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POLYMORPHISM I/D GEN ACE-1 INCREASE THE RISK OF SEVERE KNEE OSTEOARTHRITIS IN OLDER ADULT: A PRELIMINARY STUDY

M. Nasser Mustari[1], Muh. Nasrum Massi[2], Muh. Andry Usman[3], Achmad Fikry[4*], Agussalim Bukhari[5], Irfan Idris[6], Alfian Zainuddin[7], Endy Adnan[4], Syakib Bakri[4], Mizwar Hatta[8]

Department of Surgery, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[1]
Department of Clinical Microbiology, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[2]
Department of Orthopedic & Traumatology, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[3]
Department of Internal Medicine, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[4]
Department of Clinical Nutrition, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[5]
Department of Physiology, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[6]
Department of Public Health, Faculty of Medicine, Department of Internal Medicine, Hasanuddin University Indonesia[7]
Specialty & Research Laboratory. Prodia Clinical Laboratory[8]

Corresponding Author: 4*

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ABSTRACT

It has been shown that the interplay of degenerative and inflammatory processes has a role in the etiology of osteoarthritis. The Renin-Angiotensin System (RAS) has reported in knee OA pathophysiology and ACE level influenced by ACE I/D polymorphism. This study aimed to examine the influence of the interaction between age and the DD genotype of the ACE gene on knee OA severity. A total of 80 knee OA patients (mean age 62.9 ± 8.84 years) were included in this cross-sectional study. The severity of knee OA was determined based on the Kellgren–Lawrence system. The Kolmogorov–Smirnov test, chi-square test, and logistic regression multivariate analysis were employed in statistical analysis. This study included 80 female subjects: 40 subjects with mild knee OA and 40 subjects with severe knee OA. The mean age of 63.7 ± 7.7 years was found for 34 subjects (42.5%) aged ≥65 years and 46 subjects (57.5%) <65 years. Based on the ROC curve, the cut-off age is 65 years. The DD gene ACE-1 genotype were not proven to increase the risk of severe knee OA. The DD gene ACE-1 genotype increases the risk of severe OA of the knee by 4 times in subjects aged ≥65 years compared to subjects <65 years. Our study found that the DD genotype ACE-1 did not increase the risk of severe knee OA. At age 65 years, the DD genotype ACE-1 enhances the risk factors for knee OA

severity.

1. Introduction

Osteoarthritis (OA) is a leading cause of physical disability and discomfort. Despite the fact that OA has been identified for decades, the precise pathogenic processes remain unknown. It has been shown that the interplay of degenerative and inflammatory processes has a role in the etiology of osteoarthritis [1]. The incidence and severity of OA increased in an age-dependent manner [2]. The Renin-Angiotensin System (RAS) has played a major role in the pathophysiology of OA in recent years. In OA, inflammation and chondrocyte hypertrophy involve RAS-related components including renin, angiotensin-converting enzyme (ACE), Angiotensin (Ang) II, Angiotensin type 1 Receptors (AT1R) and Angiotensin type 2 Receptors (AT2R). Angiotensinogen-2, the last product of the RAS system, is a pro-inflammatory and effect-promoting active peptide. According to reports, the level of expression is associated with inflammation and the severity of arthritis [3].

The level of ACE that converts ACE1 to ACE2 is influenced by genetic variation [4]. Multiple investigations have demonstrated a correlation between ACE I/D polymorphism and ACE concentrations. ID polymorphism chromosomal The ACE1 gene contains three distinct genotypes, DD, ID, and II [4]. The DD genotype contained the highest amounts of ACE, which led to the highest conversion of ACE to ACE2. This study aimed to examine the influence of the interaction between age and the DD genotype of the ACE gene on knee OA severity.

2. METHOD

The cross-sectional study included 80 knee osteoartrithis patients and was performed at Dr Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia between July 2020 to January 2021. The inclusion criteria were knee OA patients who visited the Rheumatology and Internal Medicine outpatient department. The exclusion criteria were history of knee surgery, a history of other arthritis, and a history of knee joint trauma. All patients were evaluated for demographic data such as age, sex, body mass index (BMI), and knee OA severity.

2.1 Knee Osteoarthritis

Every patient with pain and/or stiffness in the knee joint will be further evaluated for crepitus on passive movement or knee joint enlargement. Affected knee x-ray was done in weight-bearing position. The determination of the severity of knee OA was based on Kellgren-Lawrence (KL) assessed by one orthopedist, one rheumatologist, and one radiologist. Then we will decide the severity of knee OA if two of the three specialists have the same rating. Kellgren-Lawrence (KL) classification grade 1 and 2 was classified as mild OA and KL grade 3 and 4 was classified as severe OA.

