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Level of interleukin-18 binding protein is significantly different in patients with anaphylaxis than urticaria

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Abstract

Background: Acute urticaria is a common cutaneous disease encountered in children, while anaphylaxis can show cutaneous symptoms as well as systemic symptoms. One study found that urticaria tends to precede anaphylaxis, but studies on the different role of eosinophils and related cytokines in anaphylaxis and urticaria are lacking.

Objective: The aim of this study was to compare the clinical features, total eosinophil count, serum levels of interleukin (IL)-18, IL-18 binding protein (BP), IL-1 receptor-like (RL) 1, and IL-33 and compare with tryptase to examine if any differences could be found between patients who experienced anaphylaxis and urticaria.

Methods: We included 63 patients with urticaria and 52 patients with anaphylaxis. We measured total eosinophil count and the serum levels of total IgE, tryptase, IL-18, IL-18BP, IL-1RL1, and IL-33, and we compared the differences between the groups. Lastly, receiver operating characteristic curves were constructed to determine which factors accurately diagnosed anaphylaxis.

Results: No significant differences were observed in the clinical characteristics or sensitization between urticaria group and anaphylaxis group. Laboratory findings showed that total eosinophil count and IL-18BP were significantly lower in the anaphylaxis group, compared with the urticaria group. IL-18BP showed significant correlation with tryptase. The receiver operating characteristic curve for IL-18BP for diagnosing anaphylaxis had an area under the curve of 0.530.

Conclusion: IL-18BP level was significantly different in patients with anaphylaxis compared to those with urticaria. Serum IL-18BP level may be used to differentiate between the patients with urticaria or anaphylaxis.

Key words: urticaria; anaphylaxis; interleukin; cytokine; tryptase

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Introduction

Acute urticaria is a common disease characterized by wheals resulting from intermittent activation of skin mast cells.[1] The condition is characterized by a local or generalized red, raised, itchy rash with vasodilatation, increased blood flow, and vascular permeability.[1] Anaphylaxis can show similar dermatologic symptoms as urticaria, but anaphylaxis is a systemic, life-threatening disorder. Anaphylaxis is triggered by mediators released by mast cells and basophils activated via allergic (IgE-mediated) or non-allergic (non-IgE-mediated) mechanisms.[2] Anaphylaxis is a rapidly evolving, multisystem process involving the integumentary, pulmonary, gastrointestinal, and cardiovascular systems.[2] Anaphylaxis is a serious disorder that can lead to fatal airway obstruction resulting in hypoxemia, shock, and possibly death.[2] Anaphylaxis is often diagnosed clinically, but plasma tryptase and urinary histamine levels are often elevated, allowing diagnostic confirmation.[2] A previous study found that urticaria tends to precede anaphylaxis.[3] However, studies on the different roles of tryptase, in comparison with eosinophils and related cytokines in anaphylaxis and urticaria are lacking.

One potential biomarker, interleukin (IL)-18, is a pleiotropic cytokine produced by monocytes, macrophages, dendritic

cells, keratinocytes, Langerhans' cells, and B cells, which can activate T cells and induce either T helper (Th)1 or Th2 responses, depending on the cytokine environment.[4] It has been suggested that IL-18 may play an important role either in autoimmune disorders, characterized by a predominant Th1 response, or in allergic diseases, characterized by a Th2 response.[5] In anaphylaxis, IL-18 could play a role by its ability to elevate IgE, IgG, and IgM.[6] IL-18 binding protein (BP) is an endogenous antagonist with high neutralizing capacity that inhibits the action of IL-18 by preventing interaction with its cell-surface receptors.[7]

