Association between asthma control and Interleukin-17F expression levels in adult patients with atopic asthma

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ABSTRACT

الأهداف: دراسة العلاقة بين إنترلوكين 17 (IL–17F) ومستوى السيطرة على الربو.

الطريقة: أجريت دراسة مستعرضة. أكملت عينة ملائمة من أجريت دراسة مستعرضة مكونة من 40 بالغ تم تشخيصهم بالربو التحسسي. اختير كل المشاركين من عيادة الحساسية والمناعة، البروفيسور ر.د، مستشفى كاندو العام، مانادو، إندونيسيا، خلال الفترة من أبريل 2015م و أبريل 2016م. قمنا بقياس إجمالي IL–17F المصل باستخدام طريقة فحص المناعة المرتبطة بالإنزيم؛ و تم الحصول على IL–17F باستخدام تفاعل البوليميراز المتسلسل اللحظي. قمنا بتحليل السيطرة على الربو العلاقة بين IL–17F ،وmRNA ، ومستوى السيطرة على الربو باستخدام Pearsonمعامل الارتباط (r).

النتائج: هناك علاقة موجبة قوية بين مستوى IL–17F في مصل الدم ومقياس ناتن (r=0.969)، وهو ذو دلالة إحصائية (p<0.001). أظهر تحليل الارتباط بين مستوى IL mRNA– ومقياس ناتن في ناثان وجود ارتباط إيجابي قوي (r = 0.963) ، وهو ذو دلالة إحصائية (p<0.001).

الخاتمة: تشير هذه النتائج إلى أن IL–17F يلعب دوراً هاماً في السيطرة على مستوى الربو. ومع ذلك ، فإن الدور الذي تلعبه IL–17F في مرض الربو لا يزال من الأسئلة التي يتعين الإجابة عليها.

Objectives: To investigate the correlation between Interleukin 17 (IL-17F) and the level of asthma control.

Methods: This is a cross-sectional study of 40 subjects who were diagnosed with atopic asthma. All participants were recruited from the Allergy and Immunology Clinic, Prof. R.D. Kandou General Hospital, Manado, Indonesia, between April 2015 and April 2016. Total serum IL-17F measured by using Enzyme-Linked Immunosorbent Assay methods; and mRNA IL-17F was obtained by using real-time reverse transcriptase polymerase chain reaction. Level of asthma control was quantified by using asthma control test (ACT) scoring system. The correlation between IL-17F, mRNA, and level of asthma control was analyzed by using Pearson's correlation coefficient (r).

Results: There is a strong positive correlation between IL-17F serum level and Nathan's ACT-score (r=0.969) which is statistically significant (p<0.001). Analysis of the correlation between mRNA IL-17F serum level and Nathan's ACT-score revealed a strong positive correlation (r=0.963), which is statistically significant (p<0.001).

Conclusion: These findings suggest that IL-17F plays an important role in asthma control. However, the role played by IL-17F in asthma pathogenesis are still questions to be answered.

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Asthma is an inflammatory airway disease, which ranges from a mild condition that does not interfere with daily activities to a severe, persistent disease that profoundly diminishes a patient's quality of life. Atopic or allergic asthma is a type of familial asthma characterized by an elevation in immunoglobulin E (IgE) expression levels.[1] The prevalence of asthma in Indonesia ranges from 5-7%.[2] Globally, the prevalence

of asthma ranges from 7-10%. Based on data from the National Asthma Control Program at the Center for Diseases Control and Prevention (CDC) in 2010, 18.7 million adults live with asthma, and, on average, there are 9 asthma-related deaths every day in the U.S.[3,4] Based upon the overall number of people suffering from asthma, about 70-80% have atopic asthma.[1-4] Chronic inflammation is the hallmark of asthma pathogenesis, and the regulation of cytokines plays important roles in orchestrating the inflammatory process.[5,6] Interleukin 17 (IL-17), which is produced mainly by T-helper 17 (Th17) cells, recently has been recognized as an important cytokine involved in asthma regulation.[7] While Th17 acts as the main source of IL-17, this cytokine is also expressed by basophils, mast cells, and bronchial epithelial cells, which are known to play key roles in triggering inflammation in asthma.[7] Recently, IL-17F, a subset of IL-17, has been recognized to play an important biological role in regulating asthma. As a homolog of another member of the IL-17 subfamily, studies have shown that IL-17F induces asthma-related cytokines, chemokines, and adhesion molecules in bronchial epithelial cells. Other studies have also shown a relationship between IL-17F and eosinophils, the key inflammatory cell, that play critical roles in allergic bronchial responses and remodeling of the airway.[7]

The primary objective of this study is to determine whether there is a correlation between IL-17F mRNA expression and soluble IL-17F protein with respect to the degree of asthma control in adult patients with atopic asthma. Previous studies have linked IL-17 to a greater severity of asthma, but none of these studies have related IL-17F to the level of asthma control in particular individuals.

