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Research Article

TNF-α Expression in Patients with Chronic Rhinosinusitis without Polyps

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Abstract: Background: Chronic rhinosinusitis is an inflammation of the nose and paranasal sinuses for more than 12 weeks. The inflammatory mediator process which involved include cytokines. Tumor Necrosis Factor Alpha (TNF-α) is one of the proinflammatory cytokines that found to be increased in chronic rhinosinusitis. **Objective:** To determine the TNF-α expression in patients with chronic rhinosinusitis without polyps. **Methods:** This research was analytic, with cross sectional study design, performed on 24 cases of chronic rhinosinusitis without polyp obtained by non-probability consecutive sampling. Immunohistochemical examination was performed to assess TNF-α expression in chronic rhinosinusitis without polyps. The data collected were statisically analyzed based on a significance level of p-value <0.05. **Results:** From the 24 cases, there was 21 cases that we found TNF-α overexpression (87.5%). From all the positive groups, the largest age group ≥ 40 years (100%), with the main complaints of olfactory disorders and headache (100%), TNF-α overexpression was found in the Lund-Mackay index of CT scan ≥ 6 (95,5%), and there is a correlation between TNF-α and Lund-Mackay index (p=0,004). **Conclusion:** TNF-α overexpression was found in chronic rhinosinusitis without nasal polyp, which is found mostly in age group ≥ 40 years, with the main complaints olfactory disorders and headache, and with the Lund-MacKay index of CT scan ≥ 6.
Keywords: Expression, TNF- α, chronic rhinosinusitis.

1. Introduction

Chronic rhinosinusitis is a disease that has a relatively high prevalence, often found in most populations in the world, ranging from 7-30% (Wang *et al.*, 2016). Apart from having an impact on decreasing quality of life and socioeconomic aspects, chronic rhinosinusitis that is not handled properly will cause several complications (Gianonni, 2015). Chronic rhinosinusitis initially considered only as a bacterial infection. Biopsy results from sinus tissue during surgery showed inflammatory cells and proinflammatory cytokines in active disease, one of which is TNF-α. Cytokines are molecules of peptides or glycoproteins that are released by cells and have specific effects of interaction and communication between cells (O'Hollaren, 2003; Mfuna *et al.*, 2011). TNF-α is produced by several types of cells, such as T lymphocytes, or white blood cells known as macrophages and monocytes, which are responsible for the body's immune response. TNF-α is involved in inflammation, infection and malignancy. Inflammation plays an important role in persistent chronic rhinosinusitis. TNF-α synergizes with Th1 or Th2 in inducing chronic inflammation (Bradley, 2008; Mfuna *et al.*, 2011).

This study aims to determine the Tumor Necrosis Factor Alpha (TNF-α) expression in chronic rhinosinusitis without nasal polyp.

2. Method

This research is analytic with cross-sectional study. The study samples are 24 patients with chronic rhinosinusitis without polyps who had undergone functional endoscopic sinus surgery in Department of Otorhinolaryngology-Head and Neck Surgery Haji Adam Malik General Hospital, Medan.

The maxillary or etmoidalis sinus tissue is taken and examined by immunohistochemical method in the Department of Anatomic Pathology of Faculty of Medicine, Universitas Sumatera Utara. Data processing is performed by using the Statistical Package for Social Sciences (SPSS), conducted a correlation test Spearman with a significant value limit of $p<0.05$. This study was approved by the ethics committee of the Faculty of Medicine, Universitas Sumatera Utara.

Preparation after cutting tissue: the preparation were deparaffinated with Xylol I-II-III respectively for 5 minutes, next do rehydration with multilevel alcohol (absolute alcohol, 96% alcohol, 80% alcohol, 70% alcohol, each for 4 minutes). Wash in running water for 5 minutes. Insert slides into PT Link Epitope Retrieval : set preheat 65°C, running duration 98°C for 15 minutes, then it is cooled for about 45 minutes. Limit tissue with Pap-Pen, wash with Tris Buffered Saline (TBS) pH 7,4. Blocking with endogenous peroxidase (0.5% H2O2 in methanol) for 5-10 minutes. Wash with TBS pH 7,4. Blocking non-specific activity with normal serum 3% for 15 minutes. Wash with TBS pH 7,4.

Incubate preparations with TNF-α antibody for 1 hour. Next wash with TBS pH 7,4/Tween 20 for 5 minutes. Then incubate with Santa Cruz Real Envision for 30 minutes. Wash with TBS pH 7,4/Tween 20 for 5 minutes. Furthermore, the preparation was given chromogen to be colored with DAB (Diamino Benzidine) for approximately 5 minutes. Wash with running water. Counterstain with Hematoxylin. Wash with running water. Saturated Lithium Carbonat (5% in aquadest for 2 minutes). Wash with running water. Next do dehydration with multilevel alcohol (80% alcohol, 96% alcohol, absolute alcohol each for 5 minutes).