We examined the levels of another biomarker, IL-33. IL-33 plays a central role in allergic inflammation by acting through its membrane-bound receptor, ST2 receptor (ST2L). IL-33 activity can be neutralized by a soluble spliced ST2 variant (sST2) that has been associated with allergic inflammation, but its source is not well defined.[8] A recent animal study found that IL-33 was released following mechanical skin injury, and that it enhanced IgE-mediated mast cell degranulation and promoted oral anaphylaxis by targeting mast cells.[9] Soluble suppression of tumorigenicity 2 (sST2), also known as soluble IL-1 receptor-like (RL) 1, is encoded by the IL-1RL1 gene (chr 2) and can be detected in serum. IL-1RL1 is an asthma susceptibility gene identified in genetic studies of pediatric and adult asthma patients.[10] IL-1RL1 has also been linked to blood eosinophilia, IgE (sensitization), eczema, and hay fever.[11]

Tryptase is a serine protease produced and released in large amounts by mast cells and to a lesser extent by basophils. Baseline tryptase levels have been shown to increase after mast cell activation in anaphylaxis but specificity may be limited by the fact that it can be increased in other conditions, particularly mast cell disorders such as systemic mastocytosis.[12,13] Certain mast-cell mediators including histamine, and platelet -activating factor have been purported to be elevated in anaphylaxis, but practical challenges related to the timing and handling of samples may limit the usefulness of histamine and platelet-activating factor.[14,15]

Thus, the aim of our study was to compare total eosinophil count (TEC) and serum levels of tryptase, IL-18, IL-18BP, IL-1RL1, and IL-33 in patients with urticaria and anaphylaxis and to observe any differences between them.

Methods

Patients

We included 63 patients with urticaria and 52 patients with anaphylaxis who visited Uijeongbu St. Mary's Hospital Pediatric Allergy Clinic. The diagnosis of urticaria was dependent on the recurrence of wheals with or without angioedema for less than 6 weeks. Patients with physical urticaria or other chronic inflammatory diseases including atopic dermatitis, were excluded from the study.

Definition of anaphylaxis was symptoms from at least two organ systems in line with European Academy of Allergy and Clinical Immunology (EAACI) task force position papers,[16,17] modified for children by Vetander et al.[18] This research study conformed to the standards of the Declaration of Helsinki and was approved by the Institutional Review Board of Uijeongbu St. Mary's Hospital (Protocol No. UC18RESI0066). Written informed consent was obtained from parents or guardians; assent was obtained from invited children.

Sample collection

Blood samples (2 mL) were obtained within 3 hours from each patient upon enrollment whose symptoms were present prior to treatment. Blood was allowed to clot at room temperature for exactly 60 ± 10 minutes. Then, serum isolation was carried out by centrifugation at 3,000 rpm for 10 minutes. The serum was then transferred to 1.5-mL labeled tubes, aliquoted, and stored at –70°C.

Serum IL-18 ELISA

Serum IL-18 concentration was measured by using a sandwich enzyme immunoassay with a sensitivity of 12.5 pg/mL using a commercially available human IL-18 enzyme-linked immunosorbent assay (ELISA) kit (MBL-Medical and Biological Laboratories, Nagoya, Japan), per the manufacturer's instructions. This assay uses two monoclonal antibodies against two different epitopes of human IL-18. Samples and standards were incubated in microwells coated with antihuman IL-18 monoclonal antibody. After washing, the peroxidase-conjugated antihuman IL-18 monoclonal antibody was added to the microwells and incubated. After another washing, the substrate reagent was mixed with chromogen and allowed to incubate for an additional time period. An acid solution was then added to each microwell to terminate the enzyme reaction and to stabilize color development. The optical density (OD) of each microwell was measured at 450 nm using a microplate reader. The concentration of human IL-18 was calibrated from a dose response curve based on reference standards.

IL-18BP ELISA

Detection of IL-18BP was performed with the commercial IL-18BP Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. A 96-well microtiter plate was precoated with a polyclonal-specific antibody. Then 100 µL of different standards or samples (1:2 dilutions) were added to the plate and incubated for 2 hours. After washing four times with phosphate-buffered saline containing 0.05% Tween 20 (T-PBS), 100 µL of a biotin-conjugated polyclonal-specific antibody were added to each microplate well and incubated for 2 hours. The plate was then washed four times with T-PBS and incubated with 100 µL of the working dilution of Streptavidin-horseradish peroxidase for 20 minutes. Following six washes with T-PBS, 100 µL of 3,3',5,5'-tetramethylbenzidine was added to each well and incubated for 20 minutes. The reaction was stopped by the addition of 50 µL of stop solution, and the absorbance was examined using a microplate reader at 450 nm. Each sample was measured in triplicate.

