The Association between Modifiable Risk Factor with Inflammatory Marker in Knee Osteoarthritis Women

Arnadi Arnadi[1]* , Afriwardi Afriwardi[2], Hirowati Ali[3], Roni Eka Sahputra[4]

[1]Department of Biomedical Science, Faculty of Medicine, Universitas Andalas, Padang, Indonesia; [2]Department of Physiology, Faculty of Medicine, Universitas Andalas, Padang, Indonesia; [3]Department of Biochemistry, Faculty of Medicine, Universitas Andalas, Padang, Indonesia; [4]Department of General Surgery, Faculty of Medicine, Universitas Andalas, Padang, Indonesia

Abstract

BACKGROUND: Interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are vital inflammatory cytokines in the pathophysiological process of osteoarthritis (OA). Several risk factors can increase the expression of these cytokines, such as body mass index (BMI), physical activity, and menopausal status.

AIM: This study aims to determine the relationship of modifiable factors with synovial fluid IL-1β and TNF-α levels in knee OA women.

METHODS: The cross-sectional study was conducted at outpatient clinics of orthopedic Arifin Achmad Hospital and Ibnu Sina Hospital in Pekanbaru City. A total of 93 women with knee OA were taken as samples by consecutive sampling. Data were obtained directly from respondents by conducting interviews using a questionnaire, measuring weight and height, and examining levels of IL-1β and TNF-α from the synovial fluid using the enzyme-linked immunosorbent assay method. The data were processed computerized using the Person correlation test, one-way analysis of variance, and *t*-test. The statistical analysis results were considered significant if the p-value was 0 <05.

RESULTS: The average age of subjects was 60.67 ± 9.99 years, 87.8% aged ≥ 40 years, 84.9% had menopause, and at most had moderate physical activity degrees (51.6%). The mean BMI was 27.18 ± 4.17, the average of IL-1β 424.73 ± 188.01 pg/mL, and TNF-α 105,17 ± 48.98 ng/L. There was a significant positive correlation with moderate strength between BMI and levels of IL-1β and TNF-α synovial fluid (p = 0.037, r = 0.217, and p = 0.047, r = 0.207).

CONCLUSION: BMI is a risk factor for IL-1β and TNF-α levels in synovial fluid of knee joints in women with OA, but physical activity and menopausal status are not risk factors.

Introduction

Osteoarthritis (OA) is a musculoskeletal disease characterized by the destruction of joint cartilage and narrowing of the joint space. This condition is one of the most common health problems and causes symptoms in elderly and middle-aged people. This degenerative and progressive joint disease affects about 250 million people worldwide and more than 27 million people in the United States, and about 35% of patients are over 65 years old [1].

The Framingham study reported that knee OA in adults aged 45 was 19.2%. At the same time, the results of the third survey of the National Health and Nutrition Examination Survey (NHANES III) found 37% of OA experienced by adults aged >60 years, only 50% of patients aged over 65 years who showed radiological features consistent with OA. In comparison, 10% of men and 18% of women only showed clinical symptoms of OA. An increase in OA cases is also seen in Asia. Knee OA in the Chinese population aged 60 years is 22% in men and 43% in women, and this prevalence is 45% higher than in the white US population. In a rural population in Japan, it is reported that the prevalence of knee OA in women is 30% and 11% in men. Baseline data on OA prevalence are not available in many Southeast Asian countries. In 2006, a study on the prevalence of knee OA in Thailand found that nearly 60% of men aged 40–79 years had knee OA. In contrast, an epidemiological study conducted on 170 men and 488 women aged over 40 years in Vietnam found that 31% of men and 35% of women have knee OA. These figures show that the prevalence of knee OA in Southeast Asia is comparable to that of the Japanese and Caucasian populations [2].

Along with the increase in life expectancy, by 2025, the WHO estimates that the elderly population in Indonesia will increase 414% compared to 1990. It can be understood that the older the age, the higher the likelihood of developing OA, and around 10% of OA sufferers will experience disability [3], [4]. In Indonesia, the prevalence of radiologically diagnosed knee OA reaches 15.5% in men and 12.7% in women, 5% occurs at the age <40 years, 30% at the age of 40–60 years, and 65% at the age>61 years [5]. In Indonesia, the prevalence of total OA was 34.3 million people in 2002

and increased to 36.5 million people in 2007 [6],[7]. It is estimated that 40% of the population aged over 70 years suffer from OA, and 80% of OA patients have a limited range of motion with various degrees [8]. The prevalence of OA in Riau Province is not known with certainty. The results of the study by Suari *et al.* found that the number of OA sufferers in the surgical department of Arifin Ahmad Hospital for the period January 2011–December 2013 was 198 cases of OA, the most of which were female (63.6%), the highest at age >60 years (42.4%)), and mostly attack the knee joint (83.3%) [9].

