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## **mRNA NFATC1 Expression in Genu Osteoarthritis with Effusion**

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### **ABSTRACT**

Based on a study conducted by the WHO Global Burden of Disease in 2010 stated, OA ranks 11th, the cause of disability in the world, and ranks 6th in East Asia and the Asia-Pacific countries, ranked 10th in North America, ranked 7th in Eastern Europe, and ranked 13th in Western Europe. Pathogenesis of osteoarthritis (OA) as degenerative joint disease that was once thought caused by a mechanical process. The prevalence, etiology, pathogenesis and progression of OA are still not understood until now, mainly because of the many influencing factors. This type of research is an analytic study. It was conducted in Rheumatology Polyclinic Dr. Mohammad Hoesin Palembang from February to December 2019. Affordable research population is all genu osteoarthritis sufferers with effusion who go to Dr. RSUP Mohammad Hoesin Palembang as 80 samples. Comparison of NFATC1 mRNA levels in genu osteoarthritis patients with effusion shows that there is no difference in gender and history of diabetes, whereas hypertension history and degree of osteoarthritis based on Lawrence Kalgren shows significantly different expression of NFATC1 mRNA. Age with NFATC1 mRNA expression showed no significant correlation, in contrast to BMI, Stroke Test and joint effusion volume showed a weak significant correlation, even though the direction of the correlation was negative on Stroke Test and joint effusion volume.

**Keywords:** Osteoarthritis, Effusion, mRNA NFATC1

## **Introduction**

Osteoarthritis (OA) is a degenerative joint disease that was once thought to be a pathogenesis caused by a mechanical process. The prevalence, etiology, pathogenesis and progression of OA are still not understood until now, mainly because of the many influencing factors. Without clear evidence of the cellular and molecular mechanisms that occur in OA, many opinions state that OA is considered a wear and tear process and the consequences of the aging process. Based on a study conducted by the WHO Global Burden of Disease in 2010 stated, OA ranks 11th, the cause of disability in the world, and ranks 6th in East Asia and the Asia-Pacific countries, ranked 10th in North America, ranked 7th in Eastern Europe , and ranked 13th in Western Europe. Nuclear Factor Activated T cell 1 (NFATC1) is a protein coding gene, which is part of the Nuclear Factor Activated T Cell (NFAT) group. NFAT is a group of transcription factors that are important in the body's immune response. Most of the form of NFAT is expressed in the body's immune cells. NFAT currently has an important role in regulating various systems in the body. NFATC1 is regulated by calcium signaling, which activates calmodulin (CaM) and serine / threonin phosphatase calcineurin (CN), so that NFATC1 is active and can enter the nucleus, which then occupies gene promoters that can activate the transcription of the gene to produce a protein. specific that are important for regulating systems in the body. The process of gene transcription will produce Messenger Ribonucleic Acid (mRNA) IL-1 $\beta$ . Research that analyzes the involvement of the NFATC1 gene in OA genu with effusion has never been done before, so data on gene expression and cytokines have not been published. Therefore, researchers are interested in knowing the expression of NFATC1 mRNA in patients with genu osteoarthritis effusion.

## **Methods**

This type of research is a analytic study. It was conducted at the Rheumatology Polyclinic Dr. Mohammad Hoesin Palembang from February to December 2019. Affordable research population is all genu osteoarthritis sufferers with effusion who go to Dr. RSUP Mohammad Hoesin Palembang. Inclusion Criteria : Genu osteoarthritis sufferers with effusion who have

been diagnosed based on 2010 American College of Rheumatology Criteria and Genu osteoarthritis sufferers with effusion whose aspiration of joint fluid has positive results. Patient willing to participate in research and sign informed consent. Exclusion Criteria :Genu effusion due to trauma, malignancy, autoimmune diseases (Systemic Lupus Erythematosus, Rheumatoid Arthritis), gouty arthritis, septic arthritis, refuse to take part in research. This research subjects using total sampling. All subjects who came for treatment from April 2019 to October 2019, who met the inclusion criteria were included in the study. Research variable: NFATC1 mRNA, the degree of effusion of OA genu, Age, sex, hypertension, diabetes mellitus, BMI. NFATC1 (nuclear factor activated T cell 1) is a protein coding gene. NFATC1 is a form of isoform of the NFAT (nuclear factor activated T) gene group. NFAT is a group of transcription factors that are important in the body's immune response. With Reverse Transcription Polymerase Chain Reaction (RT PCR) which goes through three stages namely RNA extraction, PCR, and Electrophoresis Obtained NFATC1 mRNA expression in units of A.U.

## Results

The largest group of women is around two thirds of the entire study sample, compared to the male group. The level of education is almost mostly tertiary education, compared to the secondary education group, namely junior high and high school, and low education which is only 11%. Patients occupations are mostly housewives, followed by state, private, peasant and teacher civil servants. The mean age of the patients was  $59.4 \pm 13.4$  years which showed the age group as a risk factor for degenerative diseases.

Table 1. Characteristics of physical examination of genu osteoarthritis patients with effusion

No.	History	Subject (n=80 )
1	Diabetes Melitus	
	Yes	5%
	No	95%
2	Hypertension	
	Yes	27,5%
	No	72,5%

Table 1 shows the history of diabetes mellitus and hypertension in genu osteoarthritis patients with effusion. Most showed no history of DM, but only a quarter had a history of hypertension. BMI of patients in this study showed more weight categories based on Asia Pacific BMI categories.

Table 2. Characteristics of physical examination of genu osteoarthritis patients with effusion

No.	Variabel	Subject (n=80 )
1	BMI (kg/m <sup>2</sup> ), Mean ± SD	24,9 ± 6,2
2	Volume Effusion (mL), Mean ± SD	14,9 ± 5,3

The volume of joint fluid aspirated in genu osteoarthritis patients was found to be 14.9 ± 5.3 mL. Characteristics of physical examination on genu effusion (table 3) show that most of them get a score of 2 based on the Stroke Test Score.

