Original Article

Polymorphism of the SMAD3 Gene rs1065080 in Elderly Patients with Severe Knee Osteoarthritis

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ABSTRACT

Background

Osteoarthritis (OA) is the most common form of arthritis and is one of the most common degenerative joint diseases in the world. One of its risk factors is related to genetics. The signal from TGF-β, which is mediated by the SMAD3 protein, plays a role in maintaining joint cartilage, so is therefore related to osteoarthritis. The objective of this study is to determine the SMAD3 gene polymorphism rs1065080 in elderly Indonesian patients with grade 4 knee osteoarthritis.

Materials and Methods

This is a descriptive observational study, with samples taken consecutively from 21 grade 4 knee OA patients. The genome was taken through saliva and isolated using the Quick-DNATM Miniprep Plus Kit from Zymo Research. RT-PCR was then conducted to determine the genotype.***Results and Discussion:*** The SMAD3 rs1065080 gene polymorphism in grade 4 knee OA in the Indonesian elderly patients resulted in the mutant genotype or GG (61.9%), the heterozygous genotype or GA (28.6%), and the normal genotype or AA (9.5%). The G allele (76.2%) was more frequent than the A allele (23.8%).

Conclusion

The GG genotype and G allele were more frequently found in the study. Analytical studies on larger samples are needed to show the relationship of the SMAD3 rs1065080 gene polymorphism to grade 4 knee OA in Indonesian elderly patients.

Keywords

Polymorphism; SMAD3; knee osteoarthritis; genes; patient

INTRODUCTION

Osteoarthritis (OA) represents the most prevalent form of arthritis and remains one of the most widespread degenerative joint disorders globally. It predominantly affects individuals over the age of 50, who commonly present with joint pain and stiffness that typically worsen with activity and improve with rest.[1] Morning stiffness lasting less than 30 minutes is a characteristic complaint frequently reported by patients with osteoarthritis.[2]

Globally, the burden of osteoarthritis is substantial, with diverse underlying etiologies that may contribute to long-term disability. In 2017, an estimated 303 million individuals were affected worldwide, imposing a significant economic impact.[3] Furthermore, osteoarthritis ranks as the fourth most prevalent condition globally, accounting for approximately 3% of total Years Lost to Disability (YLD).[4] Radiological data from Indonesia indicate that the prevalence of knee osteoarthritis among individuals aged 40 to 60 years is 15.5% in men and 12.7% in women.[5]

Several risk factors have been implicated in the development of osteoarthritis, encompassing both systemic and local factors. Systemic factors include individual-level characteristics such as age, sex, race or ethnicity, genetic predisposition, and nutritional status. In contrast,

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local factors pertain to conditions that directly affect the joint, including trauma history, joint strength, physical activity, occupation, joint alignment and instability.[6, 7]

Genetic studies has consistently demonstrated that transforming growth factor-β (TGF-β) signaling plays a crucial role in bone development, remodeling, and structural integrity.[8] This signaling pathway is primarily mediated through SMAD proteins. Previous studies have further shown that SMAD3 is essential for preserving cartilage homeostasis by suppressing hypertrophic chondrocyte differentiation and regulating extracellular matrix synthesis.[9] Consequently, disturbances within this pathway have been linked to the pathogenesis of osteoarthritis. A recent study conducted in a Chinese population reported that the GG genotype of rs1065080 is associated with an elevated risk of osteoarthritis.[10] However, evidence investigating the relationship between SMAD3 gene variants, particularly rs1065080, and knee osteoarthritis remains limited. Therefore, this study aims to investigate the SMAD3 rs1065080 polymorphism in Indonesian elderly individuals with grade 4 knee osteoarthritis.

MATERIALS AND METHODS

This is a descriptive observational study designed to investigate the SMAD3 gene polymorphism, specifically rs1065080, in elderly patients with grade 4 knee osteoarthritis. It involved an initial study of 21 elderly patients diagnosed with the condition comprising anamnesis, physical examination and supporting radiographic imaging of OA using the Kellgren-Lawrence classification.

