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THE INFLAMMATORY CYTOKINES IN OSTEOARTHRITIS AND THEIR EFFECT IN THE PROGRESSION AND PATHOGENESIS OF THE DISEASE

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ABSTRACT
Osteoarthritis (OA) is a widely prevalent condition that causes major disability globally. Osteoarthritis, often known as a condition primarily affecting the joint cartilage, can also impact the other components of the joint. The study aims to investigate the role of inflammatory cytokines in the progression and pathogenesis of osteoarthritis. The current study is a case-control trial conducted on 44 patients with osteoarthritis and 44 healthy individuals as a control group. The patients were diagnosed by a specialist physician at Al-Nasiriya Teaching Hospital between November 2023 and March 2024. 5 ml of blood was collected from each participant, the serum was separated by centrifugation and stored at -20°C. The levels of IL-1β, IL-6, IL-10 and TNF-α were measured using ELISA. The results showed that there were no statistically significant differences between the osteoarthritis and control group in age, residence and smoking. However, there were significant differences in body mass index, gender and employment status. Cytokine levels showed significant differences between the control group and osteoarthritis patients. IL-1β and IL-6 levels were increased and IL-10 and TNF-α levels were decreased in osteoarthritis patients compared with the control group. A significant positive correlation (r=0.546) was recorded between IL-1β and TNF-α. It is evident that inflammation contributes to the pathogenesis of the disease.

KEYWORDS: Cytokines; Osteoarthritis; Pathogenesis; IL-6; IL-1β; TNF-α.

INTRODUCTION
Osteoarthritis (OA) is the predominant kind of arthritis globally. Primary and secondary osteoarthritis are two distinct classifications of the condition. Osteoarthritis is commonly defined by joint pain and reduced functionality; its clinical manifestation can vary greatly, spanning from devoid of symptoms and accidental identification to a serious and chronically incapacitating disorder.[1] Osteoarthritis risk factors include aging, being female, being overweight, having certain physical traits, having weak muscles, and having a history of joint injuries from work or sports. Factors that increase the risk include injuries sustained during birth, inherited joint problems, inflammatory arthritis, infectious arthritis, Paget disease, osteopetrosis, osteochondritis dissecans, hemoglobinopathy, Wilson's disease, metabolic disorders like hemochromatosis and Ehlers-Danlos syndrome, and Marfan syndrome.[2,3] Osteoarthritis affects around 3.3 to 3.6% of the world population. Among the world most disabling diseases, it ranks eleventh, causing moderate to severe disability in 43 million people. Radiographic evidence of osteoarthritis is present in about 80% of Americans over 65, while only 60% of those people actually have symptoms. There are at least twice as many cases of radiographic osteoarthritis as there are of symptomatic osteoarthritis. There is no conclusive evidence that the patient's joint pain is due to osteoarthritis based on radiographic changes. Osteoarthritis was the second most expensive illness in the US in 2011, with about 15 billion dollars spent and around 1 million hospitalizations.[1,4] All of the tissues that make up a joint can be damaged by osteoarthritis. Mechanical stress, abnormal joint mechanics, and risk factors play a role in the development of osteoarthritis. Together, they trigger the release of proteases and pro-inflammatory markers, both of which contribute to the deterioration of joints.[5]

Surface fibrillation, irregularities, and localized erosions are common early changes in osteoarthritis that impact the articular cartilage. As the erosions advance across the joint surface, they eventually reach the bone. Under the microscope, cartilage damage causes the collagen matrix to break down, which in turn causes chondrocytes to proliferate and cluster. Osteophytes are the end result of a phenotypic shift in hypertrophic chondrocytes that start

with the development of cartilage outgrowths. Chondrocytes undergo cell death as the collagen matrix starts to degrade. Subchondral thickening and, in rare cases, bone cysts, are symptoms of collagen that has not mineralized adequately. Erosive osteoarthritis is already a rare condition, and bone erosions are extremely rarer.[6]

