Expression profiles of interleukin-6, c-reactive protein, and rheumatoid factor in pre and menopause osteoarthritis patients

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Abstract. Osteoarthritis (OA) is a disease that results from damage to the cartilage covering the bones, leading to friction between bones during movement. The inflammation in OA includes the production of several pro-inflammatory cytokines, such as interleukin 6 (IL-6). As the disease progresses, it can lower quality of life, reduce life expectancy, and create a financial burden for those affected. This study aimed to examine the effect of age on OA by looking at the expression levels of IL-6, c-reactive protein (CRP), and rheumatoid factor (RF) in participants. Methods: This descriptive cross-sectional study involved 20 female participants aged 40 and older. We measured IL-6 levels using ELISA (Enzyme-Linked Immunosorbent Assay). For CRP and RF, we used latex agglutination tests. Results: Age did not impact the occurrence of osteoarthritis (ρ value: 0.624). IL-6 levels were higher in the group without OA. Most samples showed negative results for CRP and RF. Conclusion: Elevated IL-6 levels are not exclusive to OA patients; several factors, like exercise, can also raise IL-6 levels. Most samples did not show significant systemic inflammation, as indicated by low CRP and RF levels.

1. Introduction

Arthritis is an acute or chronic inflammation of the joints with symptoms of pain, stiffness, reduced movement and joint deformity. There are two types of arthritis, namely Osteoarthritis (OA) and Rheumatoid Arthritis (RA). Osteoarthritis (OA) is a disease that arises due to damage to the cartilage tissue that covers the bones, causing the bones to rub against each other when moved. Meanwhile, rheumatoid arthritis (RA) is an autoimmune rheumatic disease and is a progressive chronic inflammatory disease that causes permanent joint damage [1].

Osteoarthritis (OA) is the most common form of arthritis in the community, affecting approximately 302 million people worldwide and being the leading cause of disability in older adults. Osteoarthritis is no longer considered solely a degenerative disease, but age remains one of its risk factors. 5.7 Approximately 50% of patients over the age of 65 exhibit radiological findings consistent with OA, while only 10% of men and 13% of women among them show clinical symptoms of OA,

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and around 10% experience disability due to OA. It is understandable that as age increases, the likelihood of developing OA also increases [1].

The prevalence and incidence of this disease vary between populations. Women are 2-3 times more likely to develop RA than men. Incidence increases with age, but there is no statistical difference in cases between women and men over the age of 70. The highest incidence of cases is in the 50-54 age group. The highest incidence of RA occurs in Northern Europe and North America compared to Southern Europe. The incidence in Northern Europe is 29 cases/100,000, 38/100,000 in North America and 16.5/100,000 in Southern Europe. The prevalence of RA is relatively constant in many populations, at 0.5-1%. The highest prevalence is reported among the Pima Indians (5.3%) and Chippewa Indians (6.8%), and the lowest prevalence is found in Chinese and Japanese populations (0.2-0.3%). The number of RA sufferers in Indonesia is not yet known with certainty, but it is currently estimated that no less than 1.3 million people suffer from RA in Indonesia, based on the global RA prevalence rate of 0.5-1% of Indonesia's population of 268 million in 2020. Data in Indonesia shows that in the Bendungan area of Central Java, the prevalence of RA is 0.34%. Data from Malang shows that among residents over 40 years of age, the prevalence of RA is 0.5% in the municipality and 0.6% in the regency [2].

Interleukin 6 is one of the pro-inflammatory cytokines produced by monocytes or macrophages. As a marker of inflammation, IL-6 plays a role in controlling differentiation, migration, proliferation, and apoptosis or cell death. In addition, IL-6 also plays a role in various metabolic processes in the body[3]. This cytokine is produced during the inflammatory process and is a stimulator of acute phase proteins that are often detected in cardiovascular disease, diabetes mellitus, osteoarthritis, and rheumatoid arthritis (RA). IL-6 plays an important role in the pathogenesis of RA, which is characterised by increased levels leading to excessive antibody production. As a result, an immune attack occurs against tissues, especially in the joints [4-5].