IL-33 and IL1-RL1 ELISA

Serum IL33 and IL1-RL1 levels were measured using commercial sandwich ELISA kits (Duo set, Catalogue number: DST200; R&D Systems). We used a dilution of 1:5 for all samples. The given limit of detection was 2000 pg/mL (range: 0–2970.322 pg/mL). All measurements were performed in technical duplicates and the respective mean values were calculated.

Serum tryptase level

Serum tryptase levels were determined using the ImmunoCAP Tryptase immunoassay (Thermo Fisher Scientific, Uppsala, Sweden). Levels > 11.4 ng/mL were considered elevated.[19] Tryptase levels were measured along with other blood sample as samples taken within the first 3 hours of anaphylaxis are considered to be a selective marker of anaphylaxis and do not require specific handling of the sample.[14] In addition, tryptase measurement is usually not part of the routine protocol in emergency departments in our country, in most cases due to financial constraints.

Measurement of Blood Eosinophils

The NE-8000 system (Sysmax, Kobe, Japan) was used to automatically count white blood cells and eosinophils in peripheral blood.

Measurement of total IgE and specific IgE to allergens

Total IgE levels were measured with the ImmunoCAP immunofluorimetric assay (Thermo Fisher Scientific, ImmunoDiagnostics, Uppsala, Sweden). Measures of total IgE levels were expressed in international units per unit volume (1 IU = 2.4 ng). The measuring range of total IgE was 2 to 5,000 IU/mL. Specific IgEs to common allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, birch, alder, oak, cow milk, hen's egg, and peanut) were measured with the ImmunoCAP immunofluorimetric assay, components, and reagents (Thermo Fisher Scientific, ImmunoDiagnostics, Uppsala, Sweden). Specific IgE levels were expressed in arbitrary units per unit volume (IU/mL). Sensitization was defined as specific IgE levels > 0.35 IU/mL. The ImmunoCAP 250 machine underwent monthly quality controls (Euro EQAS for Total IgE, Euro EQAS for Specific IgE, Phadia Quality Club for specific IgE).

Statistical analysis

Statistical analyses were performed using SPSS software, version 20.0 (IBM Inc., Armonk, NY, USA) and MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium). All continuous variables are expressed as means (SD) and categorical variables as numbers (percentage). The student T-test was used to compare the differences in laboratory findings between patients with urticaria, and patients who experienced anaphylaxis. Chi-square test was used to compare the ratio of categorical variables between the anaphylaxis group and the urticaria group. A receiver operating characteristic (ROC) curve was constructed, and the area under the curve for each parameter was compared with 0.5 to estimate the optimal cutoff levels for predicting anaphylaxis. Statistical significance was defined at $p < 0.05$ in a two-tailed test.

Results

Patient characteristics

A total of 63 patients with urticaria and 52 patients who experienced anaphylaxis were included in the study. Subject characteristics are summarized in **Table 1**. TEC and IL-18BP were significantly lower in the anaphylaxis group compared with the urticaria group while tryptase was significantly higher in the anaphylaxis group in comparison with urticaria group.