2.2 Age group

Age group were categorized into <65 years and ≥65 years based on cut-off ROC analysis.

2.3 Genomic DNA extraction and genotyping

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Peripheral blood samples were taken from all patients and controls. RNA extraction was done from blood samples. cDNA amplification from RNA extraction by RT PCR was carried out according to the method from Invitrogen. The method was performed using cDNA Synthesis – ReverTra AceTM qPCR-RT(Biorad). The genotypes of ACE gene polymorphism were determined by PCR using primers. The PCR program included the following steps: initial denaturation at 95◦C for 5 min; 35 cycles of denaturation at 95◦Cfor 30s, annealing at 58◦C for 30 s, and extension at 72◦C for 30s and a final extension at 72◦Cfor 10 min. To controlthe quality of genotyping, two independent investigators interpreted images of each gel, and at least 10% of samples were randomly selected for repeated genotyping.

2.4 Ethical consideration

All subjects included in the study signed a written informed consent. This research was approved by the Hasanuddin University Ethics Committee (reference no. 321/UN4.6.4.5.31/PP36/2021) and has been registered with the Thai Clinical Trials Registry (no. TCTR20220919005).

2.5 Statistical analysis

Statistical analysis was implemented using the statistical package for social sciences (SPSS) software, version 25.0 for Windows. Data are expressed as mean +/- SD or median (interquartile range). Both data and normality were analyzed using the Kolmogorov-Smirnov test. The chi-square test was used to evaluated significant differences between variable with normal data distribution. Statistical significance was defined as P value <0.05. Variable with p -value < 0.25 was analyzed with logistic regression multivariate analysis.

3. RESULTS

This study included 40 (50%) subjects with mild knee osteoarthritis and 40 (50%) subjects with severe knee osteoarthritis. Based on the KL classification, OA grade 1 (20 subjects) and OA grade 2 (20 subjects) were defined as having mild knee OA, whilst OA grade 3 (20 subjects) and OA grade 4 (20 subjects) were described as having severe knee OA. The subjects' mean age was 62.9 8.84 years (Table 1). There were 34 subjects aged 65 (42.5%) and 46 subjects aged 65 (57.5%). The BMI range for the subjects was 14.57–39.1 kg/m2, with a mean BMI of 27.96 4.72 kg/m2. 18 (22.5%) of the 80 subjects were non-obesity, whereas 62 (77.5%) were obese.

Table 1 Subject Characteristics

Variable	Min	Max	Mean ± SD
Age (years)	40	84	62.9 ± 8.84
Weight (kg)	35	99	66.5 ± 11.71
BMI (kg/m^2)	14.57	39.11	27.96 ± 4.72

3.1 Relationship of Polymorphism I/D gene ACE-1 on Severity of Knee OA

The genotypes of polymorphism I/D gene ACE-1 consisted of DD, ID, and II genotypes, which were categorized into two groups: subjects with DD genotypes (DD) and subjects non DD genotypes (ID+II). The distribution of DD and non-DD genotypes among patients with mild and severe OA is presented in Table 2.

It was discovered that there were 36 mild OA participants and 34 severe OA subjects among non-DD genotype subjects, while there were 4 mild OA subjects and 6 severe OA subjects among DD genotype patients. Independently, this difference was not significant (p=0.499). This may indicate that the Polymorphism I/D gene ACE-1, in this case the DD genotype, is not an independent risk factor for knee OA severity.

Table 2 Distribution of Polymorphism I/D gene ACE-1 on Knee OA Severity

	Knee OA Severity		
	Mild OA	Severe OA	P value
DD genotype	4 (5%)	6 (7.5%)	0.499
Non DD genotype (ID dan II)	36 (45%)	34 (42,5%)	

3.2 Relationship of Age on the Severity of Knee OA

In the mild knee OA group, the mean age was 61.35 8.94 while in the severe knee OA group, it was 65.10 44. To facilitate analysis, age groups were divided into two categories. The cut-off value is calculated based on an analysis of the ROC curve. With an AUC of 0.6 and a P value of 0.038, the age variable is substantially associated with the severity of knee OA, as shown by the ROC curve analysis (Figure 1). Obtaining a cutoff age of 65 years with a sensitivity of 50% and a specificity of 72.5%, the age group was separated in this study into 65 years and 65 years.