2. Method

This study is an experimental study in the form of a cross-sectional study (single-time sampling) of interleukin-6 (IL-6) levels in adult arthritis patients. Serum samples were taken directly from respondents at a single point in time. Sampling began with obtaining informed consent from respondents. The preparation stage, sample testing, and data analysis were carried out at the Integrated Laboratory, Aisyiyah University, Yogyakarta.

2.1 Research Population and Sample

The population in this study consisted of female respondents aged over 40 years at PKU Muhammadiyah Hospital, Yogyakarta. The total number of samples in this study was 20 people, selected consecutively. The inclusion criteria for respondents were female, aged over 40 years, and willing to be a respondent. The exclusion criteria were respondents who were ill and respondents undergoing routine treatment other than OA. This research has obtained ethical permission from RS PKU Muhammadiyah Yogyakarta No.00223/KT.7.4/VI/2025.

2.2 Research Procedure

IL-6 testing was carried out in accordance with the Human Interleukin 6 ELISA Kit manufacturing protocol (BT E0090Hu-48 whells).

2.3 Sample preparation

Plasma was prepared using one-tenth volume of 0.1 M EDTA as an anticoagulant. Samples were centrifuged at 3000 x g for 10 minutes. Samples were diluted 1:400 with 1X Dilution Buffer N and assayed. Undiluted samples could be stored at -20°C for up to 3 months.

Assay procedure are Prepare all reagents, working standards, and samples. Testing is carried out at room temperature (20-25°C). Add 100 microlitres of standard/sample to the well plate.Cover the plate with a 96-Well Cover Sheet, incubate for one hour at room temperature on a shaker.Discard the contents of the plate and wash with Wash Buffer. Dry the wells by inverting the plate over tissue to remove residual liquid. Add 100 microlitres of Anti-IL-6 Biotin conjugate to the wells. Cover the plate with a 96-Well Cover Sheet and incubate for one hour at room temperature on a shaker. Discard the contents of the plate and wash with Wash Buffer. Dry the wells by inverting the plate over tissue to remove residual liquid.Add 100 microlitres of Streptavidin HRP to each well.Cover the plate with a 96-Well Cover Sheet, incubate for 30 minutes at room temperature on a shaker.Discard the contents of the plate and wash with Wash Buffer. Dry the wells by inverting the plate over tissue to remove residual liquid.Add 100 microlitres of TMB substrate to each well.Cover the plate with a 96-Well Cover Sheet, incubate for 30 minutes at room temperature in a dark room.Add 100 microlitres of HRP stop solution to each well.Clean the bottom of the wells with tissue.Read the results on an ELISA reader at a wavelength of 450 nm.

