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Dynamic interaction of obesity, age, MCP-1 Level, and ACE-1 gene with the severity of knee osteoarthritis: a cross-sectional study

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Introduction: The risk factors most strongly associated with knee osteoarthritis (OA) are old age and obesity. However, few studies have evaluated the interaction between aging and obesity in conjunction with inflammatory markers and knee OA severity as part of a complete assessment of knee OA management. Therefore, this study aims to evaluate the interaction between obesity, age, inflammation [including the I/D polymorphism of angiotensin converting enzyme-1 (ACE-1)], and the severity of knee OA.

Methods: A total of 80 knee OA patients were included in this cross-sectional study. The severity of knee OA was determined based on the Kellgren–Lawrence system. All patients underwent physical and radiological examination; monocyte chemoattractant protein 1 (MCP-1) markers were measured. The parameters of the ACE-1 gene were examined with sequencing DNA.

Results: There was a significant relationship between age and severity of knee OA ($P = 0.007$), with subjects aged greater than or equal to 65 having a 3.56-fold higher risk of developing moderate to severe OA than subjects aged less than 65. There was a significant difference between body weight and knee OA severity ($P = 0.026$), in which subjects weighing greater than or equal to 60 kg had 3.14 times the risk of experiencing severe knee OA. Multivariate regression analysis indicated that age was the strongest independent variable for knee OA severity compared with body weight. MCP-1 levels were significantly higher in mild knee OA than in moderate to severe knee OA. The DD genotype of the ACE-1 gene increases the risk of severe knee OA by four times in subjects aged greater than or equal to 65 compared to subjects aged less than 65. However, the DD genotype of the ACE-1 gene does not increase the risk of severe knee OA in subjects weighing greater than or equal to 60 kg.

Conclusion: While obesity and age were found to be associated with the severity of knee OA, age emerged as the independent risk factor for knee OA severity. Furthermore, MCP-1 levels were significantly higher in cases of mild knee OA compared to severe knee OA. It was observed that the DD genotype of the ACE-1 gene increases the risk of severe knee OA in individuals aged 65 years or older.

Keywords: ACE-1 gene, age, cross-sectional study, inflammation, knee osteoarthritis, obesity

Introduction

Knee osteoarthritis (OA) is a degenerative and progressive joint disorder characterized by destruction of joint cartilage, subchondral bone, inflammation, and/or thinning of synovial tissue[1]. Knee OA prevalence is estimated at 80% of the disease's total burden and affecting at least 19% of American adults who are 45 years of age and older[2,3]. In Indonesia, knee OA prevalence is 15.5% in men and 12.7% in women aged between 40 and 60 years[4,5]. OA has a significant impact on patients' quality of life, and its high prevalence and chronic and progressive nature also have socio-economic impacts[6–8].

Obesity is another well-recognized risk factor for OA[9]. OA in weight-bearing joints is associated with trauma to the joint cartilage due to mechanical stress[10]. Various studies have shown

HIGHLIGHTS

- Obesity and advanced age were associated with knee osteoarthritis (OA).
- Obese subjects exhibited more severe than mild cases of OA.
- Subjects greater than or equal to 65 years old had a 3.6-fold higher risk of severe OA than subjects less than 65 years old.
- Inflammatory markers were higher in mild knee than moderate to severe knee OA.
- DD genotype of the angiotensin converting enzyme-1 gene increases the risk of severe knee OA in individuals aged greater than or equal to 65 years.

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that the relationship between obesity and OA is influenced not only by biomechanical factors but also by metabolic conditions, most significantly by the increased adipose tissue in obese people[11,12]. The role of inflammatory factors in the pathomechanism of knee OA has been demonstrated in several recent studies. Furthermore, aging and obesity are associated with low-grade chronic inflammation. Cytokines, including interleukin 1 (IL-1), IL-4, IL-8, tumor necrosis factor alpha, interferon, adipokines, and monocyte chemoattractant protein 1 (MCP-1) significantly contribute to the inflammatory process[9,13].