There is no therapy that can cure the effect of OA. Modifying the disease course through understanding the pathogenesis may reduce symptoms or even prevent the occurrence of OA. Inflammatory reactions during the OA process will trigger an increase in MMP3 synthesis. Biomechanical changes that occur during OA will stimulate chondrocytes to synthesize catabolic cytokines [10]. Several studies have shown that chondrocyte cells will produce several inflammatory mediators, such as interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in the joint tissue fluid of patients with OA. These cytokines will inhibit the compensatory pathways of chondrocyte synthesis required to restore the integrity of the degraded extracellular matrix (ECM) and upregulate MMP-3 of human synovium and chondrocytes [11].

Cytokines is produced by synovium and chondrocytes, especially IL IL-1β and TNF-α, plays an essential role in cartilage degradation. IL-1β is a critical cytokine for the early stages of OA. IL-1β increases the breakdown of the ECM of cartilage. It increases the collagenolytic activity of metalloproteases, and the activity of nitric oxide, which can induce chondrocyte apoptosis. Furthermore, IL-1β causes changes in cartilage homeostasis and reduces the activity of growth factors, such as TGF-beta. IL-1β will also suppress the synthesis of collagen and type II proteoglycans, inhibit the transformation of growth factor-ß to stimulate chondrocyte cell proliferation, and reduce the expression of collagen cartilage matrix types II and IX [12], [13], [14]. TNF- is secreted by the same cells in joints that synthesize IL-1β, and increased concentrations also occur in synovial fluid, synovial membranes, cartilage, and the subchondral bone layer. The effects of IL-1β and TNF-α can cause chondrocyte cells to tend to age faster and induce apoptosis [15], [16].

The development and severity of OA in all individuals are not the same, and this is still a challenge. Non-genetic factors such as obesity, history of arthrosis injury, occupational activity, sex hormone, structural changes, meniscectomy, gender, and age contribute to OA initiation and development. These factors will increase the inflammatory reaction in the joints to aggravate the clinical symptoms of OA [17]. Being overweight is one of the risk factors

for OA. Population-based studies have consistently demonstrated an association between overweight or obese and knee OA. Data from the first NHANES I showed that obese women had a nearly four times greater risk of developing knee OA than non-obese women, while obese men had almost five times the risk. In a Framingham study, overweight individuals in their thirties without knee OA were at greater risk for developing the disease later in life [18], [19].

The role of estrogen in OA has been a longstanding theme in OA research. OA symptoms are more pronounced in women than men, and most studies show a ratio of three women for every one to two men who have OA. OA often begins pre-menopausal and increases more rapidly in postmenopausal women than in men of the same age. More and more studies prove the role of estrogen in maintaining joint articular tissue homeostasis. The increasing prevalence of OA in postmenopausal women is associated with estrogen receptors (ER) in joint tissue. This association suggests a potential protective role of estrogen on OA development [20], [21].

Recent evidence suggests that OA is more common in populations who perform physically strenuous work, especially in those whose occupations involve bending the knees such as kneeling or squatting, for example, carpenters, miners, and others with a history of occupational exposure to repeated squatting or kneeling. It is estimated that 5% of cases of OA are work-related, and 20% of all knee OA symptoms can occur as a result of work that involves repetitive knee use [20].

A better understanding of the risk factors that underlie the development of OA has not been widely developed, so it is urgently needed to identify high-risk individuals in prevention and treatment efforts and to neutralize IL-1β and/or TNF- in upregulation of MMP expression, furthermore to be developed as an alternative medical therapy for OA [15].