Clearing with xylol I, II, III for 5 minutes each. Mounting with entellan and cover glass. The slides can be read directly and examined by specialists in anatomic pathology. TNF-α expression is an assessment of TNF-α protein from the results of brown color on the cytoplasm and nucleus of the cells. The immunoreactive TNF-α score is obtained by times the results of an intensity (staining) score with a broad score using a light microscope. No TNF-α overexpression is indicated with an immunoreactive score of 0-3 and TNF-α overexpression is indicated with an immunoreactive score of 4-9.

The rhinosinusitis computer tomography score according to Lund-Mackay is 0 to 24. The computer tomography score used as the cutoff point for determining the extent of sinus involvement is 6, according to the study of Ponte *et al*., (2005). Computer tomography scores were divided into 2 groups: extensive sinus involvement (score ≥6), non-extensive sinus involvement (score <6).

3. Result

There are 24 patients with chronic rhinosinusitis without polyps. TNF-α overexpression are found in 21 patients (87.5%) and no overexpression in 3 patients (12,5%).

The majority of the subjects were men, which comprises of 13 people (54,1%). The youngest age is 18 years old and the oldest age is 51 years old. The most prevalent age group of 40-49 years old comprises of 12 people (50%).

Nasal congestion is the most major complaint in 7 patients (29,2%), followed by olfactory disorders in 6 patients (25%). This is shown in table 1.

Table 1. Demographic characteristics of research subjects

Demographic characteristics	n	%
Sex		
Men	13	54,1
Women	11	45,8
Age		
<20 years old	2	8,3
20-29 years old	4	16,7
30-39 years old	4	16,7
40-49 years	12	50
≥ 50 years old	2	8,3
Chief complaints		
Olfactory disorders	6	25
Nasal congestion	7	29,2
Facial pain	4	16,7
Headache	5	20,8
Nasal discharge	2	8,3
Total	**24**	**100**

Table 2. Proportion of patient's age based on TNF-α expression

Age	Expression of TNF-α	
	Overexpression, n (%)	No overexpression, n (%)
<40 years	7 (70)	3 (30)
≥ 40 years	14 (100)	0
Total	**21 (87,5)**	**3 (12,5)**

Chronic rhinosinusitis patients aged over or equal to 40 years fully shows the results of TNF-α overexpression (100%).

Table 3. Proportion of chief complaints based on TNF-α expression

Chief complaints	Expression of TNF-α	
	Overexpression, n (%)	No overexpression, n (%)
Olfactory disorders	6 (100)	0
Nasal congestion	6 (85,7)	1 (14,3)
Facial pain	3 (75)	1 (25)
Headache	5 (100)	0
Nasal secretions	1 (50)	1 (50)
Total	**21 (87,5)**	**3 (12,5)**

Patients with chief complaint of olfactory disorders and headaches, all of them show TNF-α overexpression (100%). The main complaint of nasal congestion shows the results of overexpression in 6 patients (85,7%). The main complaint of facial pain show a result of

overexpression in 3 patients (75%), and the main complaint of nasal secretion shows the result of TNF-α overexpression in 1 patient (50%).

Table 4. Proportion of TNF-α expression based on the Lund-MacKay index of CT scan

Expression of TNF-α	Lund-Mackay Index	
	<6, n (%)	≥ 6, n (%)
Overexpression	0 (0)	21 (95.5)
No overexpression	2 (100)	1 (4.5)
Total	**2 (8.3)**	**22 (91.7)**

21 patients with Lund-Mackay index ≥ 6 showed overexpression of TNF-α (95.5%).

Table 5. Correlation TNF-α with Lund-Mackay index of CT scan in patients with chronic rhinosinusitis without polyps

	Lund-Mackay Index	
	p	r
TNF-α	0,004	0.562

Using the Spearman correlation test shows that there is a significant correlation between TNF-α expression and the Lund-Mackay index (p = 0.004). The correlation value obtained is a positive sign (0,562), meaning that there is a correlation that has a moderate strength between TNF-α expression with Lund-Mackay index and is directly proportional, the increase in TNF-α expression will be directly proportional to Lund-Mackay index.

