Association of LL-37 and IL-31 serum levels with SCORing Atopic Dermatitis (SCORAD) score in atopic dermatitis patients

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Background: Atopic dermatitis is a chronic, recurrent inflammation of the skin, accompanied by severe pruritus. Immune system dysregulation and skin barrier defects are associated with the abnormalities in atopic dermatitis. Myriad pieces of evidence have pointed to the major roles of LL-37 and interleukin-31 (IL-31) in atopic dermatitis. The studies on atopic dermatitis are still limited in Indonesia, particularly in Malang city. The objective of the present study was to understand the association of LL-37 and IL-31 serum levels with SCORing Atopic Dermatitis (SCORAD) in atopic dermatitis patients.

Methods: The present research is an analytical observational cross-sectional study with 30 atopic dermatitis patients as subjects. Through a consecutive sampling method, samples were collected from the outpatient clinics of dermatovenereology and pediatric department, Dr. Saiful Anwar General Hospital (RSSA), Malang, Indonesia. LL-37 and IL-31 serum levels were examined via Enzyme-Linked Immunosorbent Assay (ELISA) method. Subjects were allocated into two groups of atopic dermatitis severity, mild and moderate-severe, based on the SCORAD index.

Results: Based on the Pearson correlation test, there was no correlation between LL-37 serum level, IL-31 serum level and the SCORAD score (r=-0.238 with *P*=0.205, and r=0.15 with *P*=0.939).

Conclusion: LL-37 and IL-31 serum levels are not associated with atopic dermatitis severity.

Keywords: atopic dermatitis, interleukin-31, LL-37, scoring of atopic dermatitis

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INTRODUCTION

With its chronic and recurrent nature, atopic dermatitis (AD) is a major health problem, entailing psychosocial and economic burden. The clinical features of AD include eczematous skin lesions accompanied with severe pruritus symptoms, significantly affecting the patients' quality of life and that of their family [1,2]. In several developed countries, AD is one of the diseases that is largely conducive to the financial aspects of health [3].

Currently, the pathogenesis of AD is not fully understood. Various factors may be inducers of inflammation in AD, and persistent inflammation affects AD treatment. Pruritus or itchy skin is an essential factor in AD inflammation, impacting the quality of life. Antimicrobial peptides are among the components with a major role in the pathogenesis of AD. LL-37 is one of the cathelicidin antimicrobial peptides thought to be associated with inflammation in AD lesions. Dysregulation of the immune response in AD affects LL-37 expression [4,5].

There is evidence as to the involvement of Interleukin-31 (IL-31) in the pruritus mechanism. In the study conducted by Dillon *et al.* pruritus and skin lesions similar to AD occurred in transgenic

mice, indicating the overexpression of IL-31 [6]. Previous studies have further observed an increase in IL-31 serum levels in AD patients compared with healthy controls [7-9]. In their research in Makassar, Barnas *et al.* found that IL-31 serum levels were significantly higher in children with AD than in healthy subjects [10]. These levels correlated positively with the severity of AD, which is in accordance with the findings of several other studies [8,10,11]. In contrast, in another study, IL-31 serum levels were not associated with the severity of AD [12]. In a recent study by Niyonsaba et al. which investigated the association of LL-37 with IL-31, it was concluded that LL-37 is capable of inducing IL-31 mRNA expression [13].

Data from previous studies indicate that there is an association between antimicrobial peptide LL-37 and IL-31 as an inducer of pruritus based on AD severity. Several studies were recently developed to understand the ability of cathelicidin as a potential antimicrobial in the management of several skin inflammation diseases, be it topical or systemic [14-17]. There is also a study that has associated IL-31 inhibitor with AD treatment [18].

Data regarding the correlation between LL-37 and IL-31 serum levels in AD and their correlation with AD severity are required to support the previous studies. The present research aimed to understand the association between LL-37 and IL-31 serum levels in AD. As for the association with AD severity, the results of the previous studies are contradictory. In Indonesia, especially in Malang, there are no studies similar to the present research.