Genes that contribute to the inflammatory process associated with the development of OA include those within the renin-angiotensin (RA) system[14]. The cascade of the RA system begins with Angiotensinogen, which is converted by the renin enzyme into Angiotensin I (Ang-I). Angiotensin converting enzyme-1 (ACE-1) then converts Ang-I to Angiotensin II (Ang-II). Upon binding to the angiotensin receptor, Ang-II exerts biological effects such as vascular vasoconstriction and stimulation of aldosterone secretion. Ang-II is also known to have proliferative and proinflammatory effects. Several studies have demonstrated the presence of RA system components, including Renin, ACE, angiotensin II type 1 receptor, and angiotensin-1–7, in synovial tissue of individuals, indicating their involvement in the pathogenesis of inflammatory joint diseases such as rheumatoid arthritis and OA[15].

A thorough understanding of the potential mechanisms of OA is critical to optimizing the treatment and prevention of knee OA. However, a limited number of studies have evaluated the interaction of these three variables with OA as part of the complete assessment in knee OA management. Therefore, this study aims to evaluate the interaction between obesity, age, inflammation (including the I/D polymorphism of ACE-1), and the severity of knee OA.

Methods

This cross-sectional study included 80 knee OA patients and was performed at the Dr Wahidin Sudirohusodo Hospital and its networks hospital between July 2020 and January 2021. The study was reported in line with the criteria of Strengthening The Reporting Of Cohort Studies in Surgery (STROCSS)[16]. The number of samples needed in this study was calculated using the retrospective cohort formula of 80 participants. All of the subjects that met the inclusion criteria underwent all of the study investigation procedures. The inclusion criteria were knee OA patients who visited the rheumatology and internal medicine outpatient department at our institution. The exclusion criteria were a history of knee surgery, other forms of arthritis, or knee joint trauma. All patients were evaluated for demographic data, including age, sex, BMI, and knee OA severity.

Knee osteoarthritis

Patients with pain and/or stiffness in the knee joint were further evaluated for crepitus on passive movement or knee joint enlargement. X-rays were taken of the affected knee in a weight-bearing position. Determination of the severity of knee OA was based on the Kellgren–Lawrence (KL) system, and patients were assessed by one orthopedist and one rheumatologist. In cases without concordance in the grade between orthopedist and rheumatologist, the assessment was carried out by a radiologist. KL grades 1 and 2 were classified as the mild OA group, while KL grades 3 and 4 were classified as the moderate to severe OA group according to previous studies by Kim *et al.*[17].

Weight and age classification

Obesity was categorized into obese (≥ 60 kg) and nonobese (< 60 kg) patients based on cut-off receiver operating characteristic (ROC) analysis. Age groups were categorized into patients aged less than 65 years and greater than or equal to 65 years based on cut-off ROC analysis. Using the weight and age categorizations based on ROC analysis, we divided the subjects into four subgroups: Group 1 (nonobese and <65 years), (2) Group 2 (obese and <65 years), Group 3 (nonobese and ≥ 65 years), and Group 4 (obese and ≥ 65 years).

Inflammatory markers

Venous blood was collected following standard venipuncture procedures; 9 ml of whole blood was drawn into an untreated tube. MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Panel A (catalog no. HCYTA-60K; Millipore Corp.) was used to examine serum levels of MCP-1 according to the manufacturer's instructions. The MCP-1 level was measured in nmol/mg. Because there is no standard value, the MCP-1 value was categorized according to the tertile distributions.

ACE-1 gene I/D polymorphism

ACE-1 gene polymorphism manifests as the deletion (insertion)/insertion (deletion) (I/D) of 287 DNA base pairs in intron 16 of the ACE gene. There are three distinct genetic variations of the ACE-1 gene genotype: homozygous DD, homozygous II, and heterozygous ID. The I/D polymorphism of the ACE-1 gene was examined using DNA sequencing techniques.

To evaluate the interaction between ACE-1 gene I/D polymorphism and age, the subjects were divided into four subgroups: individuals with non-DD genotypes aged less than 65 years, individuals with non-DD genotypes aged greater than or equal to 65 years, individuals with DD genotypes aged less than 65 years, and individuals with DD genotypes aged greater than or equal to 65 years.