Table 3. Characteristics of effusion tests in genu osteoarthritis patients

Stroke Test Score	Subject (n=80 )
Score 1	25%
Score 2	62,5%
Score 3	12,5%

Radiological investigations based on Kallgren-Lawrence in genu osteoarthritis patients with effusions showed grade 3 and 4 results with percentages that did not differ much (table 4).

Table 4. Characteristics of kallgren-lawrence radiological investigations in genu osteoarthritis patients with effusion

Kallgren-Lawrence	Subject (n=80 )
Grade 3	45%
Grade 4	55%

Laboratory investigations showed that LED results did not increase and qualitative CRP was largely negative. The results of NFATC1 levels were  $67.8 \pm 12.8$  pg / mL.

Table 5. Characteristics of laboratory examinations of genu osteoarthritis patients with effusion

No.	Variabel	Subject (n=80 )
1.	ESR (mm/hr), Mean $\pm$ SD	10,2 $\pm$ 2,1
2.	CRP Qualitative	
	Positive	0
	Negative	100%
3.	NFATC1 (pg/mL), Mean $\pm$ SD	67,8 $\pm$ 12.8

Comparison of NFATC1 mRNA levels in genu osteoarthritis patients with effusion shows that there is no difference in sex and history of diabetes, whereas hypertension history and degree of osteoarthritis based on Lawrence Kalgren shows significantly different expression of NFATC1 mRNA. This is probably due to the large number of patients who have a history of hypertension, which is about 27.5% of the 80 samples from this study, as well as both grades on the Lawrence Kalgren, the number of samples participating is almost equal.

Table 6. Comparison of NFATC1 mRNA levels in patients with genu osteoarthritis with effusion

No.	Variabel	mRNA NFATC1 GAPDH (A.U)	<i>p</i>
1.	Gender		<i>0,615</i>
	Man	0,66 ( $\pm$ 0,531)	
	Women	0,53( $\pm$ 0,386)	
2.	Diabetes Melitus		<i>0,456</i>
	No	0,55 ( $\pm$ 0,405)	
	Yes	0,80 ( $\pm$ 0,724)	
3.	Hypertension		<i>0,015</i>
	No	0,50 ( $\pm$ 0,411)	
	Yes	0,70 ( $\pm$ 0,429)	
4.	Kalgren Lawrence		<i>0,010</i>
	Grade 3	0,68 ( $\pm$ 0,482)	
	Grade 4	0,45 ( $\pm$ 0,335)	

Table 7. Correlation of patient characteristics with NFATC1 mRNA in patients with genu osteoarthritis with effusion

No.	Variable	mRNA NFATC1
1.	Usia	$p = 0.201$ $r = -0.145$
2.	BMI	$p = 0.032$ $r = 0.239$
3.	Stroke Test	$p = 0.003$ $r = -0.333$
4.	Efusi Sendi	$p = 0.000$ $r = -0.472$

Age with NFATC1 mRNA expression showed no significant correlation, in contrast to BMI, Stroke Test and joint effusion volume showed a weak significant correlation, although the direction of the correlation was negative on Stroke Test and joint effusion volume. An increase in patient BMI shows an increase in NFATC1 mRNA expression with weak correlation strength. Conversely, an increase in stroke test and joint effusion volume will reduce NFATC1 mRNA expression with weak correlation strength.

### Discussion

In osteoarthritis, activation of catabolic enzymes such as matrix metalloproteinases (MMPs) and aggrecan will cause softening of the articular cartilage. Increased levels of IL-1 $\beta$  can stimulate catabolic enzyme expression and induce peripheral cartilage and articular cartilage bone transformation. There are studies evaluating the levels of NFAT1, IL-1 $\beta$ , TNF- $\alpha$  in articular cartilage in osteoarthritis patients and healthy people using western blot analysis. The results of the study revealed that the expression of NFAT1, IL-1 $\beta$ , TNF- $\alpha$  would increase in cartilage tissue in osteoarthritis patients. In chondrocyte cells with inactive NFAT1 levels, low IL-1 $\beta$  and MMP levels were obtained. There is a Calcineurin-NFAT pathway that plays a role in osteoarthritis. But data on the role of calcineurin-NFAT in cartilage metabolism and osteoarthritis are still very limited.

Based on a meta-analysis study conducted by Peters, MJ, Ramos, YFM, et al, 2016 regarding the association between joint effusion and in the knee and gene expression in levels in the circulation states that there is a correlation between the severity of joint effusion degree and the high expression of NFATC1 (nuclear factor of activated T cells 1). Besides functioning in the process of bone remodeling through calcium or calcineurin signals, NFATC1 has a central role in inducing genes that play a role in the immune system. Although there were no differences in the expression of NFATC1 in cartilage tissue with osteoarthritis and no osteoarthritis by microarrays analysis, detection on RT-qPCR showed a significant reduction in the amount of cartilage affected by osteoarthritis. In this studies may differ many factors that influence the expression of NFATC1 mRNA such as therapy and duration of illness, which were not examined in this study.

### **Conclusion**

Comparison of NFATC1 mRNA levels in genu osteoarthritis patients with effusion shows that there is no difference in gender and history of diabetes, whereas hypertension history and degree of osteoarthritis based on Lawrence Kalgren shows significantly different expression of NFATC1 mRNA. Age with NFATC1 mRNA expression showed no significant correlation, in contrast to BMI, Stroke Test and joint effusion volume showed a weak significant correlation, even though the direction of the correlation was negative on Stroke Test and joint effusion volume.

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