Table 1. Subject characteristics and laboratory findings

Characteristics	Urticaria (N = 63)	Anaphylaxis (N = 52)	P value
Male, n (%)	41 (56.3)	26 (50)	> 0.999
Age, y	7.5 ± 3.8	8.2 ± 4.4	0.389
Allergic disease			
Asthma	12	10	
Allergic rhinitis	25	28	
Food allergy	14	13	
Total IgE, IU/mL	416.5 ± 556.3	585.1 ± 704.8	0.185
TEC, $\times 10^4$	368.5 ± 352.8	212.2 ± 160.2	0.003
IL-1RL1, pg/mL	45.9 ± 66.0	50.3 ± 35.2	0.727
IL-18, pg/mL	78.4 ± 41.7	71.0 ± 30.4	0.361
IL-18BP, pg/mL	161.7 ± 68.2	127.1 ± 37.4	0.001
IL-33, pg/mL	241.9 ± 107.4	236.2 ± 76.3	0.745
Tryptase, ng/mL	2.7 ± 2.3	11.0 ± 3.4	< 0.001

Data expressed as mean ± SD and categorical variables as number (percentage)
Ig, immunoglobulin; TEC, total eosinophil count; IL, interleukin; RL, receptor like; BP, binding protein

Symptoms in the anaphylaxis group

Symptoms of anaphylaxis classified under different systems are summarized in **Table 2**. Respiratory symptoms included dyspnea, cough, and stridor. Gastrointestinal symptoms included abdominal pain, diarrhea, and vomiting. Cardiovascular symptoms included tachycardia and hypotension. Dermatologic symptoms included hives and angioedema.

Table 2. Symptoms in anaphylaxis group

System	Symptoms
Respiratory	18 (28.6)
Gastrointestinal	4 (6.3)
Cardiovascular	20 (31.7)
Dermatologic	10 (15.9)

Data expressed as number (percentage)

Sensitization

Sensitization was compared between the urticaria and anaphylaxis groups, but no significant difference was observed (**Table 3**).

Table 3. Comparison of sensitization between urticaria group and anaphylaxis group

	Urticaria (N = 63)	Anaphylaxis (N = 52)	P value
Inhalant	31 (49.2)	8 (15.4)	0.303
Food	10 (15.9)	10 (19.2)	
Both	5 (7.9)	14 (26.9)	
None	12 (19.0)	4 (7.7)	

Data expressed as number (percentage)

Correlation between tryptase and IL-1RL1, IL-18, IL-18BP, and IL-33

We examined whether IL-1RL1, IL-18, IL-18BP, and IL-33 showed any significant correlation with tryptase, which is known to be elevated in event of anaphylaxis. Only IL-18BP showed significant inverse correlation with tryptase.

Table 4. Correlation between tryptase and IL-1RL1, IL-18, IL-18BP, and IL-33

	IL-1RL1	IL-18	IL-18BP	IL-33
Tryptase	0.102 (0.354)	-0.078 (0.468)	-0.218 (0.020)	-0.023 (0.811)

Data expressed as r (P value)

ROC curves for IL-1RL1, IL-18, IL-18BP, IL-33, and tryptase for diagnosing anaphylaxis

ROC curves for IL-1RL1, IL-18, IL-18BP, IL-33 and tryptase yielded areas under curve (AUC) of 0.576, 0.556, 0.548, 0.530 and 0.985, respectively (**Figure 1**). We compared the sensitivity, specificity, positive predictive value, and negative predictive value of IL-18BP and tryptase for diagnosing anaphylaxis (**Table 5**).