MATERIALS AND METHODS

Data collection

This is an analytical observational cross-sectional study aimed at understanding the relationship between LL-37 and IL-31 serum levels using the SCORAD score in AD. The sampling technique used in this study was consecutive sampling. Thirty patients who visited the allergy division of dermatovenerology outpatient clinic and pediatric outpatient clinic met the inclusion criteria and were willing to sign a written informed consent; hence included as study subjects. AD diagnosis was based on health history, physical examination with typical clinical manifestations, and consecutive SCORAD assessment by two examiners on the same day. Inclusion criteria were both genders, 5–50 years of age, diagnosed with AD based on the Hanifin and Rajka criteria, with skin lesions corresponding to AD, and willing to be study subjects through a written informed consent. Exclusion criteria were: patients suffering from inflammatory diseases, either local or systemic (i.e. psoriasis vulgaris, allergic contact dermatitis, rosacea, acne vulgaris, hidradenitis supuratifa, lupus erythematosus, rheumatoid arthritis), or receiving corticosteroid and immunomodulator therapy, either topical or systemic. The subjects received antihistamines one week prior to the study and had undergone antibiotic, antiviral, and antifungal therapy from the week before. The serum levels of LL-37 and IL-31 were measured by use of ELISA method. The employed samples were the sera obtained from the centrifugation of peripheral blood at 1000 x g for 15 minutes at 4ºC. Several coagulation factors were not present in the serum sample.

Data analysis

The data were analyzed via Pearson correlation test or the Spearman test, version 24.0 of SPSS for Windows. The difference between measurements was evaluated by a significance level of $P<0.05$ and a confidence interval of 95%. Subject characteristics are illustrated in the distribution frequency table.

RESULTS

The mean values of LL-37 serum levels were 12.4±6.7 ng/ml and 9.40±6.06 ng/ml in the mild and moderate/severe AD groups, respectively. A comparison test of LL-37 serum levels indicated that there was no significant difference between the two categories with $P=0.205$ (Table 1).

The mean IL-31 serum levels of the mild and moderate-severe AD groups were respectively 399.35.37±182.33 pg/ml and 368.42±74.74 pg/ml, meaning that with a significance value of 0.953, these serum levels were normally distributed. With a significance value of 0.561, the t-test results showed no significant difference between the two groups regarding the mean IL-31 serum levels (Table 2).

Since the data distribution was normal and met the requirements for a parametric test, the association between LL-37 serum levels and

Table 1. Serum LL-37 levels based on AD severity

LL-37 Serum (ng/ml)	AD severity (n=30)		*P* value
	Mild (n=14)	Moderate-Severe (n=16)	
Mean	12.4	9.40	0.205
Range (SD)	6.7	6.06	

Table 2. Serum IL-31 levels based on AD severity

IL-31 Serum (pg/ml)	AD Severity (n=30)		*P* value
	Mild (n=14)	Moderate-Severe (n=16)	
Mean	399.35	368.42	0.561
Range (SD)	182.33	74.74	

SCORAD score was specified using the Pearson correlation test, based on which the correlation coefficient value between SCORAD and LL-37 serum levels was 0.238 with *P*=0.205 (Table 4.8), however, there was no statistically significant correlation between the two. The correlation test between LL-37 serum levels and SCORAD score further indicated their statistically insignificant correlation with the r values of -0.264 (*P*=0.159), -0.358 (*P*=0.052), and -0.092 (*P*=0.628), respectively (Table 3).

The correlation of IL-31 serum levels with SCORAD score was measured using the Pearson correlation test since the data distribution was normal and met the requirements for a parametric test. IL-31 serum levels and SCORAD scores had a correlation coefficient value of 0.15 with a significance value of 0.939 (*P*>0.05). Therefore, there was no significant correlation between IL-31 serum levels and SCORAD score. IL-31 serum levels and SCORAD components were also shown to be insignificantly correlated with the r values of -0.127 (*P*=0.503), -0.033 (*P*=0.861), and -0.008 (*P*=0.966), respectively (Table 4).