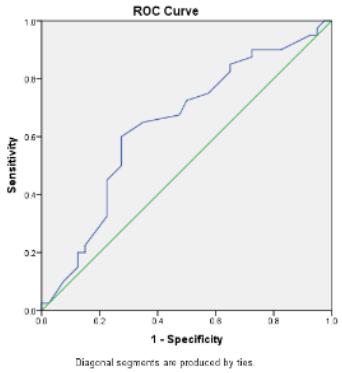

Figure 1 ROC curve of age to the degree of severity of knee OA

It was found that in subjects with mild OA there were 29 subjects aged <65 years and 11 subjects aged ≥65 years, on the other hand in subjects with severe OA there were 17 subjects aged <65 years and 23 subjects aged ≥65 years. This difference is significantly significant (P value = 0.007). This could mean that age ≥65 years is a risk factor for the severity of knee OA [OR 3.56 CI95% (1.4-9.0)] (Table 3).

Table 3 Age analysis of the severity of knee OA

Age category	Knee OA Severity		P Value	OR (95%IK)
	Mild OA	Severe OA		
<65 years	29	17	0.007	3.56(1.4-9.0)
≥65 years	11	23		

3.3 Interaction Polymorphism I/D gene ACE-1 with Age on the Severity of Knee OA

To evaluate the interaction of polymorphism I/D gene ACE-1 with age, subjects were grouped into 1) non DD genotype <65 years, 2) non DD genotype ≥65 years, 3) DD genotype <65 years, and 4) DD genotype ≥65 years. It was found that in subjects with mild OA, there were 26 subjects (65%) non DD genotype <65 years, 10 subjects (25%) non DD genotype ≥65 years, 3 subjects (7.5%) DD genotype <65 years, and 1 subject (2.5%) DD genotype ≥65 years. In the severe OA group, there were 13 subjects (32.5%) non DD genotype <65 years, 21 subjects (52.5%) non DD genotype ≥65 years, 4 subjects (10%) DD genotype <65 years, and 2 subjects (5%) DD genotype ≥65 years.

Table 4 Logistic Regression Analysis of ID ACE 1 Gene Polymorphism on Knee OA Severity

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Interaction of ACE1 ID gene polymorphisms	OR (95% CI)	P Value
II	1.00	
ID	1.12	0.806 (0.44-2.87)
DD	1.68	0.474(0.403-7.07)

Table 5 Interaction analysis of DD genotype with age on Knee OA Severity

	Knee OA Severity		P Value
	Mild OA	Severe OA	
Non DD Genotype < 65 years	26 (65%)	13 (32.5%)	0.033
Non DD Genotype ≥ 65 years	10 (25%)	21 (52.5%)	
DD Genotype <65 years	3 (7.5%)	4 (10%)	
DD Genotype ≥65 years	1 (2.5%)	2 (5%)	

*Menggunakan uji chi-square

Our multivariate study demonstrated that Polymorphism I/D gene ACE-1 increases the risk of severe knee OA. To assess the effect of the DD genotype on age, if the non DD genotype <65 years is used as a reference (OR 1.00), then patients with non DD genotype ≥65 years have an OR 4.20, DD genotype <65 years has an OR 2 .67, DD genotype ≥65 years had an OR value of 4.00 for suffering from severe knee OA (Table 7). Odds P value shows a significant result of 0.031 with an overall percentage of 66.3% and the Nagelkerke R square = 0.140.

Table 6 Multivariate Logistic Regression Analysis of Interaction of DD Genotype with Age in Knee OA Patients

Interaction of DD genotype and age	OR (95% CI)	P Value
Non DD Genotype < 65 years	1.00	0.031
Non DD Genotype ≥ 65 years	4.20	
DD Genotype <65 years	2.67	
DD Genotype ≥65 years	4.00	