Methods. The study population comprised 40 patients with atopic asthma (aged 23-60 years). Participants were residents of Manado and the surrounding regions. All participants were patients at the Allergy and Immunology Clinic at the Prof. Dr. R. D. Kandou General Hospital in Manado, Republic of Indonesia from April 2015 to April 2016. All participants were questioned in order to obtain clinical and family histories, particularly histories relating to the atopic syndrome and their degree of asthma control. Patients underwent a general physical examination, skin-prick test (SPT), and peak expiratory flow measurements. Analyses were performed on 5 mL of blood per person obtained via venous puncture. The level of asthma control was determined using the asthma control test (ACT) scoring system based on Nathan et al.[8] Total serum IgE and IL-17F levels were measured by enzyme-linked immunosorbent assay (ELISA) methods, while reverse transcriptase real time (polymerase chain reaction [PCR]) was used to determine mRNA IL-17F levels. Patients who are included in this study are those who are 18-60 years of age, was suffering for atopic asthma and agree to sign the informed consent. Patients with malignancies, sepsis, lung tuberculosis (TB) diabetes mellitus, advanced chronic kidney disease (stage 4-5), current pregnancy, and those experiencing severe stress were excluded from the study.

This study was approved by the Institutional Research Board of Prof. Dr. R. D. Kandou Manado Hospital in Manado, Republic of Indonesia. Patients gave written informed consent before participating in this study. The study was carried out according to the principles of the Declaration of Helsinki.

Assessment of clinical data. Diagnosis of asthma was based on patient's history, physical examination and spirometry evaluation especially peak expiratory flow (PEF) which is categorized according to guidelines from the Global Initiative for Asthma (GINA). Atopy asthma was determined by quantifying total serum IgE levels and defined by result ≥120 IU/ml. It was also diagnosed based upon SPT with a positive result ≥1 against housemite allergen. The ACT score test consisted of 5 questions with each question having a value range from 1 to 5 points; hence, the level of asthma control had a minimum score of 5 and maximum of 25. A score ≤15 was defined as poorly controlled, 16-19 was uncontrolled, 20-24 was well-controlled, and a score of 25 was defined as totally controlled.

Statistical analysis. This cross-sectional study examined the correlation between IL-17F mRNA expression and soluble IL-17F protein with respect to the levels of asthma control in adult patients with atopic asthma. The correlation between 2 variables was tested using either the Pearson's test if the data were distributed normally or the Spearman test if the data were not normally distributed. The distribution of the data was tested using the Klomogorov-Smirnov test. Statistical analysis was carried out by using IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.).

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Results. Demographic data and clinical characteristic of the study participants are shown in **Table 1**.

The correlation between IL-17F serum levels and Nathan's ACT score are shown in **Figure 1**. An analysis of the relationship between these 2 variables revealed a strong positive correlation (r=0.969), which was statistically significant (p<0.001, Pearson's correlation test). This result demonstrated that the higher the level of serum IL-17F, the higher the ACT score.

Table 1 - Demographic characteristic, complete blood count profile, interleukin-17F (IL-17F) expression and level of asthma control in adults with atopic asthma (N=40).

Variables	
Age, years (mean ±SD)	43.9 ± 11.0
Gender	
Female	25 (62.5)
Male	15 (37.5)
Leucocyte count, /mm^3 (mean ±SD)	8445.5 ±1744.7
Neutrophil count, /mm^3 (mean ±SD)	50.8 ± 12.8
Total IgE, IU/mL (mean ±SD)	1100.9 ± 1164.7
Nathan's ACT-Score (mean ±SD)	15.1 ± 4.7
Interleukin-17F (mean ±SD)	916.9 ± 520.4
mRNA IL-17F (mean ±SD)	14.2 ± 3.4
Degree of asthma (%)	
Intermittent	6 (15.0)
Mild persistent	8 (20.0)
Moderate persistent	13 (32.5)
Severe persistent	13 (32.5)
Level of asthma control (%)	
Controlled	7 (17.5)
Partly controlled	5 (12.5)
Uncontrolled	28 (70.0)

The correlation between serum IL-17F mRNA levels and Nathan's ACT score are shown in **Figure 2**. An analysis of the relationship between these two variables revealed a strong positive correlation (r=0.963), which was statistically significant (p<0.001, Pearson's correlation test). This result demonstrated that the higher the level of serum IL-17F mRNA, the higher the ACT score.