Another test aimed at determining the association between LL-37 and IL-31 serum level, which had a correlation coefficient of 0.005 with a significance value of 0.981 (*P*>0.05). Accordingly, no significant correlation was observed between the two serum levels.

Table 3. Association of LL-37 serum levels with SCORAD and SCORAD components

Characteristics	LL-37 serum levels	
	R	*P*
SCORAD category	-0.238	0.205
Lesion area	-0.264	0.159
Lesion intensity	-0.358	0.052
Subjective complaint	-0.092	0.628

Table 4. The association of IL-31 serum levels with SCORAD and SCORAD components

Characteristics	IL-31 serum levels	
	R	*P*
SCORAD levels	0.15	0.939
Lesion area	-0.127	0.503
Lesion intensity	-0.033	0.861
Subjective complaint	-0.008	0.966

DISCUSSION

This study showed that the LL-37 serum levels in the mild AD were relatively higher, though not significantly, compared with moderate/severe AD. Furthermore, there was no significant correlation between LL-37 serum levels and SCORAD score, indicating AD severity (r=-0.238, *P*=0.205).

LL-37 involvement in the pathogenesis of AD has not been fully elucidated yet. A study by Goo et al. indicated that the basal LL-37 expression in the skin of AD patients was not different from healthy individuals [4]. The alteration in LL-37 expression, however, was found in the skin of AD patients with lesions. Mallbris et al. indicated that the expression of LL-37 in the skin lesions of AD patients was lower than in the skin lesions of psoriasis patients [19]. Nonetheless, the expression of LL-37 in skins without lesions was similar in AD patients, psoriasis patients, and normal individuals. Based on these studies, it could be stated that the bruise in the epidermis is able to reduce LL-37 expression in AD skin, which is correlated with the dominance of the immune response of Th-2 suppressing the pro-inflammation activity of LL-37. Ballardini et al. showed that the LL-37 expression was increased in the skin of AD patients with lesions [5], an increase which was considered to be associated with the process of tissue re-epithelialization.

In a previous study by Leung et al. a significant correlation was observed between LL-37 serum levels and total SCORAD score (r=0.181; P=0.030) and objective SCORAD scores (r=0.207; P=0.013) in children. LL-37 has convoluted functions and roles [20]; other than being a pro-inflammation and anti-inflammation agent, it engages in the chemotaxis factor and wound-healing process. Such involvement in inflammation is affected by cell type and the underlying diseases. The activation of LL-37 as an immunomodulator is mediated through several different pathways. The complexity of the overlapping and simultaneous activity of LL-37 has rendered it very challenging to fully fathom its role [21].

LL-37 induction in tissues or the circulation is among these roles. Several causes of local or systemic inflammation have been associated with LL-37 expressions, such as psoriasis vulgaris, rosacea, lupus erythematous, hidradenitis supurativa, and rheumatoid arthritis. Further affecting the expression of LL-37 are inflammatory conditions, particularly on the epithelial and body mucosa surface, such as periodontitis, gastrointestinal infection, urogenital tract infection, and respiratory tract infection. LL-37 expression is balanced to maintain homeostasis. Several physiological conditions also influence the expression of LL-37, such as conception, labor, and the excessive growth of microflora. In the present research, local and systemic inflammation disease was ruled out using sample exclusion criteria; however, there exist several uncontrollable physiological conditions affecting study results. Through the secretion of endogenous glucocorticoids, psychological stress could reduce the antimicrobial peptide levels in the skin [22]. To date, there have been no studies comparing basal LL-37 expression in the circulation under various physiological and pathological conditions.