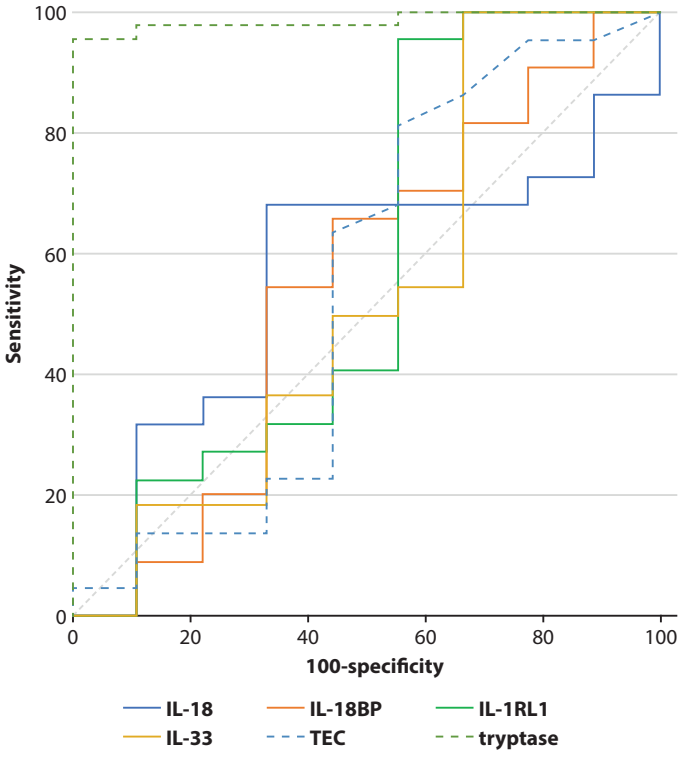

Figure 1. Receiver operating characteristic (ROC) curve of IL-1RL1, IL-18, IL-18BP, IL-33, and tryptase for diagnosing anaphylaxis (AUC = 0.576; AUC = 0.556, AUC = 0.548, AUC = 0.530, AUC = 0.985, respectively).

Table 5. Comparison of the sensitivity, specificity, positive predictive value, and negative predictive value of tryptase with IL-18BP for diagnosing anaphylaxis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Tryptase	92.3	93.7	6.3	7.7
IL-18BP	98.1	46.8	3.3	39.3

IL, interleukin; BP, binding protein; PPV: positive predictive value; NPV: negative predictive value

Discussion

In this study, we found significantly different levels of TEC, tryptase, and IL-18BP between the groups. IL-18BP and TEC was significantly lower in the anaphylaxis group compared with the urticaria group, while tryptase was significantly higher in the anaphylaxis group. No significant differences in clinical characteristics or IL-18, IL-33, and IL-1RL1 levels were observed between anaphylaxis and urticaria patients. IL-18BP levels showed significant inverse correlation with tryptase level. ROC curve showed that tryptase showed the highest AUC for diagnosing anaphylaxis.

We found that IL-18 level was not significantly different between patients who experienced anaphylaxis and urticaria. IL-18 belongs to the IL-1 family, which plays a major role in innate and acquired immunity.[20] IL-18 stimulates T cells to produce increased IFN-γ, IL-13 and IL-5, and IL-13 and IL-5, which are potent Th2 cytokines that play a key pro-inflammatory role in various allergic diseases.[20] It was reported that total IL-18 levels correlated with clinical severity scores among chronic urticaria patients who had positive autologous serum skin test (ASST) results.[21] However, conflicting results found that significantly increased levels of free IL-18 and IL-18BP were observed in chronic urticaria cases, regardless of ASST results.[22] Yet another study found no significant correlation between IL-18 and urticaria disease severity.[23] Our findings suggest that IL-18 does not play a significant role in acute urticaria or anaphylaxis.

We examined IL-18BP levels because IL-18 is known to carry out its biological functions mainly through its receptors IL-18R and IL-18BP. IL-18BP acts as a potent endogenous neutralizing antagonist of IL-18.[24] IL-18BP is known to be elevated in patients with urticaria and other inflammatory conditions to counteract the inflammatory effect of IL-18.[25] Our study found that even though IL-18 level was not significantly different between the patient groups, high IL-18BP levels could have led to decreased IL-18 activity. Since IL-18 can act as a cofactor for Th2 cell development and IgE production,[26] lower IL-18BP levels led to higher total IgE levels in the anaphylaxis group, though the effect was not statistically significant. This is possibly because IL-18 indirectly increases production of its own inhibitor in a feedback loop, through up-regulation of the major IL-18BP inducer IFN-γ.[27] Our study showed that TEC levels were significantly lower in the anaphylaxis group, similar to IL-18BP.