Methods

An observational research with a cross-sectional study design was conducted on women with a diagnosis of knee OA who were treated at the orthopedic outpatient clinic, Arifin Achmad Hospital and Ibnu Sina Hospital, Pekanbaru City. A total of 93 women with knee OA were taken as samples by consecutive sampling according to the inclusion and exclusion criteria, then signed the informed consent. Samples were women, knee OA, and were willing to be research subjects. The sample is not having a fever or suffering from an infectious disease. Exclusion criteria in this study were secondary OA, other arthritic diseases, and patients whose synovial fluid could not be collected. Data from research subjects were obtained directly from modifiable

risk factors (body mass index [BMI], physical activity, and menopausal status) and inflammatory marker levels (IL-1β and TNF-synovial fluid). The characteristics were obtained through interviews using a questionnaire and the degree of physical activity was assessed using the International Physical Activity Questionnaire [22]. BMI was determined by directly measuring the weight and height of the research subjects. Body weight was measured using a Seca digital scale with an accuracy of 0.06–0.01 kg, while body height was measured using a Stanley Malo microtoise with an accuracy of 0.1 cm. OA was diagnosed using radiological features with Kellgren and Lawrence criteria. The levels of IL-1β and TNF- were examined from the synovial fluid using the enzyme-linked immunosorbent assay (ELISA) method with manual ELISA from Bioeureaux and IL-1β reagent from Quantikine, while TNF- was examined using the Human TNF- ELISA kit issued by eBioscience. The data were processed, computerized, and analyzed using the Person correlation test, Chi-square test, analysis of variance, and T-Test. The results of statistical analysis were considered significant if $p < 0.05$. Research approval was obtained from the health and research ethics committee, Faculty of Medicine, University of Riau No.B/147/UN19.5.1.18/UEPKK/2021.

Results

The sample obtained based on the inclusion criteria was 100 people. After screening based on the exclusion criteria and completeness of the data, it was found that five people had extreme values, and two people had incomplete data. Hence, the number of samples that could be analyzed was 93 people. The description of the characteristics of the research respondents can be seen in the following table.

From Table 1, it is known that respondents are generally aged >40 years (87.8%) with an average

Table 1: Frequency distribution of research subjects based on characteristics

District	Frequency (%)
Age (years)	
<40	22.2
≥40	91 (87.8)
Occupation as	
Housewife	79 (84.9)
Government employees	12 (12.9)
Private	2 (3.2)
Education	
Low	27 (29)
Middle	13 (14)
High	53 (57)
Menopause status	
Not menopause yet	14 (15.1)
Menopause	79 (84.9)
Physical activity	
Light	10 (10.8)
Moderate	48 (51.6)
Heavy	35 (37.6)
BMI	
No risk	15 (16.1)
At risk	78 (83.9)
Mean age (years) (minimum–maximum)	60.67 ± 9.99 (34–87)

BMI: Body mass index.

age of 60.67 + 9.99 years, the most occupations are housewives (84.9%), more than half of the respondents have higher education (57%), most of them were menopausal (84.9%), and most of the research subjects had moderate levels of physical activity, as many as 48 respondents (51.6%).

Table 2: Average of body mass index, weight, levels of interleukin-1 beta, and tumor necrosis factor-alpha

Variables	Mean ± SD
BMI	4.17
Weight (kg)	64.44 ± 10.56
IL-1β (pg/mL)	424.73 ± 188.01
TNF-α (ng/L)	105.17 ± 48.98

SD: Standard deviation, BMI: Body mass index, IL-1β: Interleukin-1beta, TNF-α: Tumor necrosis factor-alpha.

In Table 2, it can be seen that the average BMI is 27.18 + 4.17, the average body weight is 64.44+10.56 kg, the mean IL-1β 424.73 + 188.01 pg/mL and the mean TNF- 105, 17 + 48.98 ng/L. To see the relationship between the two variables, bivariate analysis was performed. The relationship between BMI, body weight, and age variables with levels of IL-1β, and TNF- was analyzed using the Pearson correlation test (Figures 1 and 2).
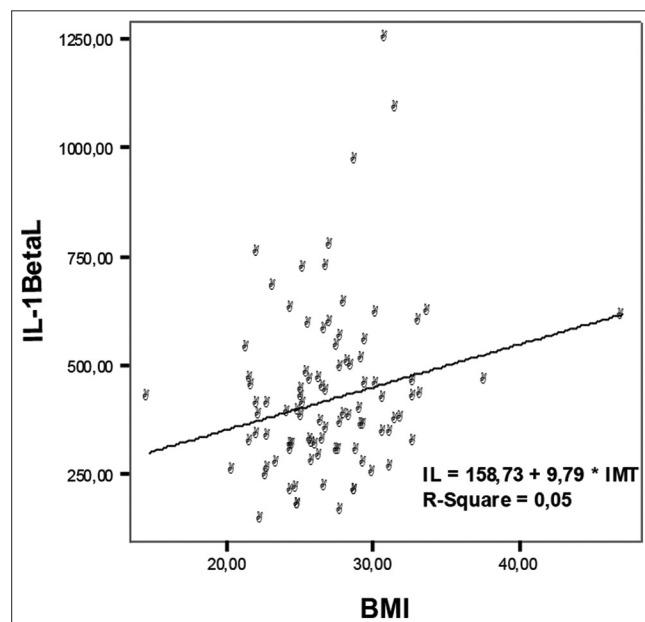