LL-37 levels in this study were between 9.40±6.06 ng/ml and 12.4±6.7 ng/ml, which is lower than the normal levels in the circulation (plasma=50-80 ng/ml) [23,24]. Certain studies in the literature state that protein components in the blood could be affected by the absence of several coagulation factors. In some other studies, it has been mentioned that the presence of serum impacts the antimicrobial activity of LL-37. However, until now, there have been no data as regards the effect of sample type on LL-37 levels in the circulation [25].

Also reviewed in the present study was the correlation between LL-37 serum levels and several clinical and laboratory factors. Myriad studies have observed that the increase in the total serum IgE levels and total eosinophils in the peripheral blood could elucidate the augment in the expression of Th-2 in AD: however, it should be borne in mind that this condition is not always consistent [26,27].

In this study, no noticeable correlation was observed between LL-37 serum levels and total serum IgE levels or the percentage of total eosinophils in the peripheral blood; accordingly, the Th-2 immune response in the circulation was not associated with the total serum IgE levels and eosinophil percentages in the peripheral blood. Currently, it is a challenging task to perform *in vivo* measurements of LL-37 expression, since it has various functions which overlap in different tissues and circulations. *In vitro* measurement is still difficult as the immune response is dynamic, and there exist certain variations between the species. There are a considerable number of biological factors affecting the LL-37 regulation expression, resulting in a huge bias in this study.

The mechanism underlying dermatitis and pruritus in AD is complicated and yet to be entirely fathomed; however, there are several pieces of evidence pointing to the role of IL-31, which is expressed at high levels in the skin lesions of AD patients [28]. IL-31 levels were measured in the serum, with results similar to that of the previous studies where the IL-31 serum levels were consistent with IL-31 mRNA level in the biopsies of skin lesions of AD patients [8]. Previous studies observed a correlation between IL-31 serum levels and AD severity, measured through the use of SCORAD method. There was a statistically recognizable association between IL-31 serum levels and SCORAD score, indicated by a 0.003 significant level [7-9], showing that mild and moderate-severe AD were no different in terms of mean IL-31 serum levels . Based on the correlation test results, the relationship between IL-31 serum levels and SCORAD score had a correlation coefficient of 0.15 with a 0.939 significance value (P>0.05). Therefore, H0 was accepted, since IL-31 levels and SCORAD score were not noticeably correlated.

The results of the present research are similar to those of several other studies, in that IL-31 serum

levels were not correlated with AD severity [10,12,29]. Interleukin-31 is a cytokine combination of four-helix bonds with the IL-6/IL-12 cytokine family through a structure and receptor complex. The IL-31 encoding gene is located on chromosome 12q24.31 [30,31]. Interleukin-31 is expressed from activated CD4 T cells andTh2 cells in particular. Recently, it has been postulated that mast cells provide an additional source of IL-31 secretion [13,31]. In AD, IL-31 is involved both in the acute and chronic phases. Many studies have observed that the dominant role of IL-31 in inflammation is mediated by Th2 and, possibly, Th1 cells [4,8,12].

A study by Perrigoue *et al.* on IL-31 and IL-31 receptors in lung inflammation, concluded that IL-31, in addition to causing inflammation mediated by Th2 cells, could also be a regulator of Th2 cells [32]. Signals from the IL-31 receptor were able to directly affect the proliferation and expression of Th2 cell cytokines. Moreover, the excessive interleukin-31 levels in transgenic mice could actively suppress the Th2 cell response, subsequently causing skin inflammation mediated by Th1 cells [32], which is among the reasons why there was no significant difference, nor any correlation, between IL-31 serum levels and AD severity. Furthermore, there are several diseases which have been associated with increased IL-31 levels, such as allergic rhinitis [33,34], chronic urticaria [35], lichen planus [36], allergic contact dermatitis [11], osteoporosis [37] and rheumatoid arthritis [38].

