

# Interferon- $\gamma$ and Interleukin-4 Expression in Chronic Rhinosinusitis

Kronik Rinosinüzitte İnterferon- $\gamma$  ve İnterlökin-4 İfadesi

Bestari Jaka BUDİMAN<sup>id</sup>, Surya AZANİ<sup>id</sup>, Effy HURİYATI<sup>id</sup>, Dolly IRFANDY<sup>id</sup>, Andani PUTRA<sup>id</sup>

## Abstract

**Introduction:** This study aims to determine the Interleukin-4 (IL-4) and Interferon- $\gamma$  (IFN- $\gamma$ ) expression in Chronic rhinosinusitis (CRS) with and without nasal polyps.

**Material and Methods:** In the study, we analyzed the findings of 27 pair pathologic specimens of patients with CRS. Concha nasalis media smear was used to determine the IL-4 and IFN- $\gamma$  expression by *real-time polymerase chain reaction* (RT-PCR). Data were analyzed by cytokine gene absolute log copy number (CN).

**Results:** Both IL-4 and IFN- $\gamma$  in patients with CRS with nasal polyps CN mean Log of CRS with nasal polyps were higher (15.28 $\pm$ 3.92 and 8.54 $\pm$ 2.96, respectively) than those of CRS without polyps (14.65 $\pm$ 4.65 and 7.57 $\pm$ 1.82, respectively), but this was not statistically significant ( $p=0.596$ ,  $0.346$  respectively).

**Conclusion:** IL-4 and IFN- $\gamma$  absolute expression level are higher in CRS with nasal polyps than those who had CRS without nasal polyps, but this was not statistically significant ( $p>0.05$ ).

**Keywords:** Chronic rhinosinusitis, IL-4, IFN- $\gamma$ , nasal polyps

## Öz

**Giriş:** Bu çalışmada nazal polipi olan ve olmayan kronik sinüzitli (KZ) hastalarda interferon- $\gamma$  (IFN- $\gamma$ ) düzeylerinin saptanması amaçlandı.

**Gereç ve Yöntemler:** Bu çalışmada KZ'li bulunan 27 çift hastanın patolojik piyesleri irdelendi. IL-4 ve IFN- $\gamma$  ölçümleri, medial nazal konkadan elde edilen sürüntü örneklerinde gerçek zamanlı polimeraz zincir reaksiyonu yöntemi kullanılarak yapıldı. Saptanan gen kopya sayılarının logaritmaları karşılaştırıldı.

**Bulgular:** Nazal polipi olan KZ hastalarında IL-4 ve IFN- $\gamma$  seviyeleri, aynı hastalığı olan nazal polipsiz olguların düzeylerine göre daha yüksek bulundu ancak, aradaki fark istatistiksel olarak anlamlı değil idi (sırasıyla,  $p=0.596$  ve  $0.346$ ).

**Sonuç:** Nazal polipi olan KZ'li hastalardaki IL-4 ve IFN- $\gamma$  düzeyleri, polipi olmayanlara göre daha yüksek olmak ile birlikte aradaki fark anlamlı değildir.

**Anahtar Kelimeler:** Kronik rinosinüzit, IL-4, IFN- $\gamma$ , nazal polip

Andalas University School of Medicine,  
Padang, Sumatera Barat 25163,  
Indonesia., School of Medicine,  
Padang, Indonesia

## Correspondence:

Bestari Jaka BUDİMAN  
Andalas University School of Medicine,  
Padang, Sumatera Barat 25163,  
Indonesia., School of Medicine,  
Padang, Indonesia  
**E-mail:** capa.journal57@gmail.com

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## Introduction

Chronic rhinosinusitis (CRS) is an inflammation of the nasal mucosa and paranasal sinus for more than 3 months accompanied by tissue remodeling. CRS belongs to the heterogeneous sinus disease group which consists of different diseases.<sup>[1]</sup> In the recent classification, CRS is divided into two groups that are CRS with nasal polyps and without nasal polyps. This classification is supported by evidence that the inflammation profile of both CRSs is different, but it is not clear whether the profile represents the difference in its etiopathogenesis.<sup>[1-3]</sup> Previously, CRS with nasal polyps was considered the last stage

of CRS without nasal polyps. Some evidence supports the separate classification based on the inflammation pathway, cytokine profile, and tissue remodeling.<sup>[4]</sup>