The study subjects were selected from an outpatient clinic in South Tangerang district, Indonesia, who had been diagnosed with grade 4 knee osteoarthritis based on the Kellgren-Lawrence radiological classification. Saliva samples were collected from the subjects to identify variations in the SMAD3 gene. The research was conducted at the Medical Diagnostic and Research Centre (MDRC) at the Faculty of Medicine, UIN Syarif Hidayatullah Jakarta. The study period lasted one year, from August 2023 to August 2024.

Sampling was conducted using the consecutive sampling technique, a non-probability sampling method in which participants are selected based on their meeting of specific criteria over a designated period. The inclusion criteria consisted of elderly patients diagnosed with grade 4 knee osteoarthritis, based on the Kellgren-Lawrence classification, and those suffering from primary osteoarthritis. The exclusion criteria referred to subjects with infectious respiratory diseases such as COVID-19 or tuberculosis, or those with a history of knee trauma or lower extremity deformities (varus, valgus).

Saliva samples were chosen for the DNA extraction due to their time efficiency, particularly for elderly populations. Saliva contains oral mucosal epithelial cells, which provide genetic material. The samples were collected by asking the patients to rinse their mouths with water before providing 10 mL of saliva in a plastic centrifuge tube.[11] The samples were then stored at -20°C before genomic DNA extraction using the Quick-DNA™ Miniprep Plus Kit from ZYMO Research. Following DNA isolation, concentration and purity were assessed using a DeNovix DS-11+ Spectrophotometer. RT-PCR reagent consists of a combination of rhAmp Genotyping Master Mix, rhAmp reporter mix, and rhAmp SNP assay Hs.GT. rs1065080.G.1, from Integrated DNA Technologies (IDT). Subsequently, RT-PCR was performed using a Light Cycler 480 II instrument to detect genetic variations in the SMAD3 rs1065080. The data were analyzed descriptively by calculating genotype and allele frequencies, together with their distribution across demographic variables (age, gender, ethnicity and occupation). The analysis was performed using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Since this was a descriptive observational study without a control group, no inferential statistical tests were applied.

Ethical clearance

This study was approved by the Ethics Committee of the Faculty of Medicine UIN Syarif Hidayatullah Jakarta University, Indonesia (Protocol Number: E-002-13-09-24).

RESULTS

Total of 21 patients were categorized according to age groupings based on the 2009 guidelines from the Indonesian Ministry of Health, namely early elderly (46-55 years), mid elderly (56-65 years), and late elderly (>65 years). Table 1 presents the distribution of SMAD3 rs1065080 genotypes (GG, GA and AA) categorized by demographic variables namely age, gender, ethnicity and occupation. The most prevalent demographic profile was late elderly (>65 years),

female, Javanese ethnicity and housewife. Additionally, analysis of the genotypes showed that the mutant genotype GG had the highest frequency (61.9%), followed by the GA genotype (28.6%), and the AA genotype (9.5%).

Table 1. General characteristics of the patients

Variable	Mutant (GG)		Heterozygous (GA)		Normal (AA)		Frequency	
Age	N	%	N	%	N	%	N	%
Early Elderly	1	4.7	0	0	1	4.7	2	9.5
Mid Elderly	3	14.28	1	4.7	1	4.7	5	23.8
Late Elderly	9	42.9	5	23.8	0	0	14	66.7
Gender								
Male	4	19	2	9.6	1	4.7	7	33.3
Female	9	42.9	4	19	1	4.7	14	66.7
Ethnicity								
Betawi	2	9.6	0	0	1	4.7	3	14.3
Javanese	4	19	3	14.3	1	4.7	8	38
Sundanese	1	4.7	0	0	0	0	1	4.8
Palembangese	0	0	1	4.7	0	0	1	4.8
Acehnese	1	4.7	0	0	0	0	1	4.8
Balinese	0	0	1	4.7	0	0	1	4.8
Minangkabau	3	14.3	1	4.7	0	0	4	19
Unknown	2	9.6	0	0	0	0	2	9.5
Occupation								
Pensioner	5	23.8	3	14.3	0	0	6	28.5
Police Officer	0	0	0	0	0	0	1	4.8
Construction Worker	0	0	1	4.7	0	0	1	4.8
Entrepreneur	0	0	0	0	1	4.7	1	4.8
Housewife	6	28.6	1	4.7	1	4.7	10	47.6
Unknown	2	9.6	1	4.7	0	0	2	9.5

Figure 1 and Table 2 display the distribution of G and A alleles in the study. The G allele is more prevalent (76.2%) than the A (23.8%). The total number of alleles observed was 42 (21 individuals having two of each). This distribution supports the fact that the GG genotype was more frequent in this study population.