Discussion. Atopic asthma has been recognized as a chronic condition characterized by Th2 regulation disorder. Although, data from several studies has shown that mechanisms underpinning the airway hypersensitivity in asthma does not depend only on Th2. Other studies show that hypersensitivity in asthma may occur even in low-Th2 cytokine conditions.[9] Woodruff et al[10] reported that not all asthmatic patients have high Th2 gene expression. In other conditions, there is a subgroup of asthmatic patients with low Th2 gene expression, who could not be differentiated from control subjects. A gene transfer study has shown that animal models suffering from inflammation and airway hyperactivity mediated by Th2, display more severe airway inflammation following Th1 gene transfer.[11] Other findings have also shown that in severe atopic asthma, multiple cytokines play an important role. These cytokines not only include Th2, but also IFN-γ and IL-17, which are the main products of Th1 and Th17.[12] This suggests that atopic asthma is a disorder with a complex immunopathogenesis involving more than just Th 2 dysregulation.

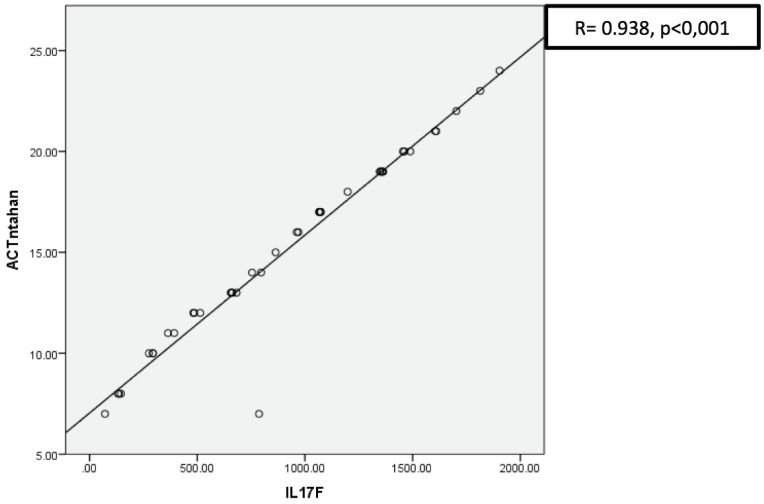

Figure 1 - Correlation between Interleukin 17 (IL-17), and Nathan's asthma control test (ACT) score (Pearson test, p<0.001).

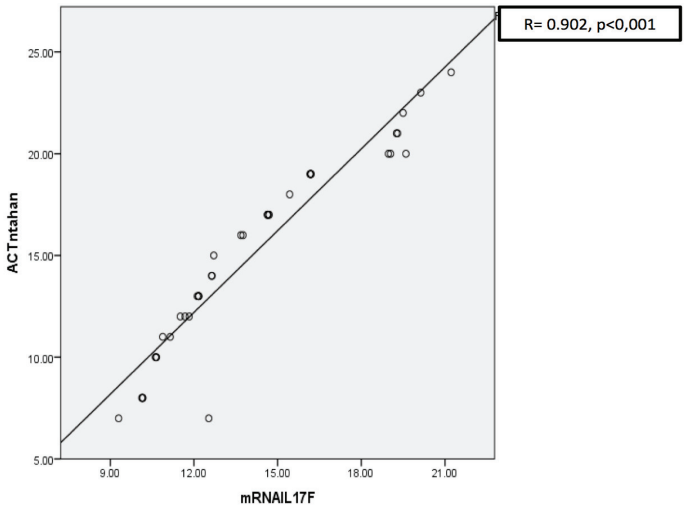

Figure 2 - Correlation between mRNA Interleukin 17 (IL-17) and Nathan's asthma control test (ACT) (Pearson test, $p<0.001$).