On the other hand, to assess the interaction between ACE-1 gene ID polymorphism and body weight, the subjects were divided into four subgroups: individuals with non-DD genotypes and body weight less than 60 kg, individuals with DD genotypes and less than 60 kg, individuals with non-DD genotypes and greater than or equal to 60 kg, and individuals with DD genotypes and greater than or equal to 60 kg.

Ethical considerations

All subjects included in the study signed a written informed consent. This research was approved by the Ethical Committee of Faculty of Medicine, Hasanuddin University (reference no. 321-/UN4.6.4.5.31/PP36/2021).

Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) version 25.0 (IBM Corp.) for Windows. Data are expressed as mean ± SD or median (interquartile range). Both data and normality were analyzed using the

Kolmogorov–Smirnov test. The χ^2-test was used to evaluate significant differences between variables with normal data distribution. Statistical significance was defined as $P < 0.05$. Variables with $P < 0.25$ were analyzed using logistic regression multivariate analysis.

Results

The characteristics of the subjects are shown in Table 1. The age range of the research subjects ranged from 40 to 84 years, with an average of 63.7 ± 7.7 years. For the determination of the elderly and nonelderly age groups, a ROC analysis was carried out, and a cut-off of 65 years in predicting the severity of knee OA was obtained, with a sensitivity of 50% and a specificity of 72.5% (Fig. 1). Consequently, subjects were divided according to age into less than 65 years (34 subjects, 42.5%) and greater than or equal to 65 years (46 subjects, 57.5%).

The body weight range of the study subjects was between 44 kg and 99 kg, with an average of 66.47 kg. A ROC analysis was performed for the determination of the obese and nonobese groups, and a cut-off body weight of 60 kg in predicting the severity of knee OA was obtained, with a sensitivity of 82.5% and a specificity of 60% (Fig. 2). Thus, in this study, the subjects were divided according to weight into <60 kg (23 subjects, 28.75%) and greater than or equal to 60 kg (57 subjects, 71.25%).

The MCP-1 values were divided into tertile categories; MCP-1 value was categorized as low if the value was less than or equal to 377 ng/ml, moderate if the value was 378–608 ng/ml, and high if the value was greater than or equal to 609 ng/ml.

Relationship between age and knee OA severity

This study identified a significant relationship between age and the severity of knee OA ($P = 0.007$). Subjects aged greater than or equal to 65 years had a 3.56-fold higher risk of developing moderate to severe OA compared with subjects aged less than 65 years (95% CI, 1.4–9.0) (Table 2).

Relationship between obesity and knee OA severity

There was a significant difference between body weight and Knee OA Severity ($P = 0.026$), in which subjects weighing greater than or equal to 60 kg had a 3.14 times risk of experiencing severe knee OA (95% CI, 1.12–8.82) (Table 3).

Interaction of risk factors with the severity of knee OA

Simple multivariate regression analysis indicated that age was the strongest independent variable for knee OA severity compared with body weight (Table 4). With group 1 as a reference (OR 1.00), group 2 had an OR of 4.58, group 3 an OR of 5.50, and group 4 an OR of 16.50 for severe degrees of knee OA (Table 5). The odds ratio increased proportionally with body weight and age, indicating that age was a stronger independent factor. The *P*-value showed a significant result of 0.004, with an overall percentage of 65% and a Nagelkerke R squared value of 0.206, meaning that 20.6% of the distribution of mild degrees of knee OA and moderate to severe degrees was explained by factors of age and body weight.