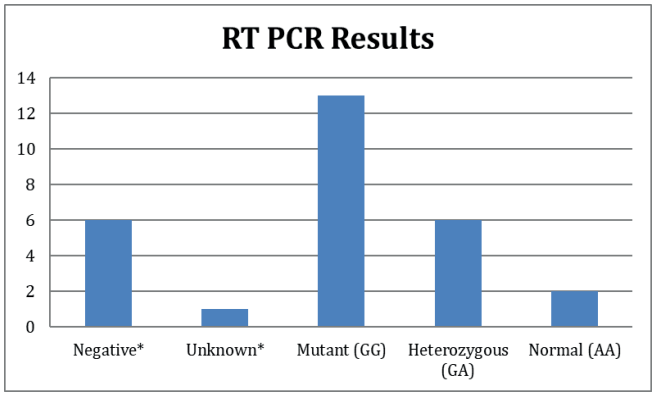

Figure 1. RT PCR Results

*The initial analysis yielded inconclusive results (negative and unknown), requiring a second RT-PCR run to confirm genotype classification.

Table 2. Characteristics of patients based on rs1065080 allele frequency

Allele	Osteoarthritis Grade 4	
	N	%
A	10	23,8
G	32	76,2
Total	42	100

DISCUSSION

Osteoarthritis is a common, chronic, progressive, and degenerative joint disease that can result in pain, functional impairment, and substantial health and

socioeconomic burdens. Globally, it is estimated that approximately 15% of the population is affected, with the condition altering the structure of articular cartilage, subchondral bone, the joint capsule, synovium, and surrounding ligaments, ultimately leading to cartilage degeneration.[12] As the disease advances, processes such as fibrillation, ulceration, and fissuring may occur, which can result in the complete loss of cartilage thickness on the joint surface.[13] Although osteoarthritis can develop in anyone, its onset is influenced by the joint's ability to tolerate mechanical loading and by structural abnormalities.[14]

Genetic research has been rapidly advancing and is increasingly connected to the development of the disease, which can involve the SMAD3 gene, which belongs to the SMAD family, which consists of at least eight different types of SMADs in humans. It is a gene that plays a role in providing signaling instructions to proteins involved in the mechanism of chemical signal transmission from the cell surface to the nucleus. The SMAD3 gene plays a crucial role in joint homeostasis and has been identified as a mediator in TGF-β (transforming growth factor β) signaling, which is involved in chondrocyte anabolism and DNA regulation.[15]

The SMAD3 rs1065080 polymorphism is located within exon 2 of the gene.[16] The allelic variants associated with this locus include the G allele, representing the dominant homozygous form (GG genotype), and the A allele, representing the minor homozygous form (AA genotype). In addition, the heterozygous GA genotype is also observed. Stratification of osteoarthritis risk in Asian populations has frequently been based on these G and A allelic variations.[15] The G allele may confer increased protein stability or stronger DNA-binding affinity, thereby enhancing the capacity of SMAD3 to interact with TGF-β response elements. Conversely, the A allele may reduce protein stability or weaken binding affinity to TGF-β response elements, ultimately diminishing transcriptional activity.

The study sample consisted of 21 patients. The researchers restricted the age characteristics to elderly individuals, but did not impose limitations on gender, ethnicity or occupation. In the study, RT-PCR was performed twice due to the initial occurrence of unknown and negative results. Negative or inconclusive results may arise from several factors, such as low DNA quality or concentration, inefficient primers, nearby polymorphisms at the target site, or DNA degradation. Since saliva samples were utilized, frequent DNA thawing could contribute to its degradation. Additionally, negative or unknown results may stem from the presence of protein inhibitors that were not fully inactivated during the extraction process. Furthermore, the study employed the rHamp SNP method, which utilizes primers and specific probes designed to bind to the mutation region. Polymorphisms in the vicinity of the target mutation can interfere with the amplification process, also potentially leading to negative or unknown results.