Interleukin 17 (IL-17) has been recognized as a cytokine that plays an important role in the pathogenesis of asthma. The primary cell producing IL-17 is Th17; however, this cytokine may also be produced from other sources, including natural killer cells, mast cells, neutrophils, and γδ T cells.[13] Currently, 6 members of the IL-17 family have been recognized, including IL-17A (may also be called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (may also be called IL-25), and IL-17F. The biological functions and regulation of IL-17A and IL-17F are better-known compared to other members of the IL-17 family. The gene for IL-17A is 50% identical to the IL-17F gene. Thus, the biological function of these cytokines is similar in many ways.[14] The pro-inflammatory function of IL-17A and IL-17F induces pro-inflammatory responses in hereditary immunity, which is mediated by tissues.

In allergic disorders, such as atopic asthma, IL-17 plays an important role in pathogenesis. A study by Bazzi et al[15] reported that asthmatic subjects (n=100) displayed a significant overexpression of IL-17A and IL-17F protein and mRNA compared to non-asthmatic subjects.

Other studies showed a more complex result. They found that IL-17F had a specificity and identity that was different from IL-17A. Despite high genetic homology, the function and regulation of IL-17A and IL-17F could not be considered identical. On one hand, mice with a IL-17A deficiency that underwent allergen exposure experienced a reduction in Th2 recruitment. On the other hand, mice with an IL-17F deficiency had significantly higher IL-4, IL-5, and IL-13 levels after allergen induction. This indicates that IL-17 has an important regulatory function in inhibiting the development of allergic asthma.[16] Other studies by Yang et al[17] in animals showed differences in the regulatory function of IL-17F. Mice with an IL-17F deficiency exposed to allergens experienced a reduction in neutrophil recruitment in their airways during the acute phase. This reduction was significant when compared to mice with an IL-17A deficiency or wild-type mice. In contrast, in a chronic asthmatic model, mice with an IL-17F deficiency showed a significant reduction in recruitment and eosinophilic degranulation compared to mice with an IL-17A deficiency and wild type mice. This result shows that in the chronic asthmatic model, IL-17A and IL-17F have different functions, and IL-17F also plays a role in inhibiting asthma.

According to the data discussed above, it could be argued that although IL-17F plays a pro-inflammatory role in atopic asthma, this cytokine also has regulatory functions that are regulated by poorly understood mechanisms. In order to investigate the role of IL-17F in atopic asthma, this study was conducted to explore the association between the soluble protein and mRNA forms of IL-17F with respect to the level of asthmatic control as measured by ACT-Nathan score. This study showed a strong positive correlation between IL-17F and the level of asthmatic control. The higher the level of soluble IL-17F protein and mRNA, the greater the amount of asthmatic control in the patient. This result is consistent with previous studies in animals that showed

a role for IL-17F in inhibiting the hypersensitivity response associated with Th2 cytokines.[16,17] High levels of soluble IL-17F protein and mRNA in subjects with controlled asthma in this study confirmed previous predictions that IL-17F may play an important role in the suppression of the Th2-mediated hypersensitivity response. However, this result is different from other studies that showed an increase in expression of IL-17F in subjects with severe asthma when compared to patients with mild asthma and limited asthmatic control.[18-21] These inconsistent results showed a complexity relating to the regulatory function of IL-17F that is not clearly understood. Despite this, previous studies connecting IL-17F and severe asthma hardly compare to this study because there is a difference in the definition of severe asthma and controlled asthma.

One of the regulatory functions of IL-17F in atopic asthma, which is recently known, is the regulation of function between Th17 as the main cell producing IL-17 and Th2, which is reciprocal. Choy et al[22] conducted a study investigating gene expression in the airway wall. This study succeeded in showing that suppression of Th2 increases Th17 gene expression and vice versa. Furthermore, the administration of steroids may cause Th2 suppression, resulting in the increased expression of Th17 in the bronchus. This can explain the positive correlation between IL-17F and the level of asthmatic control in our study. Specifically, the better the asthmatic control, the more suppression of Th2, causing increased expression of Th17 family members, including the IL-17F cytokine. However, in this study, it is unknown whether there is a relationship between IL-17F and acute asthmatic attack frequencies or airway infection episodes because the role of IL-17F has been known to induce airway hyper-responsivity and neutrophil migration to the airway.[17,23]

This is the first study investigating the relationship between IL-17F expression and level of asthmatic control. The results of this study increase our knowledge of the role of IL-17F in atopic asthma. The limitation of this study is its cross-sectional design. Thus, the conclusion that there is causality between increased IL-17F levels and the level of asthmatic control could not be determined. Furthermore, there are no data regarding the expression of Th2 cytokines in these subjects, which causes difficulty in assessing the function of IL-17F and its immunologic mechanisms in atopic asthma. Further research are needed to understand the role of IL-17F in atopic asthma.