Table 1

Characteristics of subjects

Variable	Mild OA (Mean ± SD)	Moderate to severe OA (Mean ± SD)
Age (years)	61.35 ± 8.9	65.10 ± 8.44
Weight (kg)	62.93 ± 10.36	70.12 ± 12.00
MCP-1 (ng/mL)	733.56 ± 437.13	572.22 ± 285.13

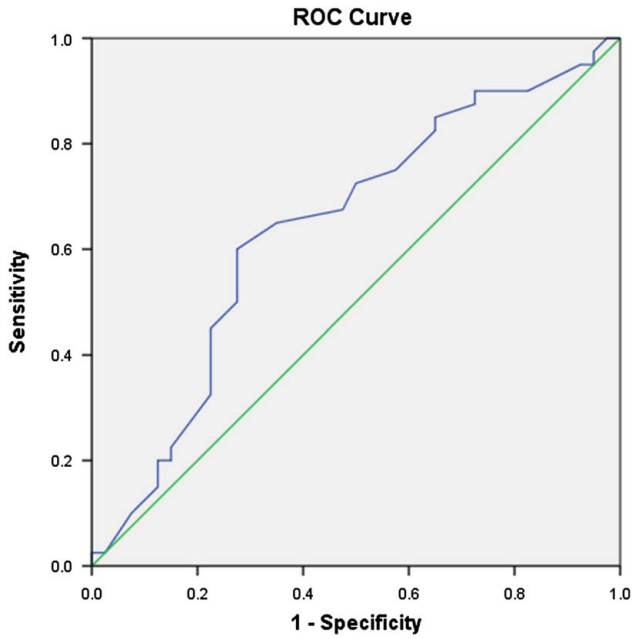

Figure 1. Receiver operating characteristic curve of age for discriminating the degree of severity of knee osteoarthritis.

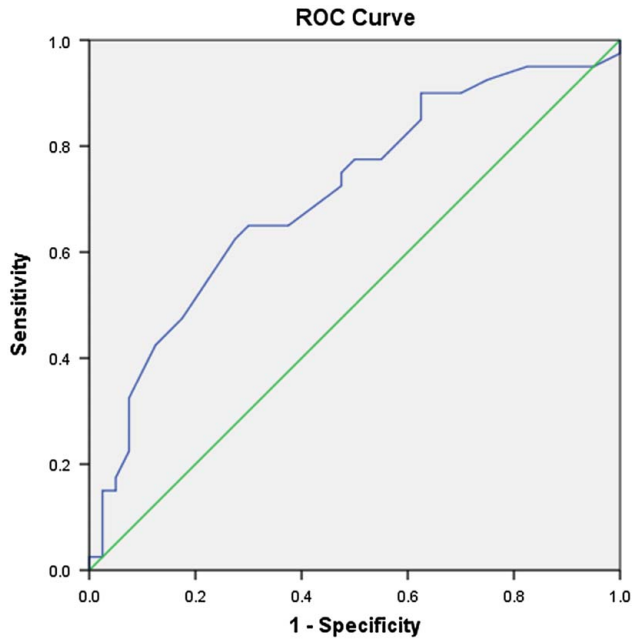

Figure 2. Receiver operating characteristic curve of body weight for discriminating the degree of severity of knee osteoarthritis.

Table 2

Relationship between age and degree of knee osteoarthritis

	Knee OA severity			
Age category (years)	Mild OA	Moderate to severe OA	*P*	OR (95% CI)
< 65	29	17	0.007	3.56 (1.4–9.0)
≥ 65	11	23		

OR, odds ratio.

Effect of age and weight on inflammatory markers

An analysis of the relationship between age and body weight on MCP-1 levels is presented in Table 6. The results showed that MCP-1 levels were not significantly different at age less than 65 years compared to greater than or equal to 65 years or at body weight less than 60 kg to greater than or equal to 60 kg.

Relationship between MCP-1 and knee OA severity

Analysis of MCP-1 compared with the severity of knee OA found that MCP-1 levels were significantly higher in mild knee OA than in moderate to severe knee OA (733.57 ± 437.14 vs. 572.22 ± 285.14 ng/ml; $P = 0.040$) (Table 7).

Interaction of age and weight with I/D Gene ACE-1 polymorphism on the severity of knee OA

This study revealed that individuals with the DD genotype exhibited a higher prevalence of severe knee OA compared to mild knee OA (60 vs. 40%). Conversely, among individuals with a non-DD genotype, mild knee OA was more prevalent than severe knee OA (51.4 vs. 48.6%). However, this difference was not found to be statistically significant ($P = 0.499$) (Table 8).