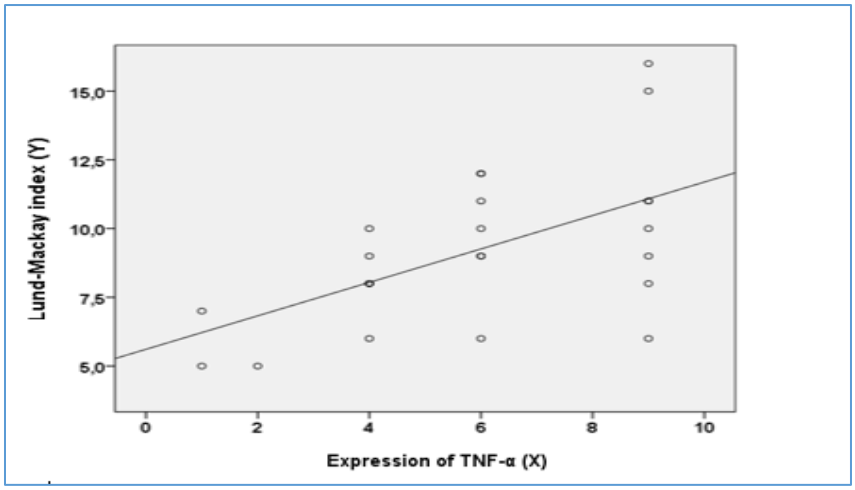

Figure 1. Scatterplot graph of TNF-α expression against the Lund-Mackay index

Discussion

In this study, table 1 shows the demographic characteristics of the study subjects, out of 24 patients, 13 people (54,1%) were men and 11 women (45,8%). Similar report published by Kim *et al.*, (2011) in Korea from 285 people studied from January to December 2008, the incidence of chronic rhinosinusitis in men was higher than women with a percentage of 50,9% and 49,1%.

The most prevalent age group is 40–49 years of age (50%) in this study. Study done by Dewi *et al.*, (2018) also reported the highest age of patients with chronic rhinosinusitis in the range of 46-60 years as many as 20 patients (37,7%) with an average age of 41.2 years.

From table 1, nasal congestion is the most prevalent chief complaint in 7 patients (29,2%). The research conducted by Sandhu *et al.,* (2017) reported from 50 people studied, as much as 96% complained nasal congestion, post nasal drip (84%) and headache (72%).

Mucosal edema and retention of secretions due to blockage of the ostium of the paranasal sinuses are key inflammatory response in chronic rhinosinusitis. Mucosal irritation of airway epithelium triggers the production of various proinflammatory cytokines, which proved to be important in the pathogenesis of chronic rhinosinusitis, such as TNF-α (Misron *et al.*, 2017). Previous research also showed a significant increase in TNF-α expression in the sinonasal mucosa of patients with chronic rhinosinusitis. TNF-α is usually not detected, but increases in serum and tissue levels are found in inflammatory and infectious conditions (Anand *et al.*, 2006; Bradley, 2008; Bruaene *et al.*, 2011).

Based on table 2, patients aged over or equal to 40 years showed the results of TNF-α overexpression (100%). Bruunsgaard *et al.,* (2000) mentions that aging is associated with an increase of several pro-inflammatory cytokines in the blood circulation, such as TNF-α, IL-6, cytokine antagonists, acute phase proteins and neopterin, and includes an increase in the level of TNF-α as well as the dissolved TNF receptor in the blood circulation. The increase of inflammatory activity in elderly has reflected an age-related pathological process.

From table 3, in this study we found the main complaint of patients namely olfactory disorders and headache, all showed TNF-α overexpression (100%). Headache on rhinosinusitis is closely connected with the process of edema or inflammation in the nasal structure, the duct nasofrontal, concha, the sinus ostium (Silberstein and Marmura, 2014). Robbins and Maides (2014) said that the cytokines involved in inflammation, in the modulation of pain threshold and also sensitization of trigeminal nerve fibers. TNF-α is involved in the activation of pain.

Tissue on the nose of patients with chronic rhinosinusitis usually have edema following a long stimulation of inflammatory cytokines. It is believed that the nasal mucosal edema and congestion obstruct the olfactory sulcus, and makes it difficult for odorants molecule to successfully achieve the olfactory mucosa, resulting in olfactory disorders. TNF-α is one of the earliest and inflammatory mediators that trigger an inflammatory response critical, and can have adverse effects on the respiratory mucosa, causing loss of ciliated cells, epithelial metaplasia and subsequent accumulation of inflammatory cells in the subepithelial layer (Wei, *et al.*, 2018; Karosi *et al.*, 2012). Correlation between TNF-α overexpression with Lund-MacKay index of CT scan is still difficult to explain. Research by Szucs *et al.,* (2002) found a significant correlation between the Lund-MacKay index with the number of inflammatory cells in sinonasal mucosa.

Positive and significant correlation between CT scan with mucosal inflammation, the possibility that the inflammatory process due to chronic rhinosinusitis is still active and stable during the study period. Inflammation of the tissues results in mucosal edema, increased secretion and microbial colonization, and is associated with lymphocyte infiltration and inflammatory cytokine expression.

Airway epithelium plays an important role in the etiology of respiratory diseases, known as a regulator of inflammation because of its capacity to produce a variety of inflammatory mediators. Infection will cause ongoing inflammation and worsen the chronic disease process (Bhattacharyya, 2008; Schleimer *et al.*, 2009; Lee dan Lane, 2011).

Conclusion

This study shows that there is TNF-α overexpression in patients with chronic rhinosinusitis without polyps and found a correlation between the expression of TNF-α with Lund-MacKay index of CT scan. This research is expected to be used as the basis for further research for the management of chronic rhinosinusitis.

Conflicts of interest

There is no conflict of interest of any kind.

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