The majority of patients in this study fell within the late-elderly age (>65 years). This finding is consistent with established evidence indicating that age-related risk factors for osteoarthritis arise from increases in both systemic susceptibility and biomechanical stress. Additionally, national data show that the prevalence of osteoarthritis in Indonesia is markedly higher among individuals over 60 years of age, reaching approximately 65%.[17]

In this study, 14 out of the 21 patients were female, supporting evidence that women have approximately twice the risk of developing osteoarthritis than men. This increased risk is often attributed to postmenopausal estrogen deficiency and lifestyle-related factors. Supporting this, previous study found that 79.2% of 72 housewife respondents in Medan were suffering from osteoarthritis, with the increased prevalence linked to gender and age, both significant risk factors.[18] Similarly, Amir et al. reported a higher proportion of female patients with osteoarthritis, suggesting that factors such as lower levels of physical activity, social isolation, immobility, and a higher prevalence of obesity among women may contribute to this disparity.[19]

In this study, the Javanese ethnic group demonstrated the highest prevalence of osteoarthritis relative to other ethnic groups. This finding may be attributable to the fact that the Javanese constitute the largest ethnic population in Indonesia and that the research was conducted on the island of Java.[20] According to census data from the Indonesian Central Bureau of Statistics, the Javanese make up 40% of the population of Indonesia.

The global average distribution of the rs1065080 genotypes is AA or wildtype (3%); GA or heterozygous (27%); and GG or mutant (70%). In this study, the prevalence of the AA genotype (9.5%) is higher than the East Asian population average (5%), while that of the

GG genotype (61.9%) slightly surpasses the East Asian population average (58%). In contrast, the proportion of the GA genotype (28.6%) was lower than the East Asian population average of 36%.[21]

The late elderly (>65 years) represented the vast majority of patients (66.7%). The GG (mutant) genotype was most commonly discovered among this age group (42.9%), showing that they are more likely to have this GG genotype than younger people. The genotype was more common in women (42.9%) than in men (19%), suggesting a link between it and a higher risk of knee OA in women. In this research, the most common employment was as a housewife (47.6%). The GG genotype was most common among this group (28.6%), which may indicate the risk factors of everyday activities that contribute to joint deterioration. The majority of patients (38%) were of Javanese descent, followed by Minangkabau (19%) and Betawi (14.3%). The Javanese ethnicity had the highest percentage of GG genotype (19%), which could be attributed to the prevalence of specific genetics in this community.

The findings of this study demonstrated that the G allele was more predominant than the A allele. The G allele represents the dominant homozygous form associated with the GG genotype, whereas the A allele corresponds to the minor homozygous form associated with the AA genotype. The combination of these two alleles results in the GA genotype, which is the heterozygous variant. In East Asian populations, the G allele exhibits a higher prevalence (76%) compared with the A allele (24%). Similar patterns have been reported in Southeast Asia, particularly in Vietnam, where the G allele frequency also exhibits a higher prevalence (70%) compared with the A allele frequency (30%). Consistent with these data, this study identified the G allele at a frequency of 76.2% (32 alleles), while the A allele accounted for 23.8% (10 alleles). Nevertheless, further research is needed, utilizing a larger sample size and controls, to better assess the correlation between the rs1065080 allele and the increased risk of osteoarthritis in the Indonesian elderly population.

CONCLUSION

The polymorphism of the SMAD3 rs1065080 gene in elderly patients with grade 4 knee osteoarthritis was observed to predominantly exhibit the GG genotype, followed by GA and AA genotypes. Based on these findings, the authors recommend conducting further investigations to explore the potential relationship between the SMAD3 rs1065080 gene and the occurrence of grade 4 knee OA. Such studies could facilitate the development of clinically significant interventions, including preventive and promotive strategies based on genetic factors, aimed at reducing the risk of knee osteoarthritis, particularly in its advanced stages.

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Conflict of interests

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