The prevalence and incidence of CRS are increasing every year. This disease not only affects life expectancy but has also become one of the most expensive diseases to treat. The U. S. A spent 5.8 Billion USD of the health budget in 1997 to treat this disease.<sup>[1,4]</sup> Between October 2011 and September 2012, there were 106 new cases of CRS; 87 cases without nasal polyps and 19 cases with nasal polyps, in Dr. M. Djamil Hospital, Padang (data of Rhinology out-patient clinic, THT-KL. Dr. M. Djamil Hospital, Padang, 2012).

The understanding of CRS pathophysiology is continuously improving, but its etiology and pathophysiology are still not clear.<sup>[2]</sup> New evidence suggests that CRS with nasal polyps has a different inflammation profile compared to that with without nasan polyp.<sup>[4]</sup> CRS belongs to the immune system disease group which is mediated by T lymphocytes and contributed to by different subsets of T lymphocytes.<sup>[5]</sup> CRS without nasal was shown to be associated with Th1 cell polarization with interferon- $\gamma$  (IFN- $\gamma$ ), transforming growth factor (TGF)- $\beta$ 1, TGF- $\beta$ 2, and IL-1 as the inflammation profile.<sup>[4,5]</sup> Th2 cell polarization with IL-4, IL-5, and IL-13 cytokines was observed in patients having CRS with nasal polyps. The latter kind of CRS is the main focus of nasal polyp research, especially in European countries.<sup>[3,5,6]</sup>

Interleukin 4 (IL-4) has an important role in immune inflammation regulation of IgE production and eosinophil activation. IL-4 stimulates the differentiation of naïve Th lymphocytes into Th2 lymphocytes; hence, it is known as Th2 inducer and serves as an important cofactor to inhibit the apoptosis of activated T cells. IL-4 is also responsible for “class-switching” of B cell immunoglobulins to IgE phenotypes.<sup>[3,6,7]</sup> IL-4 induces eosinophil migration via the elevated expression of vascular cell adhesion molecule (VCAM)-1.<sup>[8]</sup>

IFN- $\gamma$  is the Th1 cell main cytokine involved in many inflammation processes and immune system actions<sup>6</sup>. Some studies have found a decrease of IFN- $\gamma$  in nasal polyps, but its connection to the pathogenesis of nasal polyps is not clearly described.<sup>[5,6]</sup>

The inflammation pattern of CRS with a nasal polyp in the Europe is different than the type in Asia.<sup>[5]</sup> Zhang et al.<sup>[9]</sup> found a different inflammation pattern in Belgian and Chinese populations. Nasal polyp cases in Southern China and Thailand has Th1/Th7 predominance in the infiltrate, associated with the up-regulation of T-Bet mRNA, the secretion of IFN- $\gamma$ , IL-17, IL-1 $\beta$  and IL-6, and neutrophil infiltration. However, Belgian cases have Th2 cell domination, associated with the up-regulation of GATA3 mRNA, that led to the increased secretion of IL-5 and IgE, and eosinophil infiltration. These differences necessitates different therapeutical approaches. European countries and the USA have focused on the implementation of local and systemic corticosteroids as well as anti-IL-5 monoclonal antibodies. However, this concept cannot be implemented in Asian countries, as the inflammation profile is different.<sup>[9]</sup>

Non-mechanical factors, such as inflammatory mediators, are the key to understanding the etiopathogenesis of CRS. Pro-inflammatory cytokines induce persistent inflammation, which is crucial in the etiopathogenesis of CRS, with a different gene responsible for each type of CRS. Understanding the etiopathogenesis is important to determine the best course of action to treat this disease. In this study we aimed to assess the differences between gene expression of IFN- $\gamma$  and IL-4 in both types of CRS patients, qualitatively and quantitatively.

