Research

TNF-α expression in chronic rhinosinusitis with and without nasal polyps

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ABSTRACT

Background: Chronic rhinosinusitis is an inflammatory disease of the nasal and paranasal sinuses mucosa, that is caused by multifactorial factors, and has a complex and influential etiology among various microorganisms (bacteria, fungi, and viruses), environmental contamination (pollutants or cigarette smoke) and immune system instability. Tumor necrosis factor alpha (TNF-α) is one of the crucial cytokines in the inflammatory process that plays a role in chronic rhinosinusitis. **Purpose:** To compare TNF-α gene expression levels in chronic rhinosinusitis patients with and without nasal polyps. **Method:** This quantitative study included a cross-sectional design comparative analysis of 24 samples obtained from the ethmoid bulla of chronic rhinosinusitis patients. The patients were subjected to a Functional Endoscopic Sinus Surgery (FESS) approach at the Rhinology Clinic of Dr. M. Djamil Regional Hospital, Padang. Patients were selected based on inclusion and exclusion criteria. The expression of TNF-α was measured by using the Real-Time Polymerase Chain Reaction (RT-PCR). The data were analysed by SPSS with a p-value <0.05. **Result:** The average value of TNF-α expression in chronic rhinosinusitis with polyps was 4.89±12.65, while that of TNF-α expression in chronic rhinosinusitis patients without polyps was 2.77±4.22. However, there was no statistical difference between the two groups of study (p-value >0.05). **Conclusion:** This study demonstrated the increased levels of TNF-α in chronic rhinosinusitis patients with polyps compared to patients without polyps.

Keywords: chronic rhinosinusitis, polyps, RT-PCR, TNF-α

ABSTRAK

Latar belakang: *Rinosinusitis kronis merupakan penyakit inflamasi pada mukosa hidung dan sinus paranasal yang penyebabnya multifaktorial, dan mempunyai etiologi yang kompleks serta berpengaruh di antara berbagai mikroorganisme (bakteri, jamur, dan virus), kontaminasi lingkungan (polutan atau asap rokok) dan ketidakstabilan sistem kekebalan tubuh.* Tumor necrosis factor alpha (TNF-α) *merupakan salah satu sitokin penting dalam proses inflamasi yang berperan dalam rinosinusitis kronis.* ***Tujuan:*** *Untuk membandingkan tingkat ekspresi gen* TNF-α *pada pasien rinosinusitis kronis disertai polip hidung dengan tanpa polip hidung.* ***Metode:*** *Penelitian kuantitatif ini mencakup desain analisis komparatif potong-lintang dari 24 sampel yang diperoleh dari bula etmoid pasien rinosinusitis kronis. Pasien menjalani pendekatan* Functional Endoscopic Sinus Surgery (FESS) *di Klinik Rhinologi Dr. M. Djamil di Rumah Sakit Umum Daerah, Padang. Pasien dipilih berdasarkan kriteria inklusi dan eksklusi. Ekspresi* TNF-α *diukur dengan menggunakan* Real-Time Polymerase Chain Reaction (RT-PCR). *Data dianalisis dengan* SPSS *dengan* p-value *<0,05.* ***Hasil:*** *Nilai rata-rata ekspresi* TNF-α *pada rinosinusitis kronik disertai polip adalah 4,89±12,65, sedangkan ekspresi* TNF-α *pada penderita rinosinusitis kronik tanpa polip adalah 2,77±4,22. Namun, tidak ada perbedaan statistik antara kedua kelompok studi (*p-value*>0,05).* ***Kesimpulan:*** *Penelitian ini menunjukkan adanya peningkatan kadar* TNF-α *pada pasien rinosinusitis kronis disertai polip dibandingkan dengan pasien tanpa polip.*

Kata kunci: *rinosinusitis kronis, polip,* RT-PCR, TNF-α

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INTRODUCTION

Chronic rhinosinusitis is an inflammatory disease of the nasal and paranasal sinuses mucosa that persists for at least 12 weeks. There are two kinds of main symptoms, comprised of nasal congestion and viscous mucus (anterior/posterior). These symptoms could be accompanied by pain on the face, with and without olfactory disorders, and one of the nasal endoscopic symptoms, namely polyps or mucopurulent secretions from the medial meatus. Also, symptoms could be accompanied by mucosal oedema or obstruction in the medial meatus, or mucosal changes in the osteomeatal or sinus complexes, which can be evaluated by Computed Tomography Scans (CT-Scans). This entity is a multifactorial disease and has a complex and interdependent etiology among various microorganisms (bacteria, fungi, and viruses), environmental contamination (pollutants or cigarette smoke), and immune system instability. Chronic rhinosinusitis is characterized by chronic inflammation of the nasal mucosa and paranasal sinuses, the occurrence of many cytokine releases and tissue remodelling including changes in the extracellular matrix, protein deposits, and tissue structures.[1-6]