The correlation test of IL-31 serum levels with total IgE levels demonstrated a correlation coefficient of -0.137, with a significance value of 0.470 (P>0.05), hence the fact that IL-31 levels and total IgE levels were not recognizably correlated . There is no consensus as to whether the relationship between IL-31 serum levels and total IgE levels is positive or there exists no correlation at all [8,9]. In several reports in the literature, it was mentioned that the haplotype of the IL-31 gene was strongly correlated with AD, which is not mediated by the IgE sensitization process [8,39]. This was supported by previous studies investigating the polymorphisms of a single nucleotide in the IL-31 gene in the European population; the study included 690 families suffering from AD, and illustrated a significant correlation between haplotype IL-31 and non-atopic eczema. Individuals with the AA haplotype had a risk of developing an intrinsic, rather than extrinsic, AD [40].

Hong *et al.* stated that polymorphism of a single nucleotide in the IL-31 gene in extrinsic AD patients was not significantly different from healthy controls. Genotypes and alleles were able to influence IL-31 levels. Thus, the genetic polymorphism of IL-31 could regulate IL-31 levels and cause a difference in the IgE levels, thereby affecting the degree of severity. Although the single gene polymorphism of IL-31 did not correlate with the extrinsic AD, IL-31 could affect AD through the induction of eosinophils and keratinocytes in order to produce pro-inflammatory cytokines. Also, IL-31 increased CCL2 secretion by TLR-2 following the up-regulation of IL-31 receptors in keratinocytes by IFN-γ [41].

In the present study, a correlation test was conducted between LL-37 and IL-31 serum levels. Based on the Pearson correlation test, it is inferred that IL-31 and LL-37 levels had a correlation coefficient of 0.005 with a significance value of 0.981 (P>0.05). Therefore, no significant correlation existed between the two serum levels. In a recent study investigating the correlation between HBDs and LL-37 and IL-31, both serum levels induced IL-31 mRNA expression and IL-31 protein production, along with stimulating other pruritogens such as cytokines, NGF, PGE2, and LTC4. The production and secretion of IL-31 was entirely dependent on protein-G, PI3K and MAPK pathways. Human β-defensins and LL-37 recruit and stimulate mast cells to produce inflammatory mediators such as histamine and PGD2, causing pruritus. The findings of the present research are in accordance with the study by Ismail *et al.* regarding the correlation between *S. aureus* colonies and IL-31 expression [29]. In our study, an increase was observed in *S. aureus* colonization and IL-31 serum levels in AD patients, but neither were correlated. *S. aureus* colonization, in contrast to IL-31 serum levels, were positively correlated with pruritus and the degree of severity. In a study by Niyonsaba *et al.* it was illustrated that alongwith T cells, mast cells are conducive as a source of pruritogenic factors [13]. Despite the fact that the concentration of IL-31 produced by HBDs was lower compared with the IL-31 levels required for LL-37 to play its role, IL-31 was able to synergize with other molecules so as to stimulate its biological functions [8,13,29].

In the present investigation, there was no

significant difference between the mild and moderate-severe AD groups in as far as LL-37 levels and IL-31 serum levels are concerned. Based on SCORAD score, neither LL-37 nor IL-31 serum levels directly affected disease severity in AD patients.

Among the limitations of our research, mention can be made of genetic factors, predisposition factors, exacerbation factors and comorbid factors in each study subject, all of which play important parts in AD and the measured parameters. Secondly, the subjective nature of one of the measurement methods was a yet another concern. Last but not least, the study subject size was relatively small, and unable to fully depict a balanced proportion in each study group.

It is indispensable to further the research into the comparison of AD patients and healthy controls as far as IL-31 and LL-37 serum levels are concerned. In order to obtain more in-depth results on the role of IL-31 and LL-37 in the pathogenesis of AD, further research needs to be carried out on the correlation between IL-31 and LL-37 serum levels and total IgE levels and the relationship between specific IgE and intrinsic or extrinsic AD. Additionally, studies on these serum levels based on DA severity have to consider the genetic factors and eliminate confounding factors.

Conflict of Interest: None declared.

