		44.4	75.2	3.1	160.5
	Severe	61.2±9.2				43.1	43.1

Data presented as mean ±SD **P-value is significant at <0.05* ***P-value is highly significant at ≤0.001*

ROC curve illustrates the potential of IL-6 as predictors of early stages of osteoarthritis (non-severe).At a cut-off value of 41.7 the sensitivity of IL-6 for presence of early stages osteoarthritis was 64% and the specificity was 20% (AUC=0.324). Figure (4).

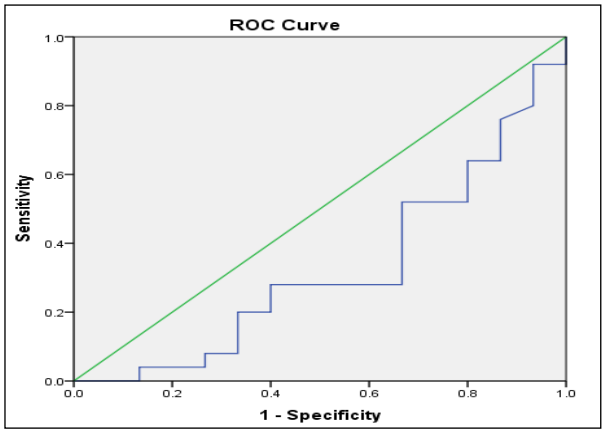

Fig. 4: Receiver operator characteristic curves: prediction of early stages osteoarthritis (non-severe) by IL-6 level (regarding to K-L score)

Table (8) summarizes the overall goal of the work and describes the importance of serum IL-6 assessment and its association of IL-6 with disease activity, severity, disability, early development of osteoarthritis and early skeletal osteoporosis in OA patients based on WOMAC, M.HAQ and Kellgren and Lawrence (K-L) scores.

A significant relationship between IL6 and M-HAQ with high sensitivity (82.8%) and specificity (99.9 %) Table (8).

Table 8: **Sensitivity and specificity of different clinical assessment scores**

IL-6	WOMAC score	M-HAQ score	K-L score
Sensitivity	45.5%	82.8%	64%
Specificity	14%	99.9%	20%

DISCUSSION

Osteoarthritis is a degenerative articular disease of unknown origin. The available evidence suggests that alterations in cartilage are the events leading to the onset of this pathology, and are followed by synovial and subchondral bone damage [1].

A study done by Livshits et al.,[21] revealed that, IL-6 is a potential biomarker for OA development and may provide useful information in the prediction of disease outcome.

In the current study, serum levels of IL-6 were measured in 60 subjects; 40 patients of OA and 20 healthy controls. This study compared the serum concentrations of IL-6 in patients with OA to those in controls and investigated the correlation between serum levels of IL-6 and the clinical parameters that indicated the disease activity and disability of OA according to WOMAC, M.HAQ and K-L scores.

In this study the mean age of the studied group with OA was 54.1 years. This agreed with previous studies of *Hess et al.*[16], who reported that aging is a risk

factor for OA, and the mean age was 53.9 years and Eberly et al.[17]; reported higher mean age (58.6years) of 355 osteoarthritis patients.

We observed a female predominance (85%) among cases with 15% of cases were males. This agreed with Eberly et al.[17] and Hess et al.,[16] who also reported female counts were higher than males, confirming the female hormonal role in the pathogenesis of OA.

Moreover, in our study there was no statistically significant difference between cases and controls regarding smoking, residence, the CRP level and associated chronic diseases. This was against the study of Pearle et al.[18]*;* who reported that 57% of patients with idiopathic OA had inflammatory infiltrates. The mean CRP level in patients with inflammatory infiltrates was significantly higher than those without inflammation *(p value 0.003).* Also, *Mabey et al.,* (19); reported that OA patients had significantly higher circulating CRP than controls *(p value>0.001).*

Our work showed that cases had significant higher levels of IL-6 levels than controls *(P-value<0.001),* which agreed with the studis of Tsuchida et al.,[20], Livshits et al.,[21] and Zhao et al.[22]; who reported that circulating levels of IL-6 were consistently and significantly higher in subjects diagnosed as having OA. We reported that the mean of WOMAC score was 63.7%, while previous studies of Dowding et al. [23] and Lenguerrand et al. [24]; which documented the mean of WOMAC score as 46.0% for the former and 44% for the later reported lower means for the same score.