3. Results and Discussion

3.1 Results

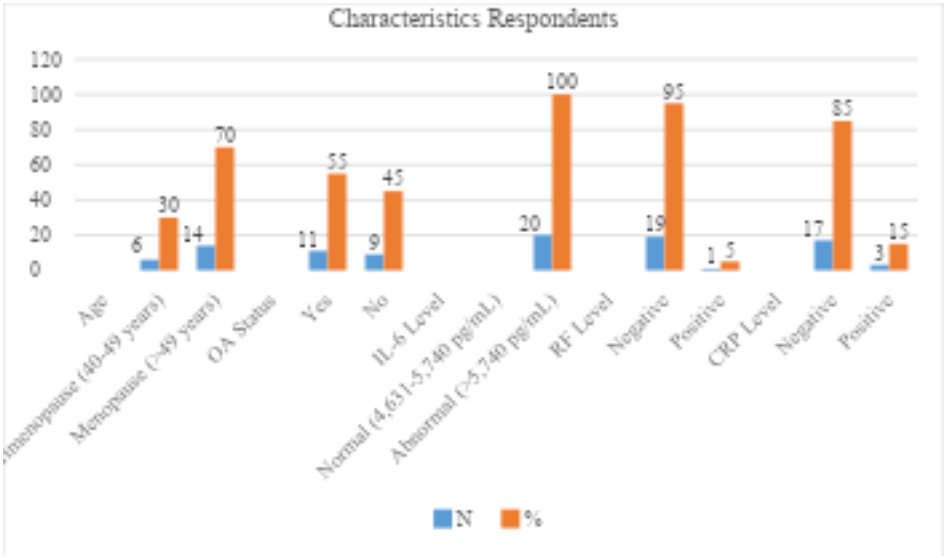

Diagram 1. Frequency Distribution of Respondent Characteristics

Based on Diagram 1, it is known that 70% of respondents were aged >50 years, had osteoarthritis status of 55%, and all research respondents (100%) had abnormal IL-6 levels. Normal IL-6 values in the range of 4.631-5.740 pg/ml became the threshold used as a marker for healthy individuals.

Tabel 1. The Effect of Age on Osteoarthritis (OA)

Age	Osteoarthritis				*P-value*
	Yes	%	No	%	
Menopause	7	63,6	7	77,8	0.642
Premenopause	4	36,4	2	22,2	

Based on Table 1, it is known that most respondents in this study were of menopausal age (>49 years), namely 14 individuals (70%). Furthermore, 7 menopausal respondents suffered from OA. In the perimenopausal age group, 4 respondents experienced OA, while the other 2 respondents were normal. The Fisher's exact test yielded a p-value of 0.642 for the age group, indicating that age did not affect OA status because it exceeded the significance value of 0.05 (p>0.05).

Tabel 2. Mean IL-6 Levels in Osteoarthritis and Non-Osteoarthritis Groups

IL_6 Level			
OA	**Mean**	**N**	**Std. Deviation**
Yes	184.3364	11	118.69974
No	198.6167	9	102.21535
Total	190.7625	20	108.93976

Table 2 shows that the average IL-6 level in respondents who did not have osteoarthritis was higher than in the group of respondents who had osteoarthritis.

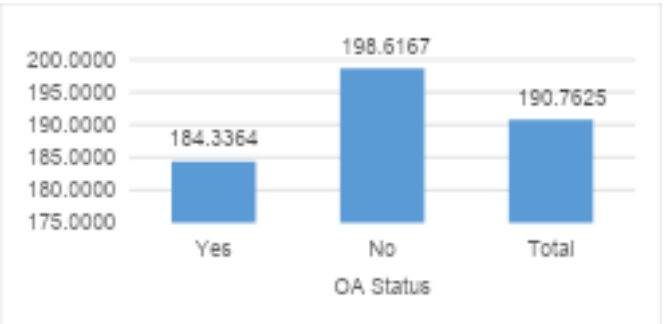

Diagram 2. Mean IL-6 Levels in Osteoarthritis and Non-Osteoarthritis Groups

3.2 Discussion

As the most common chronic degenerative joint disease, osteoarthritis (OA) is a major cause of pain and physical disability that can affect millions of people worldwide. Joint inflammation occurring in most OA patients is associated with the pro-inflammatory mediator Interleukin-6 (IL-6), which is actively involved in the development of the disease. Increased IL-6 levels in the serum or synovial fluid of OA patients correlate with the incidence and severity of the disease. IL-6 induces the activation of Vascular Endothelial Growth Factor (VEGF) and angiogenesis in the synovium and increases vascular permeability, resulting in synovitis. IL-6 also induces osteoclast differentiation, causing bone resorption and increasing the production of matrix metalloproteinases, resulting in cartilage degradation[6]. Several of the above factors can increase the inflammatory process in the synovial tissue, causing OA pain[7] .

This study involved 20 respondents from PKU Muhammadiyah Hospital in Yogyakarta in June 2025. Based on the research data in Table 1, it can be seen that all respondents were female. Research shows that women have a higher incidence and worse clinical progression of OA compared to men[8]. Globally, women account for 60% of all OA sufferers, with a greater difference after the age of 40 [13]. Higher risk in women is possible due to several factors such as anatomical differences and joint alignment, muscle strength, hormonal influences, obesity, and genetic influences [9]. In this study, 11 respondents (55%) had OA.