REFERENCES

1. Kabashima K. New concept of the pathogenesis of atopic dermatitis: interplay among the barrier, allergy, and pruritus as a trinity. J Dermatol Sci. 2013;70(1): 3-11.
2. Watson W, Kapur S. Atopic dermatitis. Allergy Asthma Clin Immunol. 2011.7(suppl 1): S4. doi: 10.1186/1710-1492-7-S1-S4.
3. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy. 2014;69(1): 3-16.
4. Goo J, Ji JH, Jeon H,, et al. Expression of antimicrobial peptides such as LL-37 and hBD-2 in nonlesional skin of atopic individuals. Pediatric Dermatol. 2010;27(4): 341-348.
5. Ballardini N, Johansson C, Lilja G, et al. Enhanced expression of the antimicrobial peptide LL-37 in lesional skin of adults with atopic eczema. Br J Dermatol. 2009;161(1):40-47.
6. Dillon SR, Sprecher C, Hammond A, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol. 2004;5(7): 752-760.
7. Ezzat MH, Hasan ZE, Shaheen KY. Serum measurement of interleukin 31 (IL-31) in paediatric atopic dermatitis: elevated levels correlate with severity scoring. J Eur Acad Dermatol Venereol. 2011;5(3): 334-339.
8. Kim S, Kim HJ, Yang HS, et al. IL-31 serum protein and tissue mRNA levels in patients with atopic dermatitis. Ann Dermatol. 2011;23(4): 468-473.
9. Raap U, Wichmann K, Bruder M, et al. Correlation of IL-31 serum Levels with severity of atopic dermatitis. J Allergy Clin Immunol, 2008;122(2): 421-423.
10. Barnas SM, Tabri F, Ilyas FS. Interleukin 31 serum pada dermatitis atopik anak. Makassar: Hasanuddin University; 2011.
11. Neiss MM, Peters B, Dreuw A, et al. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. J Allergy Clin Immunol. 2006;118(4): 930-937
12. Luzenczyk KS, Przytuska AS, Zeman K. Correlation between serum interleukin-31 level and the severity of disease in children with atopic dermatitis. Postepy Dermatol Alergol. 2013;30(5);282-285
13. Niyonsaba F, Ushio H, Hara M, et al. Antimicrobial peptides human b-Defensins and Cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. J Immunol. 2010;184: 3526–3534.
14. Does AM, Bergman P, Agerberth B, et al. Induction of the human cathelicidin LL-37 as a novel treatment against bacterial infections. J Leukoc Biol. 2012;92(4): 735-742.
15. Reinholz M, Ruzicka T, Schauber J. Cathelicidin LL-37: An antimicrobial peptide with a role in inflammatory skin disease. Ann Dermatol. 2012;24(2): 126-134.
16. Choi K, Mookherjee N. Multiple immune-modulatory functions of cathelicidin host defense peptides. Front Immunol. 2012;3(149): 1-4.
17. Novak N, Simon D. Atopic dermatitis – from new pathophysiologic insights to individualized therapy. Allergy. 2011;66(7): 830-839.
18. Venereau E, Diveu C, Grimaud L, et al. Definition and characterization of an inhibitor for interleukin-31. J Biol Chem. 2010;285(20): 14955-63.
19. Mallbris L, Carlén L, Wei T, et al. Injury downregulates the expression of the human cathelicidin protein hCAP18/LL-37 in Atopic dermatitis. Exp Dermatol. 2009;1(5): 442-449.
20. Leung TF, Ching KW, Song APS, et al. Circulating LL-37 is a biomarker for eczema severity in children. J Eur Acad Dermatol Venereol. 2012; 26(4): 518-522.
21. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. J Immunol. 2013;191(10): 4895-901.
22. Aberg KM, Radek KA, Choi EH, et al. Psychological stress downregulates epidermal antimicrobial peptide expression and increases severity of cutaneous infections in mice. J Clin Invest. 2007;117(11):3339-3349.
23. Ramos R, Domingues L, Gama M. LL-37, a human antimicrobial peptide with immunomodulatory properties. Science against microbial pathogen: communicating current research and technological advances. Sci Against Microb Pathogens. 2011; P:915-925.