Figure 1: Correlation of body mass index with interleukin-1 beta and tumor necrosis factor-alpha levels

In Figures 1 and 2, it can be seen that the higher the BMI of the research subjects indicating the higher the levels of I IL-1β and TNF-α in synovial fluid. There is a significant positive correlation with moderate strength between BMI with levels of IL-1β and TNF- synovial fluid of research subjects (BMI with IL-1β $p = 0.037$, $r = 0.217$ and BMI with TNF- $p = 0.047$, $r = 0.207$).

In Table 3, it can be seen that the average levels of IL-1β and TNF-α were greater in research subjects with light physical activity than moderate and heavy physical activity. Synovial fluid IL-1β and TNF- levels in subjects who have not menopause are higher than those who have menopause, but at risky BMI, synovial fluid IL-1β and TNF- levels are higher
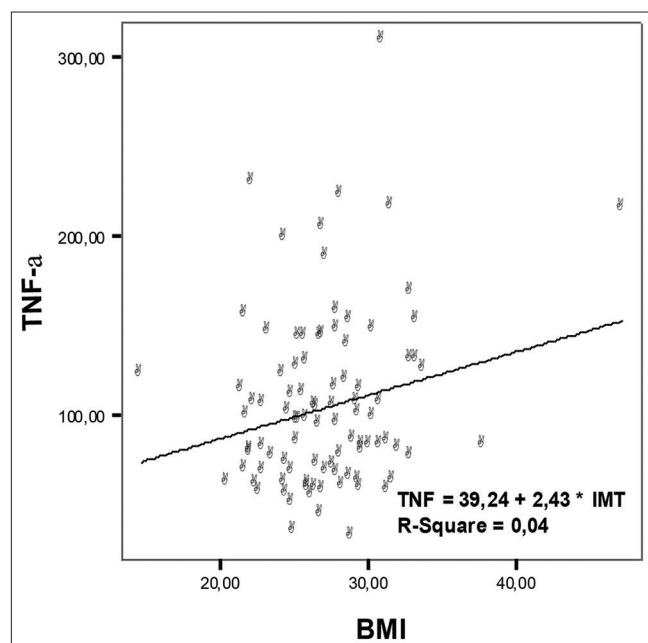
Figure 2: Correlation of body mass index with interleukin-1 beta and tumor necrosis factor-alpha levels

than those without risk. There was no difference in levels of IL-1β and TNF- synovial fluid based on the degree of physical activity, menopausal status, and BMI at risk (p > 0.05).

Table 3: Relationship of physical activity, menopause status, and body mass index at risk with levels of interleukin-1 beta and tumor necrosis factor-alpha synovial fluid

Variables	Mean ± SD	
	IL-1β	TNF-α
Physical activity		
Light	443.03 ± 145.24	110.19 ± 48.76
Moderate	416.04 ± 192.79	101.29 ± 49.89
Heavy	431.42 ± 196.04	109.05 ± 48.76
p	0.89	0.74
Menopausal status**		
Not menopause yet	436.79 ± 254.92	109.03 ± 66.21
Menopause	422.59 ± 175.59	104.48 ± 45.79
p	0.79	0.75
BMI		
No risk	377.22 ± 144.96	99.21 ± 45.38
At risk	433.87 ± 194.65	106.31 ± 49.84
p	0.29	0.61

**t-test. P<0.05 (significant). SD: Standard deviation, BMI: Body mass index, IL-1β: Interleukin-1beta, TNF-α: Tumor necrosis factor-alpha.