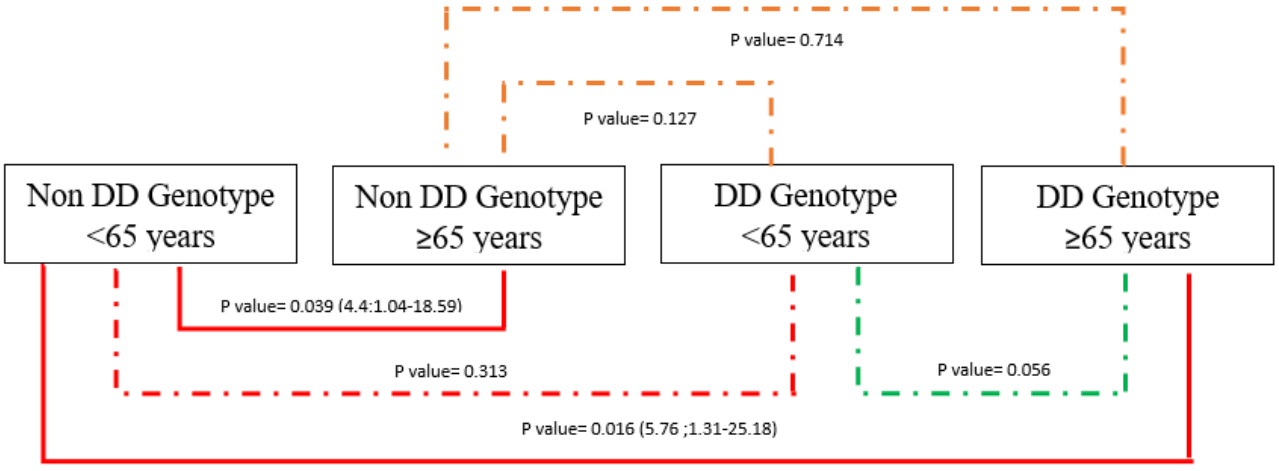

Figure 2. Relationship between DD genotype and age on the severity of knee OA

4. DISCUSSION

Age is a well-known risk factor for the severity of knee OA. [5] reported a 1.6-fold increased risk of knee OA worsening for every five-year increment. This is attributed to the aging of chondrocytes (chondrocyte

senescence), bone changes such as osteophyte production, nervous system degeneration, the addition of fat cells, and a decrease in muscle mass will induce joint degeneration simultaneously [6], [7].

In addition to aging, inflammation is crucial to the pathophysiology of osteoarthritis. ACE is an essential enzyme in the RAS and a potent proinflammatory mediator. In inflammation, high local ACE causes an increase in Ang II concentration, which increases vascular permeability by increasing the production of prostaglandin and VEGF, so contributing to inflammation [8]. Various molecules, including cytokines, chemokines, and MMPs, secreted by chondrocytes, synoviocytes, and invading immune cells, regulate the joint anabolism and catabolism process. Activated cells will release elastase to breakdown type II collagen crosslinks and proteoglycan in articular cartilage [9].

The relative expressions of ACE in the synovial tissue of patients with severe OA were substantially greater than mild OA [10]. Genetic variation greatly influences ACE levels. Expressions of ACE mRNA were low in patients with the II genotype, moderate in those with the ID genotype, and high in those with the DD genotype [11].

A meta-analysis study reported that DD genotype and D allele carriers, the Polymorphism I/D gene ACE-1 were reported to be associated with the incidence of osteoarthritis [4]. Although it is related to the incidence of OA, our study found that the DD genotype was not significantly related to the severity of knee OA. Similar findings were reported by [12] in the Kuwati Arab population which has a very high incidence of the D allele. This is due to the disease's phenotypic not always manifesting. No single gene determines the phenotype, there is always the role of interaction between genes. Another gene was discovered to be important and play a substantial influence in the OA phenotype (bone).

Intriguingly, our study discovered that the interaction between DD genotype and age increased knee OA phenotypic severity. Angiotensin-converting enzyme 2 (ACE2), which was discovered as a homologue of ACE, serves as a physiological counterweight by regulating angiotensin II (Ang II) levels homeostasis. Angiotensin metabolism is counterbalanced by ACE producing Ang II and ACE2 eliminating it by conversion to Ang-(1-7) [13]. The levels of ACE2 expression differed between age groups, according to a study. It is more prevalent in newborns and early children and decreases with age [14]. The decrease in ACE2 expression associated with aging is caused by an increase in RAS signaling throughout the body [15]. The findings of this work can serve as a theoretical foundation for studying the pathophysiology of OA, and RAS components may be a potential therapeutic target for patients with knee OA of advanced age and a DD genotype. This was preliminary study, additional data such case inflammatory markers, angiotensinogen II levels have not evaluated to prove further ACE and inflammatory role in OA pathogenesis. Further study is needed.

5. CONCLUSION

Our study found that the DD genotype ACE-1 did not increase the risk of severe knee OA. At age 65 years, the DD genotype ACE-1 enhances the risk factors for knee OA severity.

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