In addition, there was no statistical significant difference between different WOMAC score grades regarding the ESR and BMI, but cases with severe scores had significant higher mean IL-6 and age than who had moderate score. This was on the contrary of Orita et al. [25]; who reported that IL-6 exhibited a non-significant correlation with the total WOMAC scoring method.

Our work showed that most OA patients (60.0% of cases) had moderate K-L score, 37.5% had severe score and only 2.5% had mild score. This was in consistent with Joseph et al.[26]; who investigated 141 osteoarthritis patients finding that majority of the subjects (46.8%) had moderate score (K-L2), followed by 29.1% had severe score (K-L3), then 24.1% had mild scores (K-L0/ K-L1).

We observed no statistically significant difference between different K-L scores regarding the ESR, CRP levels and BMI, but cases with severe scores had significant higher mean age and higher mean IL-6 levels than who had mild score. This was in accordance with Zhao et al.[22]; who found that comparison among OA patients with different K-L grades indicated that IL-6 in plasma was significantly associated with the severity of OA.

It was found that M.HAQ showing high sensitivity of IL-6 (82.8%) and the specificity was 99.9% that indicate its role in diagnosis and prediction of early stages of osteoarthritis.

In conclusion, the serum level interleukin-6 in osteoarthritis patients is related to the disease activity and disability which make it a useful biomarker for diagnosis of OA. It can be also used for following up of the disease as it is an important predictor for disease progression.

Recommendations:

More research is necessary to evaluate whether targeting IL-6 or its pathway may be shifted from an inflammatory marker for only diagnosis to a target for treating inflammatory diseases including OA and to be an effective way to decrease symptomatic cartilage defects in OA, also to improve symptoms of the disease. This will be performed by measuring IL-6 in patients before and after treatment. Second, further researches may be done to examine associations between more than one inflammatory cytokine at once such as the relation between levels of IL-6, IL-10 and TNF-α and osteoarthritic changes. A third point of further prospection is to measure cytokine in synovial fluid rather than levels in serum which may represent more appropriate and sensitive measure for inflammatory markers in OA.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Rahmati M, Mobasheri A, Mozafari M.: Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges Bone. 2016; 85, 81–90 .
2. Koskinen A, Vuolteenaho K, Moilanen T, Moilanen E.: Resistin as a factor in osteoarthritis: synovial fluid resistin concentrations correlate positively with interleukin 6 and matrix metalloproteinases MMP-1 and MMP-3. Scand. J. Rheumatol. 2014; 43: 249-253 .
3. Klein-Wieringa IR, de Lange-Brokaar BJ, Yusuf E, Andersen SN, Kwekkeboom JC, Kroon HM, et al.: Inflammatory cells in patients with end-stage knee osteoarthritis: a comparison between the synovium and the infrapatellar fat pad. J Rheumatol. 2016; 43(4):771–8.