## Methods

In this study, 27 pair nasal samples from patients who have a diasnosis of CSR with or without nasal polyps were analyzed. The samples were taken from the patients who were admitted to THT KL outpatient clinic. Concha nasalis media smears were used to analyze the expression of IL-4 and IFN- $\gamma$ . Sterile cotton swabs were pressed against the mucus of the nasalis concha media and smeared back and forth 3 times. The pressure was adjusted in order to prevent rupture of the nasal blood vessel. This step was assisted with a nasendoscopy. Concha nasalis media smears were put into 1 mL phosphate buffer saline (PBS) in tubes and transported to the Biomedical Laboratory, Medicine Faculty, Andalas University for analysis.

EPOS 2012 criteria were used to diagnose CSR patients: aged 18–60 years old; having 1st or 2nd degree nasal polyps; and did not receive any anti-allergic drugs during the washout period prior to the smear (chlorpheniramine

for 3 days; cetirizine, fexofenadine, and loratadine for 5 days; and corticosteroid for 2 weeks prior the smear). All patients gave written consent to be included as research subjects.

### IFN- $\gamma$ and IL-4 expression

Every sample was amplified by Light Cycler<sup>®</sup> TaqMan DNA Master Mix kit (Roche) using a Real-Time PCR machine (LightCycler<sup>®</sup> 2.0-Time PCR System Real, Roche). The primers and probes were intended to target IFN- $\gamma$  and IL-4 genes.

**IFN- $\gamma$  primer:** F464: CTAATTATTCGGTAACT-GACTTGA 75 F 600; R 900, R538: ACAGTTCAGC-CATCACTTGGA, P491:6Fam-TCCAACGCAAAG-CAATACATGAAC-Tamra-p.

**IL-4 Primer:** ACAGGAGAAGGGACGCCATGAAGC-CCTACAGACG,

**Probe:** TCCTCACAGCAACGAAGAACCACACA

Gene expression analysis was performed in two ways: Relative quantification CT Method ( $\Delta\Delta C_t$ ) by Livak & Schmittgen, and absolute quantification.

### Statistical Analysis

Data were manually analyzed and presented in a table. Absolute gene expression analysis was based on Log Copy Number (CN) from the equation in the standard curve (Table 1). The non-paired t-test was used to analyze the differences. The data were considered significant if  $p < 0.05$  was achieved.

**Table 1.** IL-4 and IFN- $\gamma$  mean expression based on Log CN in CRS with and without nasal polyp (mean  $\pm$  standard deviation)

	Log CN IL-4	P	Log CN IFN- $\gamma$	p
CRS with nasal polyp	15.28 $\pm$ 3.92	0.596	8.54 $\pm$ 2.96	0.346
CRS without nasal polyp	14.65 $\pm$ 4.65		7.57 $\pm$ 1.82	

CN: Copy number

## Results

### General Characteristics

Table 2 describes the general characteristics of all subjects in this study, based on their gender.

**Table 2.** Subjects general characteristic

Characteristic	CRS with nasal polyp (n=27)		CRS without nasal polyp (n=27)	
	number	%	number	%
Gender				
Male	13	48.15	6	22.22
Female	14	51.85	21	77.78

### IL-4 and IFN- $\gamma$ expression in CRS patients with and without nasal polyp

Relative gene expression was calculated from the ratio of gene expression from CRS with nasal polyps compared to CRS without nasal polyps after each gene was normalized by housekeeping genes (Table 3).