IL-33 and IL-1RL1 levels were not significantly different between the groups. A recent study with a mouse model of atopic dermatitis found that IL-33 promoted anaphylaxis by targeting mast cells.[9] IL1-RL1 is also known as suppression of

tumorigenicity 2 (ST2). IL-1RL1 is a key molecule in the Th2 -mediated allergic inflammation process.[27] Among the four known isoforms of ST2: a longer membrane-anchored form, a shorter soluble form (sST2), and two variant forms, sST2 and ST2 are particularly relevant for the regulation of allergic airway inflammation in mice.[28] It has been shown that sST2 antagonizes ST2, suppressing the IL-33-mediated activation of the nuclear factor κB pathway, as well as downstream effects such as Th2 cytokine production, thereby attenuating allergic symptoms in mice.[29] sST2 inhibits Th2 immunity by acting as a decoy receptor for IL-33.[29] It appears that IL-33 and IL-1RL1 do not play different roles in anaphylaxis or urticaria.

Sensitization to inhalants or food was compared between the anaphylaxis group and the urticaria group. A previous study from Korea found that food was the most common anaphylaxis trigger (74.7%), followed by drugs and radiocontrast media (10.7%).[30] Similarly, another study found that food was the most frequent cause of urticaria (n = 236, 37.8%), followed by changing one's environment (n = 172, 27.6%).[31] A study from Southern Europe reported that respiratory infections were the most common urticaria triggers, while food allergens were the least.[32] There appears to be regional differences in the causes of urticaria, and our study was consistent with previous reports from Korea.

Cardiovascular symptoms were most common in anaphylaxis cases, followed by respiratory symptoms. Anaphylaxis, by definition, involves at least two organ systems or sudden changes in vital signs. Skin and mucosal changes are usually, but not always, present, whereas hypotension and shock are not mandatory for diagnosis.[2] Interestingly, skin involvement was considerably lower than the results from a recent study.[33] This may be due to the fact that we enrolled consecutive patients with urticaria and anaphylaxis, and those with skin involvement tended to be included in the urticaria group. This could have compromised the ability of IL-18BP for differentiating urticaria and anaphylaxis.

IL-18BP levels showed significant inverse correlation with tryptase. Tryptase is known to be elevated in cases with anaphylaxis. Other biomarkers, such as chymase, carboxypeptidase A3, CCL-2, and platelet activating factor (PAF) have been studied in the diagnosis of anaphylaxis, but no study has studied the role of IL-18BP in anaphylaxis.[34,35] Our study found that IL-18BP level was significantly different in patients with urticaria and anaphylaxis.

Using the ROC curve, we compared whether IL-1RL1, IL-18, IL18BP, or IL-33 can diagnose anaphylaxis more accurately than tryptase. We found that tryptase remains the most accurate but IL-18BP may act as a significant biomarker for diagnosing anaphylaxis. To the best of our knowledge, this is the first study to examine whether any cytokine acts as a diagnostic marker for anaphylaxis. Further studies with a larger study population that investigate the role of IL-18BP or that discover other novel biomarkers for anaphylaxis would be valuable.

Our study has a number of limitations. First, our study's anaphylaxis group had a relatively small sample size. Second, a previous study found that IL-18 correlated with urticaria severity, but we were unable to assess urticaria or anaphylaxis severity. Thirdly, as mentioned earlier, dermatologic involvement was considerably lower in the anaphylaxis group because of the manner of subject enrollment. Finally, we did not measure Th2 cytokines, such as IL-13 and IL-5, which could have clarified the roles of IL1-1RL1, IL-18, IL-18BP, and IL-33. Nevertheless, our results are the first to show that IL-18BP level was significantly different in anaphylaxis in comparison with urticaria.

Conclusion

In conclusion, IL-18BP may be used to differentiate patients with urticaria and anaphylaxis. Further studies in-vitro, ex-vivo and in-vivo are needed to prove causality and may reveal novel biomarkers that can accurately predict which urticaria patients are more likely to progress to anaphylaxis.

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None

Conflict of interest

None

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