Discussion

Obesity and overweight are vital risk factors for OA, primarily knee OA. In obesity, skeletal muscle becomes loaded with intramuscular fat, which is associated with elevated levels of systemic pro-inflammatory biomarkers. Low-grade systemic inflammation is considered a hallmark of obesity. It is manifested by an increase in IL 1β and TNF-α as a local inflammatory response in the synovial fluid of joints affected by OA [23]. This study found that 83.9% of the study subjects had a risky BMI (obesity and overweight) with a mean BMI of 27.18 + 4.17. The average BMI

in this study was higher than that of Munthe (2017) at RSU UKI Jakarta with an average BMI of 25.6 and the study of Samma *et al*. (2019) on 60 OA patients at the Orthopedic Department of Wahidin Sudirohusodo Hospital with an average BMI of 26.7 ± 1.8. This result is lower than the study of Afolabi *et al*. in outpatients at the Universiti Sains Malaysia Hospital HUSM surgery poly. Afolabi's *et al*. study reported that the BMI of patients with OA was 40.97 + 3.59. The difference in these results was due to the different criteria used in the sample, wherein our study, all study subjects were women, while the other studies consisted of men and women. Besides that, the research design is also different, Afolabi *et al*. has a cohort study design, and our study is a cross-sectional study. The difference in location and lifestyle will also affect BMI in each study. Obesity causes changes in the structure and composition of joint cartilage. The process of cartilage damage will occur and cause abnormal joint cartilage formation and activate inflammatory mediators that damage the knee joint enzymatically. This will increase the production of pro-inflammatory cytokines. Then adipose tissue will produce adipokines that trigger synovial inflammation, cartilage degradation, and bone matrix remodeling [24], [25], [26]. Adipose tissue also secretes leptin; increasing leptin levels will increase the synthesis of pro-inflammatory cytokines and cause accelerated cartilage degradation. The results of our study found that there was a significant positive correlation with moderate strength between BMI with IL-1β and TNF-α. The higher the BMI, the higher the IL-1β and TNF-α. This result is different from the study of Vidal P in the Caucasian population in Lausanne, Switzerland (2003-2006), which reported that there was no significant relationship between IL-1β levels and BMI. Still, on the contrary, there was a substantial relationship between TNFα levels based on BMI in the female population [27].

TNFα, together with IL-1β, is considered critical inflammatory cytokines involved in the pathophysiological processes occurring during OA. It is one of 19 ligands in the TNF superfamily. TNFα is secreted by the same cells in joints that synthesize IL-1β, and increased concentrations were also observed in the same elements, such as synovial fluid, synovial membrane, cartilage, and the subchondral bone layer [16], [28].

The relationship between physical activity and OA risk is still being debated among researchers. In general, physical activity can increase the risk of OA. The Framingham study reports that people who engage in relatively high activity levels have a threefold greater risk of developing knee OA than sedentary people. Similar findings were also reported in another study in which women who had a high level of physical activity had a high prevalence of hip OA, but, on the other hand, long-distance running and jogging did not increase the risk of OA [4].

Our study found that 51.6% of research subjects had moderate physical activity (MPA), but there was no relationship between physical activity and levels of IL-1β and TNF-α. This result is different from the experimental study conducted by Castrogiovanni *et al*. on experimental animals, which proved that MPA could decrease the expression of biomarkers related to OA (IL-1β and TNF-α) and increase the expression of chondroprotective (IL-4, IL-10, and lubricin) [29]. This represents a beneficial effect of MPA on the synovium and on cartilage preservation. Exercise therapy can decrease cytokines and inhibit inflammatory factor-mediated cartilage degradation. This difference in results may be due to differences in study subjects, where our study was conducted on women with knee OA with a cross-sectional study design, whereas study of Castrogiovanni *et al* was an experimental study conducted on animals. The pattern of physical activity in Castrogiovanni *et al*. studies is provide MPA which is proven to save the function of type B synoviocytes, in our study, all of whose movements were carried out irregularly, repeatedly, and prioritized the knee as body weight support. Walking and running are the dominant movements carried out by housewives, where this will place repeated loads on the knees, thereby increasing the risk of OA, besides that other risk factors are also difficult to control in studies with human subjects. Women are more at risk of suffering from OA with a more severe degree than men. The increased risk of OA in women increases around menopause because estrogen deficiency makes postmenopausal women more vulnerable. Hormonal factors may play a role in OA development in this condition. The hormone estrogen has been shown to exhibit anti-inflammatory and antinociceptive properties during inflammation. Estrogen prevents lipopolysaccharide-induced microglial toxicity by attenuating the release of TNF-α and IL-1β [28]. In this study, we did not find a significant difference in levels of IL-1β, TNF- between postmenopausal and postmenopausal women. This is in contrast to the Women's Health Initiative data. Women undergoing estrogen replacement therapy were 15% less likely to require knee or hip arthroplasty than those who did not use the treatment. A study by Shivers *et al*. (2015) reported that estradiol significantly decreased bone marrow-injected proinflammatory TNF-and IL-1β levels [30].