**Table 3.** The expression ratio of each gene (IL-4 and IFN- $\gamma$  gene) in CRS with nasal polyp group to CRS without nasal polyp (mean ratio  $\pm$  standard deviation)

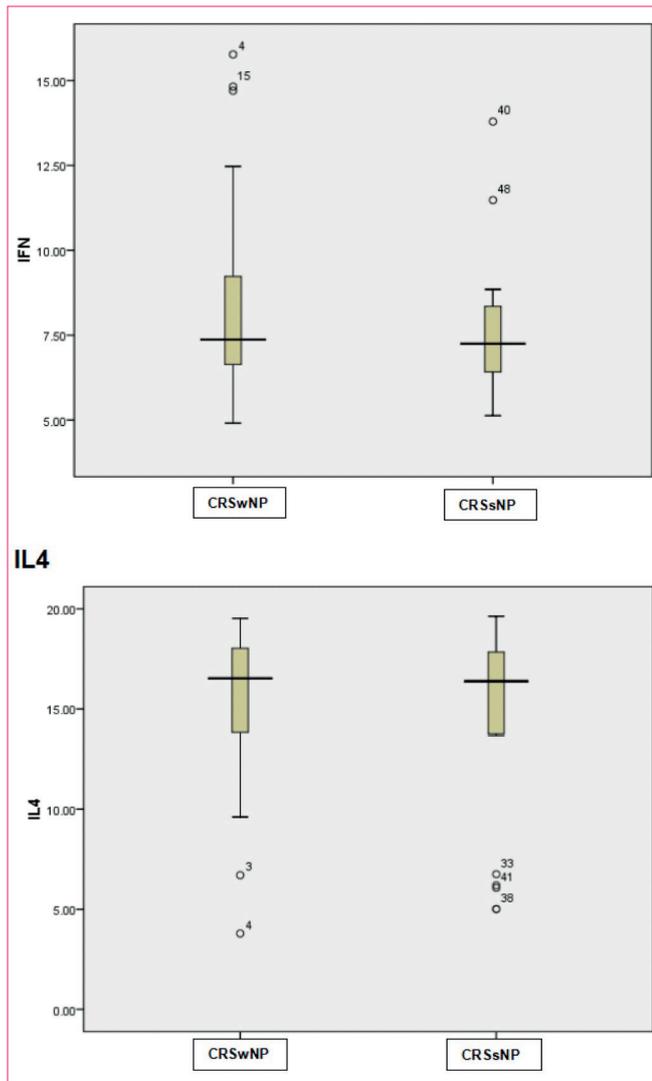
Gene	Mean Ratio
IL-4	3918237687.32 $\pm$ 19801377953.08
IFN- $\gamma$	36227.60 $\pm$ 130372.70

## Discussion

### IL-4 and IFN- $\gamma$ expression in CRS patients with and without a nasal polyp (Figure 1)

IL-4 absolute expression was higher in the CRS with nasal polyps than that of those without, but the difference was not statistically significant. Zhang et al.<sup>[10]</sup> found different results in China; there was a significant increase in IL-4 mRNA and protein expression along with the up-regulation of TLR expression. These up-regulations signify the innate immune system response in the epithelium of the nasal mucosa. Zhang et al.<sup>[10]</sup> concluded that the nasal mucosa in patients with CRS with nasal polyp is more sensitive to airborne microorganisms than in patients with CRS without nasal polyps, and suggested the predominant involvement of Th2 lymphocytes and IL-4 in the inflammation process.

The role of IL-4 in CRS with nasal polyp related to the innate immune system has been described.<sup>[11]</sup> IL-4, along with TLR2, TLR3, TLR4, and TLR5 ligands, stimulates fibroblasts in the polyps to secrete TARC (Thymus and activation-regulated chemokine), a chemoattractant that induces the migration of inflammatory cells, especially Th2. Th2 cells secrete cytokines, like IL-4 and IL-13,



**Figure 1.** IFN $\gamma$  and IL-4 levels of Chronic rhinosinusitis patients with nasal polyps (CRSwNP) and chronic rhinosinusitis patients without nasal polyps (CRSsNP)

which induce eotaxin release in structural cells, such as fibroblasts, and cause tissue eosinophilia.<sup>[11]</sup>