The average age of respondents in this study was 52.15 (SD = 3.99), with the majority (70% of respondents) entering menopause (>49 years). This is in line with the study, which states that the accumulation of senescent cells due to the natural ageing process of organisms plays a role in the manifestation of OA. In addition to cellular ageing, the onset of mitochondrial dysfunction and oxidative stress also contribute to the development of OA with advancing age. Furthermore, numerous studies have shown that the increased incidence of OA in postmenopausal women is caused by a deficiency of the hormone oestrogen, which is involved in the pathogenesis of this disease [10].

As one of the steroid hormones, oestrogen plays an important role in reproductive and endocrine health in women. Relation to OA, oestrogen has been proven to protect against the degradation of cartilage. Therefore, the decrease in oestrogen levels that occurs from the onset of menopause is often associated as a potential factor in the increased risk of OA in women. In addition, oestrogen is also associated with pain in OA. Research shows that post-menopausal women with lower oestrogen levels report more severe OA pain [11].

Table 2 data shows that OA conditions are not only found in the menopausal group (7 respondents) but also in the perimenopausal age group (4 respondents). Research shows that a sudden decrease in oestrogen during the perimenopausal period can trigger arthralgia or arthritis, making this age group a common time for women to begin experiencing musculoskeletal symptoms. Estrogen can influence inflammatory responses and disease progression in perimenopausal women with KOA through the regulation of inflammatory responses, inhibition of cell ageing and apoptosis, and modulation of neurotransmitters. Respondents in the perimenopausal age group were all over the age of 30. This is in line with research, which shows that there is no burden of disease due to OA before the age of 30. This is because younger age groups are less prone to muscle weakness and degenerative changes [12].

The IL-6 test results in this study can be seen in Table 1. All respondents showed IL-6 levels exceeding the normal limit (<5 pg/mL) in healthy individuals, even though there were no indications of OA. The average IL-6 level in the respondents in this study was 176.48 pg/mL (SD = 110.19 pg/mL). When differentiated by age category, the mean IL-6 in the perimenopausal group was 130.99 pg/mL (SD = 115.70 pg/mL), while the menopausal group was higher at 180.77 pg/mL (SD = 111.94 pg/mL). This is in line with the results of a study, which showed that IL-6 levels increase in postmenopausal women compared to perimenopausal women. This increase in cytokines is caused by a decrease in oestradiol, the type of oestrogen most produced by the ovaries in women with normal menstrual cycles. In menopausal women, ovarian follicle activity decreases until atresia occurs, and eventually no follicles remain to produce oestrogen hormones [13].

The increase in IL-6 in the non-OA group may also be due to the effects of regular exercise performed by some non-OA respondents. Exercise induces significant physiological changes in the immune system, including characteristic cytokine responses. Most notable is the significant increase in IL-6 originating from the muscles, which, although traditionally considered a potent pro-inflammatory cytokine, IL-6 helps orchestrate the anti-inflammatory immune response during exercise. Although IL-6 and

pro-inflammatory cytokines are involved in various chronic musculoskeletal conditions, the exercise-induced increase in IL-6 does not cause inflammation in these conditions, with exercise instead providing beneficial effects [14].

Research data shows that the average IL-6 level in the group of respondents with OA was 184.34 pg/mL (SD = 118.70 pg/mL). This value was 5% lower than that of the group of respondents without OA, who had an IL-6 level of 198.62 pg/mL (SD = 102.22 pg/mL). Based on the results of data analysis, it can be seen that the group of respondents without OA also had high IL-6 levels. This could be influenced by several factors, one of which is age. According to, IL-6 levels increase by 0.05 pg/mL per year of age in healthy individuals. Therefore, the older a person is, the higher their IL-6 levels are likely to be [15]. Cytokine IL-6 is produced by various human cells, one of which is adipocytes during their differentiation stage. Furthermore, IL-6 is also regulated hormonally, suppressed by glucocorticoids, and stimulated by catecholamines and insulin in physiological concentrations. This may explain the presence of IL-6 in the blood of healthy individuals [6-10].The limitation of the study, which did not directly measure cytokines in the synovium, may also explain the high IL-6 levels in the non-OA group. This suggests that IL-6 may be measured as an inflammatory response to other pathological conditions, not just OA.