Conclusion

The conclusions in this study are most of the study subjects had a risky BMI (obesity and overweight), there is a positive correlation with moderate strength between BMI and BW with levels of IL-1β and TNF-α in knee joint synovial fluid, there was no difference in levels of IL-1β and TNF-in knee joint synovial fluid based on physical activity, menopausal status and BMI at risk. This study is the first research conducted in Riau Province, Indonesia, and is expected to be an input in designing obesity prevention patterns for women at risk of OA. OA is multifactorial, while we only looked at two risk factors, so it is recommended to conduct further research with a more complete risk factor, including genetic aspects.

Acknowledgment

References

1. Mora JC, Przkora R, Almeida YC. Knee osteoarthritis: Pathophysiology and current treatment modalities. J Pain Res. 2018;11:2189-96. https://doi.org/10.2147/jpr.s154002
   PMid:30323653

2. Nguyen TV. Osteoarthritis in southeast Asia. Int J Clin Rheumatol. 2014;9(5):405-8.

3. Indonesian Rheumatology Association. Rekomendasi Perhimpunan Reumatologi Indonesia untuk Diagnosis dan Penatalaksanaan Osteoartritis. Tempat Tidak Diketahui. PBIRA Diunduhdari; 2014. Available from: http://www.reumatologi.or.id/reurek/download/24 [Last accessed on 2017 Sep 10].

4. Zhang Y, Vasheghani F, Li YH, Blati M, Simeone K, Fahmi H, *et al*. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. Ann Rheum Dis. 2015;74:1432-40. https://doi.org/10.1136/annrheumdis-2013-204599
   PMid:24651621

5. Grottle M, Hagen HB, Natvig B, Dahl FA, Rvien TK. Prevalence and burden of osteoarthritis: Results from a population survey in Norway. J Rheumatol. 2008;35(4):677-84.
   PMid:18278832

6. Soeroso J, Isbagio H, Kalim H, Broto R, Pramudiyo R. Osteoartritis. In: Sudoyo AW, Setiyohadi B, Alwi I, Simadibrata M, Setiati S, editors. Buku Ajar Ilmu Penyakit Dalam. 4th ed. Jakarta: Pusat Penerbitan Ilmu Penyakit Dalam FKUI; 2006. p. 1195-20110.

7. Riset Kesehatan Dasar 2013. Available from: http://www.depkes.go.iddownloadriskesdas2013Hasil%20Riskesdas%202013.pdf.AdobeReader [Last accessed on 2014 Apr 11].

8. Irwan K. Angka Harapan Hidup Lansia. Fakultas Kedokteran. Indonesia: Universitas Indonesia; 2013.

9. Suari BA, Ihsan M, Burhanuddin L. Gambaran penderita osteoartritis di bagian bedah RSUD arifin achmad periode Januari 2011-Desember 2013. JOM FK. 2015;2(2):1-10. https://doi.org/10.26891/jik.v5i2.2011.101-110

10. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res. 2004;427:S27-36. https://doi.org/10.1097/01.blo.0000144854.66565.8f

PMid:15480070

11. Bleasel JF. Aetiological pathway. Osteoarthritis and inflammatory arthritis: Osteoarthritis. Aplar J Rheumatol.1998;2:148-51.

12. De Isla NG, Stoltz JF. *In vitro* inhibition of IL-1β catabolic effects on cartilage: A mechanism involved on diacerein anti-OA properties. Biorheology. 2008;45(3-4):433-43. https://doi.org/10.3233/bir-2008-0503

PMid:18836243

13. Jacques C, Gosset M, Berenbaum F, Gabay C. The role of IL-1 and IL-1Ra in joint inflammation and cartilage degradation. Vitam Horm. 2006;74:371-403. https://doi.org/10.1016/s0083-6729(06)74016-x

14. Fernandes JC, Martel-Pelletier J, Pelletier JP.The role of cytokines in osteoarthritis pathophysiology. Biorheology.2002;39(1-2):237-46.