4. Wang CT, Lin YT, Chiang BL, Lin YH, Hou SM.: High molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early osteoarthritis. Osteoarthritis Cartilage. 2006; 14:1237-1247.
5. Lee YA, Choi HM, Lee SH, Yang HI, Yoo MC, Hong SJ. et al.: Synergy between adiponectin and interleukin-1beta on the expression of interleukin-6, interleukin-8, and cyclooxygenase-2 in fibroblast-like synoviocytes. 2012; Exp. Mol. Med. 44: 440-447.
6. Grassel S, Beckmann J, Rath B, Vogel M, Grifka J, Tingart M.: Expression profile of matrix metalloproteinase-2 and -9 and their endogenous tissue inhibitors in osteonecrotic femoral heads. Int. J. Mol. Med. 2010; 26: 127-133 .
7. Kwan TS, Padrines M, Theoleyre S, Heymann D, Fortun Y.: IL-6, RANKL, TNF-alpha/IL-l: inter relations in bone resorption pathophysiology. Cytokine Growth Factor Rev.; 2004; 15:49–60 .
8. De Benedetn F, Rucci N, Del Fattore A, Peruzzi B, Paro R, Longo M, et al.: Impaired skeletal development in interleukin-6-transgenic mice, a model for the impact of chronic inflammation on the growing skeletal system. Arthr Rheum. 2006; 54:3551–3563.
9. Le Goff B1, Blanchard F, Berthelot JM, Heymann D, Maugars Y.: Role for interleukin-6 in structural joint damage and systemic bone loss in rheumatoid arthritis. Joint Bone Spine.; 2010; 77(3):201-205.
10. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, et al.: OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage. 2008;16(2):137-162.
11. Michael CN, David TF and Gayle L.: A knee health study, the osteoarthritis initiative protocol for the cohort study (OIA protocol), Osteoarthritis Initiative, 2006; vol. 1, page 14.
12. Blackburn H. and Jacobs D.: "Commentary: Origins and evolution of body mass index (BMI): continuing saga" (PDF). International Journal of Epidemiology. 2014; 43 (3): 665–9 .
13. Bellamy N.: WOMAC Osteoarthritis Index User Guide. Version V. American College of Rheumatology, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). 2002.
14. Lubeck DP and Fries JF.: Health status among persons infected with human immunodeficiency virus. A community-based study. Med Care. 1993;31:269–276
15. Bonnie B, and James FF.: The Stanford Health Assessment Questionnaire: Dimensions and Practical Applications. Health Qual Life Outcomes, 2003; v.1; 1-20.
16. Hess SR, O'Connell RS, Bednarz CP, Waligora AC, Golladay GJ, Jiranek WA.: Association of rapidly destructive osteoarthritis of the hip with intra-articular steroid injections. Arthroplasty Today; 2018; 4(2):205-209.
17. Eberly L, Richter D, Comerci G, Ocksrider J, Mercer D, Mlady G, et al.: Psychosocial and demographic factors influencing pain scores of patients with knee osteoarthritis. 2018.
18. Pearle AD, Scanzello CR, George S, Mandl LA, DiCarlo EF, Peterson M, et al.: Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. Osteoarthritis Cartilage; 2007; 15(5):516–523.
19. Mabey T, Taleongpong P, Udomsinprasert W, Jirathanathornnukul N, Honsawek S.: Plasma and synovial fluid autotaxin correlate with severity in knee osteoarthritis. Clin Chim Acta. 2015; Apr 15; 444:72-7.
20. Tsuchida AI, Beekhuizen M, Rutgers M, van Osch GJ, Bekkers JE, Bot AG, et al.: Interleukin-6 is elevated in synovial fluid of patients with focal cartilage defects and stimulates cartilage matrix production in an in vitro regeneration model. Arthritis Res Ther. 2012; Dec 3;14(6): R262.16.
21. 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 Rheum. 2009; 60:2037–2045.
22. Zhao XY, Yang ZB, Zhang ZJ, Zhang ZQ, Kang Y, Huang GX, et al.,: CCL3 serves as a potential plasma biomarker in knee degeneration. Osteoarthritis Cartilage. 2015; 23(8):1405-11.
23. Dowding C, Dobransky JS, Kim PR, Beaulé PE: Metal on Metal Hip Resurfacing in Patients 45 Years of Age and Younger at Minimum 5-Year Follow-Up. The journal of arthroplasty. 2018 Jun; 1. pii: S0883-5403(18)30525-4.
24. Lenguerrand E, Beswick AD, Whitehouse MR, Wylde V, Blom AW.: Outcomes following hip and knee replacement in diabetic versus nondiabetic patients and well versus poorly controlled diabetic patients: a prospective cohort study. Acta Orthop. 2018 May; 14:1-7.

25. Orita S, Koshi T, Mitsuka T, Miyagi M, Inoue G, Arai G, et al.: Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskeletal Disorders 2011; 12:144.

26. Joseph GB, Nevitt MC, McCulloch CE, Neumann J, Lynch JA, Heilmeier U, et al.,: Associations between Molecular Biomarkers and MR-based cartilage composition and Knee Joint Morphology: Data from the Osteoarthritis Initiative, Osteoarthritis and Cartilage. Osteoarthritis Cartilage. 2018; 26(8):1070-1077.