Meza et al.<sup>[8]</sup> also described that IL-4, even in minuscule concentrations (0.1 ng/mL), is the main cytokine that stimulates the secretion of eotaxin-2, a potent eosinophil chemoattractant, from nasal polyps. There is no significant difference between nasal polyp patients with a history of atopy and those without.<sup>[8]</sup> IL-4 also stimulates mucus production and takes part in tissue remodeling by stimulating fibrosis, fibroblast proliferation, and collagen synthesis. IL-4 mRNA-expressing cells are increased in number in nasal polyps than in normal patients, without considering the atopy status. Anti-IL-4 implementation is expected to decrease the inflammation process in the polyp.<sup>[7]</sup>

The data showed no significant difference in IFN- $\gamma$  absolute expression between CRS with nasal polyps and without. This result corresponds with the result in China, where there was no observable difference in IFN- $\gamma$  expression in CRS with polyps, without polyps, and the controls.<sup>[12]</sup> The superantigen theory of *Staphylococcus aureus* (*S. aureus*) states that the exotoxin or enterotoxin of *S. aureus* plays a role in CRS with nasal polyp inflammation mechanisms, including the lymphocyte local expansion, followed by Th1 and Th2 cells cytokine release.<sup>[12]</sup>

*S. aureus* is a commonly found bacteria in CRS patient culture.<sup>[13,14]</sup> Based on the THT-KL clinic of M. Djamil Hospital data from October 2011 to September 2012, *S. aureus* is the most commonly found pathogen bacteria in the CRS patient secretion culture at our institution. Superantigen (SAg) of *S. aureus* directly damages the nasal mucosa epithelial tissue and alters the sodium and chloride transfer in the epithelial cells. Intra-epithelial *S. aureus* will continuously secrete enterotoxins and induce local eosinophilic inflammation.<sup>[14]</sup> The SAg is a highly potent T lymphocyte activator, 1000 times stronger than conventional antigens, and creates massive T cell response and cytokine release.<sup>[14]</sup> SAg may bind to the lateral aspect of MHC class II molecules and bypass the internal APC process, which is different than the role of conventional antigens.<sup>[13,14]</sup>

*S. aureus* enterotoxins B elevate the secretion of Th1 and Th2 cell cytokines (i.e., IFN- $\gamma$ , IL-4, IL-5, IL-10 dan IL-13) in the mucosal polyp, with relatively higher Th2 cytokine secretion.<sup>[14]</sup> Cho et al.<sup>[13]</sup> observed different result in Korea, with up-regulation of the IFN- $\gamma$  gene and increased T cell infiltration after mucosal polyp exposure to SAg *S. aureus*, compared to the control. Cho et al.<sup>[13]</sup> reported that IFN- $\gamma$  is the main cytokine of Th1 in CRS patients with nasal polyp inflammation after exposure to SAg *S. aureus*. Soyka et al. (2012) mentioned that disruption of epithelial integrity by IFN- $\gamma$  and IL-4 in vitro suggests a possible role for these proinflammatory cytokines in the pathogenesis of patients with CRS.<sup>[15]</sup> A defective epithelial barrier was found in CRS patients with nasal polyps along with a decreased expression of TJ (tight junction) proteins.<sup>[15]</sup>

This study cannot conclude the relative gene expression due to the oversized gene ratio. The relative gene expression means that the expression ratio of each gene in CRS with nasal polyp group to CRS without nasal polyp after normalization of each gene by housekeeping

gene GAPDH, is based on Livak & Schmittgen method. This oversized result may be caused by (1) the variation in GAPDH expression of each subject; (2) bad primer and probe design; (3) unreplicated observations; and (4) the possibility of human error, such as in pipetting.<sup>[16]</sup>

In order to determine the complete inflammation pattern of each CRS classification, some factors that up-regulate the gene expression of IFN- $\gamma$  and IL-4 are suggested to be analyzed, such as: (1) the role of bacterial, super-antigen, biofilm, virus, and fungal; (2) the severity of clinical symptom; (3) the infiltration profile of inflammatory cells to the tissue; (4) Th1 and Th2 cells infiltration; (5) the expression of Th1 and Th2 transcription factors; and (6) the role of the local innate immunity system of nasal mucosa.

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