PMid:17027524

15. Melo-Florián A. IL-1 and its role in osteoarthritis. Open J Med. 2011;1(1):3.

16. Wojdasiewicz P, Poniatowski LA, Szukiewiczet D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm. 2014;2014:581459. https://doi.org/10.1155/2014/561459

PMid:24876674

17. Loeser RF. Pathogenesis of Osteoarthritis. Official Reprint from UpToDate. Available from: https://www.uptodate.com [Last accessed on 2021 Dec 21].

18. Anderson J, Felson DT. Factors associated with osteoarthritis of the knee in the first national health and nutrition examination Survey (HANES I). Evidence for an association with overweight, race, and physical demands of work. Am J Epidemiol. 1988;128(1):179-89. https://doi.org/10.1093/oxfordjournals.aje.a114939

PMid:3381825

19. Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis: The Framingham study. Ann Int Med. 1988;109(1):18-24. https://doi.org/10.7326/0003-4819-109-1-18

PMid:3377350

20. Charles BJ, David CS, David MH. Matrix metalloproteinses: A review of their structure and role in acute coronary syndrome. Department of Internal/Cardiology. Cardiovasc Res. 2003;59(4):812-23.

PMid:14553821

21. Georgief T, Ivanova M, Kopchev M, Velikova T, Miloshov A, Kurteva M, *et al*. Cartilage oligomeric protein, matrix metalloproteinase-3, and Coll2-1 as serum biomarkers in knee osteoarthritis: A cross-sectional study. Rheumatol Int. 2018;38(5):821-30. https://doi.org/10.1007/s00296-017-3887-y

PMid:29164307

22. IPAQ Research Committee. Guidelines for the Data Processing and Analysis of the International Physical Activity Questionaire (Short form), Diunduh Dari; 2004. Available from: http://www.Ipaq.Ki.Setanggal [Last accessed on 2015 Apr 20].

23. Vincent HK, Heywood K, Connelley J, Hurley RW. Weight loss and obesity in the treatment and prevention of osteoarthritis PMR. 2012;4(5):S59-67. https://doi.org/10.1016/j.pmrj.2012.01.005

24. Munthe RV, Hendrika W, Gurusinga NY. Relationship between body mass index (BMI) and Knee osteoarthritis at the UKI general hospital, Jakarta in 2017. Int J Health Sci Res. 2017;11(10):365. https://doi.org/10.52403/ijhsr.20211047

25. Afolabi HA, Zakaria Z, Hashim MN, Vinayak R. Body mass index and predisposition of patients to knee osteoarthritis. Obesity Med. 2019;16(8):100143. https://doi.org/10.1016/j.obmed.2019.100143

26. Marques-Vidal P, Bochud M, Bastardot F, Lüscher T, Ferrero F, Gaspoz JM, *et al*. Association between inflammatory and obesity markers in a swiss population-based sample (CoLaus Study). Obes Facts. 2012;5:734-44. https://doi.org/10.1159/000345045

PMid:23108472

27. Piccioli P, Rubartelli A. The secretion of IL-1β and options for release. Sem Immunol. 2013;25(6):425-9. https://doi.org/10.1016/j.smim.2013.10.007

PMid:24201029

28. Smith JA, Das A, Butler JT, Ray SK, Banik NL. Estrogen or estrogen receptor agonist inhibits lipopolysaccharide induced microglial activation and death. Neurochem Res. 2011;36(9):1587-93. https://doi.org/10.1007/s11064-010-0336-7

PMid:21127968

29. Castrogiovanni P, Rosa MD, Ravalli S, Castorina A, Guglielmino C, Imbesi R, *et al*. Moderate physical activity as a prevention method for knee osteoarthritis and the role of synoviocytes as biological key. Int J Mol Sci. 2019;20(3):511. https://doi.org/10.3390/ijms20030511

PMid:30691048

30. Shivers KY, Amador N, Abrams L, Hunter D, Jenab S, Jenab VQ. Estrogen alters baseline and inflammatory-induced cytokine levels independent from hypothalamic-pituitary-adrenal axis activity. Cytokine. 2015;72(2):121-9. https://doi.org/10.1016/j.cyto.2015.01.007

PMid:25647266