While inflammation and edema of the synovium do occur, their significance is comparatively lower than in inflammatory arthritis. Soft tissue structures, including ligaments, the joint capsule, and the menisci, are also detrimentally affected. Advanced osteoarthritis is characterized by the presence of calcium phosphate and calcium pyrophosphate dihydrate crystals. Although their precise role remains uncertain, they have the potential to exacerbate inflammation in the synovium.[7] Tumour necrosis factor (TNF-α), a potent pro-inflammatory cytokine, exerts diverse effects on various cell types and plays a crucial role in the initiation of chronic inflammatory disorders such as osteoarthritis. Increasing evidences indicate that both the soluble and transmembrane variants of TNF-α contribute to the inflammatory response. In contrast to soluble TNF-α, which operates outside the cells that produce it, transmembrane TNF-α functions by transmitting signals directly between cells. Experimental animals genetically modified to produce transmembrane TNF-α, exhibited symptoms of arthritis, including swelling and inflammation of the synovial joints.[8,9] Tumor necrosis factor-alpha is produced by T-helper 1 cells and its functions to recruit inflammatory cells, thicken the epidermis, and activate synovial fibroblasts.[10]

Furthermore, various other cytokines such as IL-1β, IL-6, IL-15, IL-17, IL-18, IL-21 were associated with osteoarthritis (OA).[11] While the effectiveness of suppressing these proinflammatory cytokines in the treatment of osteoarthritis (OA) has been investigated in a small number of human trials and animal experiments which offered evidences for this correlation. Cytokines such as IL-1, IL-6, and TNF-α stimulate synovial fibroblasts to enhance the synthesis of cathepsins and matrix metalloproteinases (MMPs), which then break down collagen and proteoglycan. Joint erosion occurs as a result of the breakdown of bone and cartilage. Osteoclasts play a crucial role in osteoarthritis (OA) by activating TNF-α, which subsequently stimulates the growth of new blood vessels (angiogenesis) and the rapid expansion of synovial tissue.[12] The aim of the current study is to analyze the concentrations of certain cytokines in osteoarthritis (OA) and their role in the advancement and development of the illness.

MATERIALS AND METHODS

This trial is a case-control trial carried out on (44) patients with osteoarthritis and (44) age-matched healthy persons as control group. Patients were diagnosed by specialist physician during their visit to Al-Nassiriya Teaching hospital through the period from November 2023 to March 2024. Verbal consent was taken from participants for blood collection and information recording. 5 ml blood sample was taken from all participants. Sera were isolated in special laboratory tubes and kept at -20 °C until use. 2 ml of blood was placed in an EDTA tube for measuring ESR levels. Anti-CCP (U/mL), CRP (mg/L), RF (IU/mL), and ANA levels were determined using a Cobas device according to the recommendations of (Roche, Germany). TNF-α, IL-10, IL-6 and IL-1β levels were calculated using ELISA test (Sunlog, China).

Statistical analysis

The statistical analysis was carried out by using SPSS (version 26) and using dependent t-tests (two-tailed) and independent t-tests (two-tailed) for normally distributed variables, whereas the Mann-Whitney and Wilcoxon test was used for those variables that were not normally distribute. Chi square test was used for percentages. P value < 0.05 in any parameter was considered significant.

Ethical approval

All participants who are going to be part of this study were properly informed and gave their verbal permission. This research was registered in, and approved by the postgraduate studies of Southern Technical University- Basrah. The committee on publication ethics at the Al-Naseriah teaching hospital, Thi-qar, Iraq, also gave its approval to carry out the study.

RESULTS

Socio-demographic and anthropometric characteristics

Table (1) described the socio-demographic characteristics in the OA and control groups, it shows a non- significant difference in age, residence and smoking between OA and control groups. While there are significant difference in the BMI, sex and employment status.

Table 1: Demographic proprieties of OA patients and healthy control.