In conclusion, there is a strong positive correlation among IL-17F protein/mRNA serum levels (r=0.969) and the Nathan ACT-score (r=0.963). These findings suggest that IL-17F plays an important role in asthma control. How IL-17F plays this role in asthma pathogenesis remains to be answered.

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References

1. Global Initiative for Asthma. Global strategy for asthma management and prevention [Updated 2015]. Available from URL: http://ginasthma.org/wp-content/uploads/2016/01/GINA_Pocket_2015.pdf
2. Sundaru H, Sukamto. Asma bronkial. In: Sudoyo AW, Setiyohadi B, Alwi I, Simadibrata M, Setiati S. editors. Buku Ajar Ilmu Penyakit Dalam edisi ke 6. Jakarta: Internal Publishing; 2014.
3. Lazarus S. Emergency Treatment of Asthma. ***N Eng J Med*** 2010; 363: 755-764.
4. Centers for Disease Control and Prevention. Data from the CDC National Asthma Control Program 2012 [Internet]. Atlanta, GA. [cited 2015 Oct 6]. Available from: http://www.cdc.gov/asthma/pdfs/investment_americas_health.pdf
5. Ten Hacken NH, Oosterhoff Y, Kauffman HF, Guevarra L, Satoh T, Tollerud DJ, et al. Elevated serum interferon-γ in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. ***Eur Respir J*** 1998; 11: 312-316.
6. Holgate ST. Innate and adaptive immune responses in asthma. ***Nature Medicine*** 2012; 18: 673-685.
7. Ota K, Kawaguchi M, Matsukura S, Kurokawa M, Kokubu F. Potensial Involvement of IL-17F in Asthma. ***J Immunol Res*** 2014; 2014: 602846.
8. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, et al. Development of the Asthma Control. A survey for assessing asthma control. ***J Allergy Clin Immunol*** 2004; 113: 59-65.
9. Sterk PJ, Lutter R. Asthma phenotyping: TH2-high, TH2 low and beyond. ***J Allergy Clin Immunol*** 2014; 133: 395-396.
10. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. ***Am J Respir Crit Care Med*** 2009; 180: 388-395.
11. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-spesific Th1 cells fail to counterbalance Th2 cell-induced airway hyperactivity but cause severe airway inflammation. ***J Clin Invest*** 1999; 103: 175-183.
12. Kim YM, Kim YS, Jeon SG, Kim YK, Immunopathogenesis of allergic asthma: more than the Th2 hypothesis. ***Allergy Asthma Immunol Res*** 2013; 5: 189-196.
13. Pappu R, Carrozzi VR, Sambandam A. The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. ***Immunology*** 2011; 134: 8-16.
14. Jin W, Dong C. IL-17 cytokines in immunity and inflammation. ***Emerging Microbes and Infections*** 2013; 2: 1-5.
15. Bazzi MD, Sultan MA, Tassan AI, Alanazi M, Al-Amri A, et al. Interleukin (IL)-17A and IL-17F and Asthma in Saudi Arabia: mRNA transcript level and gene Polymorphisms. ***African Journal of Biotechnology*** 2013; 12: 3615-36210.
16. Chang SH, Dong C. IL-17F: regulation, signaling and function in inflammation. ***Cytokine*** 2009; 46: 7-11.

17. Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L et al. Regulation of inflammatory responses by IL-17F. ***J Exp Med*** 2008; 205: 1063-1075.
18. Sorbello V, Ciprandi G, Stefano AD, Massaglia GM, Favata G, Conticello S, et al. Nasal IL-17F is related to bronchial IL-17F/neutrophilia and exacerbations in stable atopic severe asthma. ***Allergy*** 2015; 70: 236-240.
19. Alyasin S, Karimi MH, Amin R, Babaei M, Darougar S. Interleukin-17 gene expression and serum levels in children with severe asthma. ***Iran J Immunol*** 2013; 10: 179-187.
20. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. ***Respiratory Medicine*** 2010; 104: 1131-1137.
21. Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, Lemiere C, et al. TH17-associated cytokine (IL17-A and IL-17F) in severe asthma. ***J Allergy Clin Immunol*** 2009; 123: 1185-1187.
22. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. ***Sci Transl Med*** 2015; 7: 301ra129.
23. Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK. Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. ***Am J Respir Crit Care Med*** 2005; 171: 12-18.