To evaluate the impact of the interaction between the ACE-1 gene I/D polymorphism and age on the severity of knee OA, the non-DD genotype aged less than 65 years was used as the reference (OR 1.00). Patients with the DD genotype aged less than 65 years had a 2.67 times higher risk, while those with DD genotypes aged greater than or equal to 65 years had a 4.00 times higher risk. Non-DD genotypes aged greater than or equal to 65 years had a 4.20 times higher risk of developing severe knee OA (Table 8). Age emerged as the primary factor influencing the incidence of severe knee OA, but the presence of the DD genotype also contributed to its development. The *P*-value indicated a significant result of 0.031, with an overall percentage of 66.3% and a Nagelkerke R square of 0.140, suggesting that the DD genotype and age accounted for 14% of the distribution of mild and severe degrees of knee OA.

Table 3

Relationship between BMI and degree of knee osteoarthritis

	Knee OA severity			
BMI category (kg/m^2)	Mild OA	Moderate to severe OA	*P*	OR (95% CI)
< 60	16	7	0.026	3.14 (1.12–8.82)
≥ 60	24	33		

OR, odds ratio.

Table 4

Multivariate logistic regression analysis of age and weight

Variable	OR (95% CI)	*P*
Age	4.032 (1.496–10.864)	0.006
Weight	3.677 (1.214–11.139)	0.021

Table 8 demonstrates that body weight plays a dominant role in the incidence of severe knee OA. However, the presence of the DD genotype also influences its development, albeit without statistical significance ($P = 0.136$).

Discussion

There was a significant relationship between age and OA severity ($P < 0.01$). Subjects aged greater than or equal to 65 years had a 3.56-fold higher risk of developing moderate to severe OA compared with subjects aged less than 65 years. Cho *et al.*[18] reported an additional 1.6-fold increased risk of worsening knee OA for every 5 additional years. This is because, with increasing age, joint changes occur, such as degenerative changes in joint cartilage, the formation of osteophytes, the addition of fat cells, and decreased muscle mass, leading to joint destruction, and decreased joint function[19,20]. In addition, the aging process has been linked to low-grade chronic inflammation[21]. Chondrocytes are the main component of joint cartilage on the bearing joint surfaces, and chondrocyte senescence occurs with age[20]. In elderly age, there is also an increase in proinflammatory cytokines and matrix metalloproteinase production, which stimulates joint degradation, increases susceptibility to cell death, decreases matrix synthesis, decreases resistance and tensile strength, and changes mechanical properties[19]. These conditions further cause increased joint cartilage damage. Moreover, joint cartilage metabolism may be affected by estrogen deficiency in post-menopausal women, who show an increased incidence of OA[22]. In this study, the estrogen hormone factor had no effect because all study subjects were greater than 50 years old.

In this study of 80 subjects, subjects who weighed greater than or equal to 60 kg were 3.14 times more likely to develop severe degree knee OA compared with those weighing <60 kg. Raud *et al.*[23] reported a correlation between BMI and the severity of knee OA. Similarly, Holliday *et al.*[24] reported that subjects with a BMI greater than 30 kg/m^2 were 7.48 times more likely to have knee OA. The risk of damage to a weight-bearing joint is influenced by the length of exposure to the load. Biomechanical and metabolic mechanisms are responsible for the association between obesity and OA[25]. Excessive load changes the composition, structure, and mechanical properties of hyaline cartilage,

Table 5

Multivariate logistic regression analysis of the interaction of age and obesity between groups

Age and obesity interaction	OR (95% CI)	*P*
Group 1	1.00	0.004
Group 2	4.58 (0.87–23.98)	
Group 3	5.50 (0.78–38.69)	
Group 4	16.50 (2.81–96.61)	

Table 6

Relationship between age and body weight on MCP-1 levels

Variable	MCP-1 (ng/ml)	P
Age (years)		
< 65	675.88 ± 426.73	0.528
≥ 65	621.78 ± 296.03	
Weight (kg)		
< 60	616.61 ± 294.56	0.586
≥ 60	667.53 ± 405	

which causes erosion of the articular cartilage surface[9]. Furthermore, metabolic changes occurring with obesity have been shown to play a role in the pathogenesis and aggravation of OA. Obesity is known to be a micro-inflammatory condition because adipose tissue produces adipokines such as leptin, tumor necrosis factor alpha, and IL-6, which affect cartilage homeostasis and cause inflammation[26,27].