According to the latest guidelines, the use of antibiotics in chronic rhinosinusitis is allowed if this entity meets three symptom criteria and signs of bacterial infection from the following five standards, such as: 1)one-sided mucopurulent nasal discharge; 2)one-sided facial pain; 3)high body temperature (38^{o}C); 4)double sickening symptoms, or condition worsened after an examination; and 5)increased levels of C-reactive Protein (CRP), and Blood Sedimentation Rate, and given according to the antibiotic resistance culture test.[7]

Tumor necrosis factor alpha (TNF-α) is a cytokine that is primarily secreted by monocytes and macrophages. This cytokine works on the pathophysiological mechanisms that affect homeostasis in various tissues. TNF-α plays an important role in the inflammatory process underlying the occurrence of chronic rhinosinusitis.[4,8-10] Also, TNF-α has crucial functions as an endogenous alarm that coordinates gene expression, cellular activity and provides an inflammatory response to infection, injury, or irritation. On the airway tract organ, TNF- α is released by bronchial epithelial cells in inflammatory conditions and affects the airway mucosa that causes loss of ciliary cells, epithelial metaplasia, and accumulation of inflammatory cells in the sub-epithelial layer. Increased expression of TNF-α is also associated with the morphological and functional changes related to the integrity of the respiratory mucosa and olfactory system. TNF-α concentrations increase significantly in patients with asthma, chronic rhinosinusitis, and cystic fibrosis compared with normal patients.[10-12]

The study conducted by Charlemwatanachai et al.[13] showed an association between intramucous microorganisms and inflammatory patterns in chronic rhinosinusitis patients. The ability of *S. aureus* to enter and survive inside host cells can contribute to the development of persistent disease or chronic infections, that can lead to infection or spreading to deeper tissues. Invasion into vascular endothelial cells is the most decisive stage of *S. aureus* infection because it can promote the production of the proinflammatory cytokines. According to Rocha-de-Souza et al.[14], mast cells infected with *S. aureus* will release two cytokines, one of which is TNF-α.

This study aimed to compare the differences in TNF-α gene expression among chronic rhinosinusitis patients with and without polyps.

METHOD

This was a quantitative research study with a cross-sectional comparative design, including 24 samples. The samples were taken from chronic rhinosinusitis patients. The ethmoid bulla isolation was performed by Functional Endoscopic Sinus Surgery (FESS) in the Rhinology Clinic of Dr.M. Djamil Regional Hospital, Padang. The technique was a consecutive sampling of patients who were willing and submitting written consents. The sample inclusion criteria were chronic rhinosinusitis patients aged 18 to 60 years. In patients with bilateral chronic rhinosinusitis, samples were taken on the more severe side based on computed tomography imaging. The sample exclusion criteria were chronic rhinosinusitis patients with severe persistent allergic rhinitis, or solitary rhinosinusitis patients. There was a total of 10 minimum samples in this study. This research was approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang.

RT-PCR

Ethmoid bulla in intraoperative samples were put down into a microtube containing 1 ml of phosphate-buffered saline (PBS). RNA samples were isolated within 24 hours. Respondents' data were recorded in the research form. Two primers were used in this study, namely TNFα-Reverse: 5'-CAC TGA AAG CAT GAT CCG GG-3', and TNFα-Forward: 5'-GGC TGA TTA GAG AGA GGT CC-3'. RNA was extracted from ethmoid sinus mucosal cells and isolated by using TRIzol® Reagent. The initial stage was started by RNA precipitation from the samples (106 cells or <10 mg tissue), adding 5–10 µg of RNase-free glycogen as a carrier for the aqueous phase. Then, 0.5 mL of 100% isopropanol was added to the aqueous phase per 1 mL of TRIzol® Reagent for homogenization, incubation at room temperature for 10 minutes followed by centrifugation at 12,000 x g for 10 minutes at 4°C, and an RNA wash.

RNA washing was carried out by removing the supernatant from the tube, and then the pellet was washed with 1 mL of 75% ethanol per 1 mL of TRIzol® Reagent used in the initial homogenization. The sample was then centrifuged at 7,500 x g for 5 minutes at 4°C, and the supernatant was discarded. The pellet was dried for 5–10 minutes and was used to make cDNA using the iScript TM cDNA Synthesis Kit. This kit provided a solution that was sensitive and easy to use for quantitative reverse transcription PCR (RT-qPCR). The iScript was a modification of the optimum reverse transcriptase Moloney Murine Leukemia Virus (MMLV) for the synthesis of broad and dynamic RNA inputs. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) was used as a control of cDNA formation.

The gene was amplified using an RT-PCR machine. At this stage, SSoFastTMEvaGreen® Supermix liquid was used. The supermix was a combination of all components (except primers and templates) which could be used for real-time quantitative PCR (qPCR). All components were liquefied at room temperature by reversing the tube several times to ensure homogeneity according to the desired gradient concentration. After that, H_2O was added until a final 10-µl volume was achieved.

Data collection of RT-PCR was carried out by two approaches, namely relative quantification and absolute quantification. Relative quantification could be done with two models, namely the relative standard curve method and the comparative CT method (ΔΔCt). The ΔΔCt method was used if the efficiency of the gene was almost the same,

and this method did not require a normal curve. In this study, TNF-α gene expression was analysed by the comparative CT method (ΔΔCt).