Variables		Control group n=44	Osteoarthritis n=44	P. value*
Age (years)		47.41 ± 14.51	52.00 ± 8.06	0.18NS*
BMI (kg/m2)		26.98 ± 4.11	31.81 ± 6.02	<0.001*
Sex	**Male**	24	8	<0.01**
	Female	20	36	
Residence	**City**	36	37	0.70NS**
	Village	8	7	

Smoking	**Smoker**	7	4	0.50NS**
	Non-smoker	40	37	
Employment Status	**Employee**	27	12	<0.05**
	Private Job	0	0	
	Housewife	12	27	
	Not working	5	5	

* t-test test ** Chi-square test NS: Non-Significant

Comparison of cytokine levels between control groups and Osteoarthritis patients

In comparing the mean levels of cytokines between the control group and osteoarthritis patients, IL-1β levels were increased in the osteoarthritis group (19.56 ± 8.46 pg/mL) compared to (12.01 ± 2.56 pg/mL) in the control group (P<0.001). IL-6 levels, were also increased in the patients compared to the control group (5.22 ± 1.54 VS. 4.10 ± 1.64 pg/mL, P<0.01). IL-10, was declined among osteoarthritis patients (13.29 ± 5.05 pg/mL) versus (16.62 ± 4.22 pg/mL) for the control group (P<0.01). TNF-α levels in osteoarthritis patients were lower (12.81 ± 4.00 ng/mL) compared to the control group (16.41 ± 5.96 ng/mL) (P<0.01). These data indicate important changes in the cytokine environment in osteoarthritis patients compared to controls.

Table 2: The cytokine levels between control groups and Osteoarthritis patients.

Parameters	Control group (n=44) Mean±SD	OA group (n=44) Mean±SD	P. value
IL-1β (pg/mL)	12.01 ± 2.56	19.56 ± 8.46	<0.001
IL-6 (pg/mL)	4.10 ± 1.64	5.22 ± 1.54	<0.01
IL-10 (pg/mL)	16.62 ± 4.22	13.29 ± 5.05	<0.01
TNF-α (ng/mL)	16.41 ± 5.96	12.81 ± 4.00	<0.01

Table (3) A significant positive correlation between IL-1β and TNF-α (r=0.546) was recorded in osteoarthritis patients, while there is non-significant correlation among other parameters.

Table 3: Correlation of the study markers among osteoarthritis patients.

Parameters		Age	BMI	IL-6	IL-1β	IL-10
BMI	**Pearson Correlation**	.077				
	Sig. (2-tailed)	.618				
IL-6	**Pearson Correlation**	.060	.309			
	Sig. (2-tailed)	.733	.070			
IL-1β	**Pearson Correlation**	-.121	.073	.216		
	Sig. (2-tailed)	.458	.656	.234		
IL-10	**Pearson Correlation**	.142	-.151	-.231	-.227	
	Sig. (2-tailed)	.407	.379	.219	.189	
TNF-α	**Pearson Correlation**	-.062	.046	.091	.546**	-.116
	Sig. (2-tailed)	.722	.791	.645	.001	.542

DISCUSSION

IL-1β and IL-1α are produced as pro-proteins that are tethered to the cytoplasm in the absence of signal peptides. Unlike pro–IL-1α, the biologically inactive pro–IL-1β requires caspase 1, for processing. The physiological impacts of IL-1β depend on its processing by caspase 1, which is linked to its release. In addition, IL-1 undergoes a similar process. The IL-1 biological action is dependent on a two-step process. These effects are activated by the 'inflammasome' complex: first, mRNA induction occurs, followed by the processing of the pro-molecule before it is secreted by the cells.[13]

Our results revealed higher levels of IL-1β in individuals with OA compared to healthy control, and this is agree with Lee et al.[14] and Yang et al.[15] In osteoarthritis (OA), IL-1β triggers harmful processes, including the deterioration of cartilage, via activating the mitogen-activated protein kinase (MAPK) signaling pathway. P38 MAPKs, extracellular signal-regulated kinases (ERKs), and c-Jun N-terminal kinases (JNKs) are supplementary constituents of the MAPK signaling pathway. The activation of ERK by IL-1β leads to the dysregulation of aggrecan genes and a decrease in the production of type II collagen. Consequently, the rate at which cartilage extracellular matrix (ECM) is synthesised is decreased. Moreover, the JNK signaling pathway blunts the function of SOX-9, therefore impeding the production of collagen.[16] The cytokine interleukin-6 (IL-6) is a significant contributor to the inflammatory response in osteoarthritis (OA). Additional cytokines belonging to the IL-6 family, such as oncostatin M and adiponectin (a member of the adipokine family), also significantly contribute to this immune response. Acquiring a comprehensive understanding of the molecular mechanisms involved in the interaction between IL-6-