In this study, with the group aged less than 65 years and weighing less than 60 kg as a reference, the subjects aged greater than or equal to 65 years and weighing greater than or equal to 60 kg were 16.5 times more likely to have a severe degree of OA of the knee. Loeser[19], in his review, stated that there are several risk factors for the severity of knee OA, including aging factors that affect joint function and joint tissue, as well as local factors such as obesity, joint injuries, joint alignment, and joint anatomy. Those results agree with the findings in this study that age and weight were associated with an increase in the severity of knee OA. This is understandable because these two factors independently affect the severity of knee OA, so the interaction between the two will further increase the risk of knee OA severity.

The role of inflammation in knee OA pathogenesis and progression was also evaluated. Giordano *et al.*[28] reported a significant difference in 15 out of 92 inflammatory markers between knee OA patients and healthy subjects; however, their study did not evaluate severity. In a prospective study, Pan *et al.*[29] reported that elevated levels of IL-6 at baseline increased the risk of developing a worsening pain trajectory and more lateral tibial cartilage volume loss; however, their study did not evaluate IL-6 levels at progression. A meta-analysis study showed that MCP-1 expression levels were higher in OA patients than in healthy controls, suggesting that MCP-1 may be crucial to the development of OA[30]. Interestingly, the present study found that MCP-1 was lower in severe knee OA than in mild knee OA. This may be caused by the study participants consuming non-steroidal anti-inflammatory drugs or steroids independently or as prescribed by doctors in patients with severe knee OA due to disturbing pain complaints. These two types of drugs are known to suppress the inflammatory process, both systemically and locally. The statistically significant increase in MCP-1 serum levels among patients with OA supports the inclusion of this metric as a preliminary screening method for anti-inflammatory drug administration.

Table 7

Relationship between inflammatory markers and degree of knee osteoarthritis

	Knee OA severity		
Variable	Mild OA	Moderate to severe OA	P
MCP-1 (ng/ml)	733.57 ± 437.14	572.22 ± 285.14	0.040*

*Note: Significant.

Table 8

The effect of age and weight interaction with the I/D Gene ACE-1 Polymorphism on the severity of knee OA

	Knee OA severity		
Variable	Mild OA (%)	Moderate to severe OA (%)	P
Genotype			
DD	4 (40)	6 (60)	0.499
non-DD (II and ID)	36 (51.4)	34 (48.6)	
Age category (years)			
Genotype non-DD & < 65	26 (68.4)	13 (31.6)	0.033
Genotype non-DD & ≥ 65	10 (31.3)	21 (68.8)	
Genotype DD & <65	3 (42.9)	4 (57.1)	
Genotype DD & ≥ 65	1 (33.3)	2 (66.7)	
Weight category (kg)			
Genotype non-DD & <60	14 (70)	6 (30)	0.136
Genotype DD & <60	2 (66.7)	1 (33.3)	
Genotype non-DD & ≥ 60	22 (44)	28 (56)	
Genotype DD & ≥ 60	2 (28.6)	5 (71.4)	

Note: chi-square test.