24. Mendes-Samperio P. Recent advences in the field of antimicrobial peptides in inflammatory diseases. Adv Biomed Res. 2013;2: 50.

25. Lunblad R. Considerations for the use of blood plasma and serum proteomic analysis. Int J Genomics Proteomics. 2003.1(2).

26. Bacharier LB, Geha RS. Molecular mechanisms of IgE regulation. J Allergy Clin Immuno. 2000;105(2 part 2.): 547-58.

27. Dhar S, Malakar R, Chattophaday S, et al. Correlation of the Severity of Atopic Dermatitis with Absolute Eosinophil Counts in Peripheral Blood and Serum IgE levels. Indian J Dermatol Venereol Leprol. 2005;71(4): 246-249.

28. Stander S, Luger TH. Itch in atopic dermatitis – pathophysiology and treatment. Acta Dermatovenerol Croat. 2010;18(4): 289-296.

29. Ismail EA, Ibrahim HA, Farid CL, et al. The relation between Interleukin-31 serum levels and staphylococcus aureus skin colonization in pediatric atopic dermatitis. J Egypt Women Dermatol Soc. 2014;12(1): 38–43.

30. Park K, Park JH, Yang QJ, et al. Transcriptional activation of the IL31 gene by NFAT and STAT6. J Leukoc Biol. 2011;91(2): 1-13.

31. Zhang Q, Putheti B, Zhou Q, et al. Structures and biological functions of IL-31 and IL-31 receptors. Cytokine Growth Factor Rev. 2008;19(5-6): 347–356.

32. Perrigoue JG, Li J, Zaph C, et al. IL-31–IL-31R interactions negatively regulate type 2 inflammation in the lung. J Exp Med. 2007;204(3): 481-7.

33. Liu W, Luo R, Chem Y, et al. Interleukin-31 promotes helper T cell type-2 inflammation in children with allergic rhinitis. Pediatr Res. 2015;77(1-1): 20-8.

34. Baumann R, Rabaszowski M, Stenin I, et al. The release of IL-31 and IL-13 after nasal allergen challenge and their relation to nasal symptoms. Clin Transl Allergy. 2012;2(1): 13.

35. Crisan IG, Bocsan CI, Vesa SC, et al. Correlations between serum levels of IL-17, IL- 4, IL-31, IFN-gamma and etiological factors in patients with chronic spontaneous urticaria. Aquac Aquar Conserv Legis. 2014;6(1): 25-30.

36. Kubiak KW, Kobuszweska A, Reich A. IL-31 is overexpressed in lichen planus but its level does not correlate with pruritus severity. J Immunol Res. 2015: 1-6.

37. Ginaldi L, De Martinis M, Ciccarelli F, et al. Increased levels of interleukin 31 (IL-31) in osteoporosis. BMC Immunol. 2015;16: 60.

38. Yu JI, Park YR, Lee SS, Chae SC. Polymorphisms of interleukin-31 are associated with anti-CCP levels in females with rheumatoid arthritis. J Genet. 2014;93(3): 813-818.

39. Nobbe S, Dziunycz P, Muhleisen B, et al. IL-31 expression by inflammatory cells is preferentially elevated in atopic dermatitis. Acta Derm Venereol. 2012;92: 24–28.

40. Schulz F, Marenholz I, Folster-Holst R, et al. A common haplotype of the IL-31 gene influencing gene expression is associated with nonatopic eczema. J Allergy Clin Immunol. 2007;120(5): 1097-102.

41. Hong CH, Yu HS, Ko YC, et al. Functional regulation of interleukin-31 production by its genetic polymorphism in patients with extrinsic atopic dermatitis. Acta Derm Venereol. 2012;92(4): 430-2.