The results of this study show that only 1 out of 20 samples (5%) were RF positive. This figure is much lower than the prevalence of RF positivity in patients with rheumatoid arthritis (RA), which usually reaches 60–80%. This indicates that most of the samples in this study did not exhibit the autoimmune activity characteristic of RA. RF is not a specific biomarker and can be found in other conditions, even in healthy individuals. Therefore, these results emphasise that RF interpretation must be cautious and always correlated with clinical examinations and other biomarkers. Biologically, RF is an autoantibody primarily directed against the Fc fragment of IgG. RF formation occurs due to chronic stimulation of the immune system, for example due to persistent antigen exposure. These antibodies can form immune complexes with IgG, which then precipitate in the synovial tissue and trigger inflammation. This process contributes to the pathogenesis of RA. However, not all RA patients produce high levels of RF, and not all individuals with positive RF develop RA. This explains why only a few samples in this study showed positive results, as age also plays an important role in RF levels. Some studies report that RF can be found in 5–10% of healthy elderly individuals, even without symptoms of RA [7]. In addition, RF can also appear in chronic infections such as tuberculosis, hepatitis C, or HIV, as well as in other autoimmune diseases such as systemic lupus erythematosus. Thus, positive RF results in this study do not always indicate RA, but may be a reflection of a non-specific immune response due to other factors.

The examination method also affects RF results. Qualitative tests based on latex agglutination or simple nephelometry often have limitations in terms of sensitivity and specificity. In contrast, quantitative methods based on ELISA are capable of detecting RF levels at low concentrations with higher accuracy. Because this study used qualitative methods, it is possible that the actual number of RF-positive cases is higher but undetected. Previous research by confirmed that RF sensitivity increases when combined with anti-CCP (anti-citrullinated protein antibody), which has a specificity of nearly 98% for RA. Thus, the low RF positivity rate in this study may be due to the characteristics of the sample, which did not have active RA, limited detection methods, or biological variations between individuals. This further reinforces that RF should not be used as a single marker, but rather combined with other biomarkers for more accurate interpretation.

In this study, CRP was examined qualitatively and semi-quantitatively, with 2 samples testing positive in the qualitative examination and only 1 sample testing positive in the semi-quantitative examination. These findings indicate variations between examination methods and the biological dynamics of CRP in the body. CRP is an acute phase protein produced by hepatocytes in response to pro-inflammatory cytokines, particularly IL-6, IL-1β, and TNF-α [15]. CRP levels typically begin to rise within 6–8 hours after the onset of inflammation, peak within 48 hours, and then decline rapidly as the inflammation subsides. Variations in results between qualitative and semi-quantitative tests can be explained by differences in method sensitivity. Qualitative tests based on latex agglutination are more prone to producing false-positive results, particularly in conditions with high immunoglobulin levels or elevated RF [14-15].

Meanwhile, semi-quantitative tests are more accurate because they provide estimates of CRP levels. The results of this study indicate that only a small proportion of samples actually have significantly high CRP levels. Previous studies have confirmed that CRP is more useful as a marker of inflammatory activity than as a diagnostic tool. reported that CRP levels are closely correlated with the severity of rheumatoid arthritis and the progression of joint damage [15]. This is consistent with the findings of this study, in which most samples did not show positive results, meaning that they were not in the acute inflammatory phase. In addition to disease factors, CRP levels can also be influenced by non-specific factors. Several studies have shown that obesity, metabolic syndrome, and smoking can increase CRP levels even in the absence of significant infection or inflammation. These factors may explain why there are differences in results between samples even though the main clinical conditions are not uniform.

The results of this study also show that although CRP and RF are often tested together, they have different functions. RF better describes the presence of an autoimmune response, while CRP better reflects active inflammation. Thus, the combination of the two can help to understand the patient's immunological status more comprehensively. However, the low positive rate in this study indicates that most samples did not experience significant systemic inflammation. The study adds that CRP has important prognostic value in various acute and chronic inflammatory conditions, including cardiovascular disease. Therefore, even though only a few samples showed positive CRP in this study, clinical interpretation remains important because an increase in CRP indicates the presence of an active inflammatory process.

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