Previous studies have examined the impact of the ACE-1 gene I/D polymorphism on the incidence of knee OA, with some researchers suggesting that the DD genotype is a risk factor for knee OA[14,31–33]. However, another study reports that the DD genotype is not a factor[34], and some studies even propose that the II genotype is a risk factor for knee OA events[35]. The disparities in these findings may be attributed to variations in race and the selection of research subjects. Moreover, numerous genetic factors contribute to the pathogenesis of knee OA. These include genes involved in the structure of the extracellular matrix cartilage (e.g. *Collagen Type II Alpha 1 Chain* [*COL2A1*]), genes associated with bone mass (e.g. *vitamin D receptor, Calmodulin [CALM]-1, CALM2, calcitonin-related polypeptide alpha, estrogen receptor alpha, bone morphogenetic protein-2, bone morphogenetic protein-5, osteoprotegerin,* and *leptin*), genes involved in inflammation (e.g. *Interleukin 1 Receptor Type 1 [IL1R1], Interleukin 4 Receptor, Prostaglandin-endoperoxide synthase 2, phospholipase A2 group IVA, Cyclooxygenase-2,* and *ADAM Metallopeptidase Domain 12*), as well as other genes such as *Thioredoxin domain-containing protein 3, Ras Homolog Family Member B, ß-amyloid-binding protein-like protein-2, cold-induced autoinflammatory syndrome 1, CD36, nuclear receptor co-repressor 2, Endothelial cell differentiation gene-2, Iodothyronine Deiodinase 2,* SMAD3, *Paired Like Homeodomain 1, Threose nucleic acid,* and genes in the RA system, including *ACE-1* and *ACE-2* genes[36]. The results of this study indicate no significant difference ($P = 0.499$) in the severity of knee OA between subjects with the DD genotype and those with non-DD genotypes. However, the interaction between genetic factors and age exhibits a significant relationship with the severity of knee OA. Therefore, it is believed that environmental factors play a crucial role in the disease phenotype.

The interplay between genetic and environmental factors plays a crucial role in determining the phenotype of the disease[37]. The provided data demonstrates that in individuals aged greater than

or equal to 65 years, genetic factors can increase the risk of severe knee OA. Advancing age leads to degenerative changes in joint cartilage and bone turnover, including the development of osteophytes, alterations in the nervous system, increased fat cell accumulation, and decreased muscle mass, all of which collectively contribute to a decline in joint function[19,38]. Chondrocytes, the essential components of joint cartilage, undergo senescence with aging[38]. Age-related changes in joints involve increased cytokine and matrix metalloproteinase production, which promote joint degradation, heightened susceptibility to cell death, reduced matrix synthesis, diminished resilience and tensile strength, altered mechanical properties, and the potential activation of inflammatory signals[19].

This study had some limitations. The sample size was small. The study subjects might have used nonsteroidal anti-inflammatory drugs and/or steroids before or at the time of sampling. Inflammatory markers from synovial fluid were not assessed. Correlations were not carried out for sex, pain symptoms, functional impairment, or genetic characteristics (e.g. *vitamin D receptor, CLAM1, CLAM2, COL2A1, IL1R1,* and *ACE-2* genes).

Conclusions

Although both obesity and age were associated with knee OA severity, only age, as an unmodifiable risk factor, was the independent risk factor for knee OA severity. MCP-1 was significantly higher in mild knee OA than in severe knee OA. It was observed that the DD genotype of the ACE-1 gene increases the risk of severe knee OA in individuals aged 65 years or older.

Ethical approval

All procedure for human experiment has been approved by Health Research Ethical Committee of the Faculty of Medicine, Universitas Hasanuddin, Number: 321/UN4.6.4.5.31/PP36/2021.

Consent

Written informed consent was obtained from all the subjects for participation and publication of the data. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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None.

Author contribution

M.N.M., M.N.M., M.A.U., A.B., and I.I.: wrote the manuscript and participated in the study design; M.N.M., E.A., I.I., and A.F.: drafted the manuscript; M.N.M. and A.F.: checked the manuscript and made corrections; A.A.Z., M.N.M. and A.F.: performed bioinformatics analyses and revised the manuscript; M.N.M. and M.H.: provided the overall guidance and support; M.N.M., M.A.U., A.B., and S.B.: critically reviewed the manuscript. All authors read and approved the final manuscript.

Conflicts of interest disclosures

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

This study has been registered with the Thai Clinical Trials Registry no. TCTR20220919005. https://www.thaiclinicaltrials.org/show/TCTR20220919005.

Guarantor

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Data availability statement

NA.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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