type cytokines and the soluble form of the IL-6 receptor (sIL-6), the membrane form of the IL-6 receptor (mIL-6Rα/gp130), and other comparable receptors present on cell membranes is crucial.[17]

The current results showed a significant increase in interleukin-6 levels in the osteoarthritis group compared to the control group, and these results are consistent with Livshits et al.[18] Considering that IL-6 is the main tissue affected in osteoarthritis (OA), previous research have mostly focused on investigating its effects on cartilage structure. While the specific mechanisms by which IL-6 affects cartilage remain unclear, it has been observed to possess both catabolic and protective characteristics. Initial studies focused on the traditional signaling pathway of IL-6 and usually determined that IL-6 had beneficial effects, such as activating tissue inhibitor of metalloproteinases (TIMPs).[19] Furthermore, although it did not impact the synthesis of proteoglycan in bovine or human chondrocytes, the commonly used IL-6 signaling route only slightly enhanced the production of proteoglycan in human osteoarthritis chondrocytes. Evidence of adverse consequences of conventional IL-6 signaling in cartilage has also been demonstrated. Protein production was suppressed by IL-6 in both human cartilage explants and rabbit chondrocytes.[20] Generously synthesised by several immune cells, regulating cytokines such as IL-10 function as anabolic agents. Resident on both immune and non-immune cells, the interleukin 10 receptor (IL-10 R1 and R2 subunits) is The study revealed a notable decrease in interleukin 10 levels among the older adult patient group compared to the control group. These results are consistent with the findings of Li et al.[21] Despite the lack of definitive findings, IL-10 has been studied for its potential to decrease inflammation and safeguard cartilage in the management of osteoarthritis (OA). IL-10 exacerbates inflammation by enhancing the activity of B cells and the quantity of Fc receptors on antigen-presenting cells. Occasionally, this can counteract its potent anti-inflammatory properties. This, in addition to the short half-life of IL-10, has been cited as a potential explanation for the relatively underwhelming outcomes.[22]

IL-10 plays a vital role in protecting cartilage. It enhances collagen type II and aggrecan synthesis, which are crucial proteins in the cartilage extracellular matrix. Additionally, it alters the metabolic processes of proteoglycans, reduces the activity of MMPs, and inhibits the apoptosis of chondrocytes, similar to IL-4.[23] Thyrotropin-alpha (TNF-α) is a potent cytokine crucial for the body's response to inflammation of its tissues. Therefore, it contributes to the process of cell differentiation, population growth, and programmed cell death. Biomedical researchers have discovered that the protein TNF-α has the ability to induce cell death and induce necrotic processes, leading to the shrinkage of specific tumor forms. Interleukin-1 (IL-1) is widely believed to be the primary determinant of inflammation and the development of arthritis. By comparison to the control group, our findings revealed a significant elevation in TNF- levels among those with OA. These findings align with the results reported by Moelants et al.[24] and Koyama et al.[25] Both IL-6 and TNF-α played crucial roles as mediators in the advancement of osteoarthritis. Hence, both anti-cytokine therapy and the cytokines themselves have been thoroughly studied in relation to osteoarthritis (OA), both in clinical and experimental settings.[26]

CONCLUSION

The results indicate significant changes in cytokine levels between OA patients and controls, reflecting a pivotal role of inflammation in disease progression. Increased levels of IL-1β and IL-6 and decreased levels of IL-10 and TNF-α may contribute to the inflammatory process and joint deterioration. These findings support the hypothesis that cytokine balance is a key factor in OA pathogenesis, which may open the way for new targeted therapies.

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