The comparative CT method (ΔΔCt) was developed by Livak and Schmittgen.[15] This method of analysis did not require a normal curve and nor the efficiency must be similar between the genes. This calculation approach was more comprehensive than other calculations because there was no normal curve analysis.

The calculations used to get the ratio were divided into four stages, namely: 1)Calculation of the mean and standard deviation of CT/CP values of the control sample group, both the target gene (TNF-α) and housekeeping gene (GAPDH); 2)Calculation of ΔCt treatment and control groups: ΔCt was calculated by reducing the mean group Ct treatment with the housekeeping gene (GADPH); 3) Calculation of ΔΔCt (ΔΔCt value obtained from the reduction of the ΔCt treatment group with ΔCt control); and 4)Calculation of the expression ratio (expression ratio calculated by formula= $2^{-\Delta\Delta Ct}$).

RESULT

In this study, there were 6 males and 7 females experiencing chronic rhinosinusitis with nasal polyps. Whereas, there were 3 males and 8 females experiencing chronic rhinosinusitis without nasal polyps. Furthermore, the average age of chronic rhinosinusitis with nasal polyps was 23–45 years, and the average age of chronic rhinosinusitis without nasal polyps was 36–63 years.

Based on the observed results (Table 1), most of the respondents experienced nasal congestion, cold, and downwards flowing mucus in the throat. Approximately 100% of individuals with chronic rhinosinusitis with nasal polyps and 90.90% of chronic rhinosinusitis without nasal polyps reported a smelling disturbance. Furthermore, the complaint of cheek fullness in chronic rhinosinusitis patients with polyps was 53.8%, and that in chronic rhinosinusitis patients without polyps was 72.72%. More importantly, TNF-α expression in chronic rhinosinusitis patients with polyps and without polyps was 4.89±12.65 and 2.77±4.22, respectively. Statistical analysis showed no significant difference (p >0.05) (Table 2).

Table 1. Characteristics of the respondents

Characters	Chronic rhinosinusitis with nasal polyps		Chronic rhinosinusitis without nasal polyps	
	f	%	f	%
Male	6	46.15%	3	27.27%
Female	7	53.84%	8	72.72%

Table 2. The expression of TNF-α in chronic rhinosinusitis with and without nasal polyps

The expression of TNF-α in chronic rhinosinusitis with nasal polyps	The expression of TNF-α in chronic rhinosinusitis without nasal polyps
1.00	0.07
0.59	0.04
4.45	2.11
1.80	2.26
0.78	2.55
0.49	0.89

0.45	15.03
0.61	1.52
1.81	3.94
46.85	1.36
0.79	0.67
2.89	
1.07	

DISCUSSION

Chronic rhinosinusitis is the second most chronic disease reported in the United States, and is associated with a significant decrease in quality of life. The pathogenesis of chronic rhinosinusitis is not well understood, but it is known that chronic rhinosinusitis leads to dysfunctional interactions between hosts and the environment. The normal mechanical barrier of the sinonasal mucosa consists of pseudostratified epithelium, motile cilia, respiratory epithelial cells joined with apical tight junctions, and a mucus layer.[16]

In this study, a group of chronic rhinosinusitis patients with polyps or without polyps was more common in women than men. The experts confirmed a higher prevalence of chronic rhinosinusitis in female than male,[17,18] which was consistent with our results. The same results were also obtained by Reh et al.[19] who found that the ratio of women (62.8%) was greater than that of men (55.6%).

The RT-PCR results showed the mean of TNF-α expression in chronic rhinosinusitis patients with polyps was higher than that of chronic rhinosinusitis patients without polyps. Van Zele et al.[20] in their study also stated that the level of proinflammatory mediators in chronic rhinosinusitis patients was significantly higher than that of controls. TNF-α is the most activated proinflammatory gene in chronic rhinosinusitis.[16]

These cytokines could stimulate and enhance expression of inflammatory-mediated enzymes, cyclooxygenase 2 (COX-2) and nitric oxide synthase 2 (NOS-2). However, the role of nitric oxide in inflammation was complex, while its high concentration and cross-talk with COX-2 showed a high inflammatory response. COX-2 played an important role in autophagy and the inflammatory process induced by tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β).[21] Plewka et al.[21] demonstrated that the highest TNF-α expression was found in the vascular endothelium. TNF-α had an important involvement in the development of nasal polyps, and its level significantly increased in eosinophilic polyps.

An understanding of the role of TNF-α in the formation of nasal polyps is fundamental given the identification of TNF-α involvement in the pathogenesis of other conditions, which is also reflected by the success of anti-TNF antibody therapy.

In conclusion, the results showed that increased TNF-α expression levels was found in patients with chronic rhinosinusitis with polyps compared to those with chronic rhinosinusitis without polyps. Further research is needed to determine the role of inflammatory mediators in chronic rhinosinusitis with and without polyps.

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