

Serum Human β -Defensin 2 Is Increased in Angioedema Accompanying Chronic Spontaneous Urticaria

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Keywords

Chronic spontaneous urticaria · Antimicrobial peptide · Human β -defensin 2 · Angioedema

Abstract

Introduction: Chronic spontaneous urticaria (CSU) is a common cutaneous disease caused by mast-cell degranulation. Human β -defensin 2 (HBD2) is a well-known antimicrobial peptide that is also a pruritogen inducing vascular permeability via non-IgE-mediated mast-cell degranulation. **Objective:** We investigated the associations between serum HBD2 levels and the clinical characteristics of CSU patients. **Methods:** Serum samples from 124 CSU patients and 56 healthy controls were screened for the levels of HBD2 and translationally controlled tumor protein (TCTP) by using ELISA. The urticaria activity score over 7 days (UAS7) was used to measure disease activity in CSU patients. Accompanying angioedema was self-reported. **Results:** Serum HBD2 levels were higher in the CSU group than in healthy subjects (median [interquartile range], 84.1 [43.5, 142.5] vs. 59.5 [26.7, 121.5], $p = 0.034$). In CSU patients, serum HBD2 level was negatively correlated with the peripheral basophil percentages (Spearman's $\rho = -0.229$, $p = 0.01$) and vitamin D levels

(-0.262 , $p = 0.02$), but positively correlated with TCTP levels (0.252 , $p = 0.006$). In CSU patients, HBD2 level was higher in those with than without angioedema (101.7 [50.9, 184.2] vs. 66.7 [37.9, 132.0], $p = 0.019$). It did not differ by aspirin hypersensitivity or atopy status, or autologous serum skin test positivity. **Conclusion:** A known mast-cell degranulator, HBD2 was elevated in the sera from CSU patients compared to healthy controls and may be involved in the pathogenesis of accompanying angioedema.

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Introduction

Chronic spontaneous urticaria (CSU) is a common cutaneous disease characterized by the repeated appearance of transient hives, angioedema, or both, persisting for >6 weeks attributable to either the development of mast cell-activating autoantibodies or unknown causes [1]. Degranulation of cutaneous mast cells plays a central role in histamine, chemokines, cytokines, and protease releases. The subsequent production of arachidonic acid metabo-

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lites and platelet-activating factors trigger vasodilation and sensory nerve stimulation.

Angioedema occurs in deeper skin layers than do wheals, associated with fewer mast cells and sensory nerve endings. Therefore, the lesions exhibit little or no pruritus; any swelling may be described as painful or burning. Angioedema has been observed up to 70% of CSU patients [2, 3], often in the face, eyelids, lips, and oral cavity. Angioedema is associated with the severity of urticaria in various studies and negatively impacts on the quality of life [3, 4]. About 10% of CSU patients mainly suffer from angioedema without wheals [5].

Human β -defensin 2 (HBD2) is a cysteine-rich, cationic 41-amino-acid peptide produced by epithelial cells, keratinocytes, and macrophages that are stimulated by bacteria, fungi, and cytokines [6–8]. The principal function of HBD2 is antimicrobial; the peptide kills bacteria and fungi. In vitro, HBD2 promotes inflammation by recruiting CD4+ T lymphocytes and macrophages via interaction with the chemokine receptor C-C chemokine receptor 2 (CCR2) and CCR6 [9]. HBD2 has been reported to induce mast-cell degranulation via interaction with the Mas-related G protein-coupled receptor-X2 (MRG-PRX2) [10]. HBD2 serves as a pro-inflammatory pruritogen, interacting with Toll-like receptor 4 to activate MRGPRX2, in turn triggering histamine-independent itching in patients with skin diseases [11, 12]. β -defensins increase vascular permeability in vivo [12].

HBD2 and human β -defensin 3 (HBD3) are produced principally by keratinocytes in the skin; however, the serum level of HBD2 is significantly increased in patients with cutaneous diseases including atopic dermatitis [13] and psoriasis [14]. In the present study, we sought to measure serum levels of HBD2 in CSU patients and to explore their associations with clinical characteristics, such as disease activity, the autologous serum skin test (ASST) results, accompanying angioedema, and peripheral basopenia.

Materials and Methods

Study Subjects

We enrolled 56 healthy controls (NCs) and 124 active CSU patients treated at Ajou University Hospital in Korea. We followed the evidence-based practice guidelines for CSU treatment in Korean adults [15]. Serum samples from CSU patients were collected after antihistamines had been paused for at least 5 days, and the samples were stored at -70°C . CSU disease activity was assessed by reference to the urticaria activity score over 7 days (UAS7). Atopy was defined as a positive skin prick test response to at least one common inhalant allergens on skin prick tests with common in-

halant allergens (pollens of alder, birch, oak, grass mixture, mugwort, and ragweed, cat or dog fur, or *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, or *Aspergillus niger*, or *Alternaria* spp.). Written informed consent was collected from all study subjects. The study was approved by our Institutional Review Board (approval no. AJIRB-BMR-SMP-18-74).

Assessment of Clinical Parameters

ASST was performed and interpreted in accordance with EAA-CI/GA²LEN guidelines [16]. Antinuclear antibody was detected using an indirect fluorescent antibody technique (Fluoro HEPA-NA test, Medical and Biological Laboratories, Nagoya, Japan). Serum levels of total IgE were measured using an ImmunoCAP system (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Serum levels of 25-hydroxy vitamin D were analyzed using a competitive chemiluminescent immunoassay (ADVIA Centaur Vitamin D Total Assay, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Vitamin D deficiency was defined as a 25-hydroxy vitamin D level <20 ng/mL [17].

Measurement of HBD2 and TCTP Levels

Levels of HBD2 and translationally controlled tumor protein (TCTP) were measured using commercial ELISA kits (MyBioSource, Inc., San Diego, CA, USA) according to the manufacturer's instruction. Serum samples were diluted to within the detection range of HBD2 (3.12–100 ng/mL).

Statistical Analyses

Statistical analyses were performed using IBM SPSS, version 22 software for Windows (IBM Corp., Chicago, IL, USA). Categorical variables were analyzed using the Pearson's χ^2 test. Continuous variables that were normally distributed are presented as means $\pm$ standard deviations and were compared using the t test, variables that were not normally distributed are presented as medians and interquartile ranges and were compared using the Mann-Whitney U-test. One-way ANOVA with post hoc Dunnett T3 test was used for between-group comparisons of HBD2 levels. Spearman correlations were derived between HBD2 levels and other clinical and laboratory parameters. The significance level for all analyses was set to $p < 0.05$.

Results

Clinical Characteristics of the Study Subjects

The demographics of the study subjects are summarized in Table 1. A total of 124 CSU patients (aged 38.6 ± 10.3 years, 80 females) and 56 NCs (aged 40.3 ± 10.9 years, 32 females) were enrolled. Atopy rate and the mean total IgE level were higher in CSU patients than in NCs. Of the CSU patients, 48 (38.7%) had allergic rhinitis. The mean urticaria duration and the UAS7 were 25.5 months and 28.4, respectively. The ASST-positivity was 49.6%, and the prevalence of angioedema was 41.9% in CSU subjects.

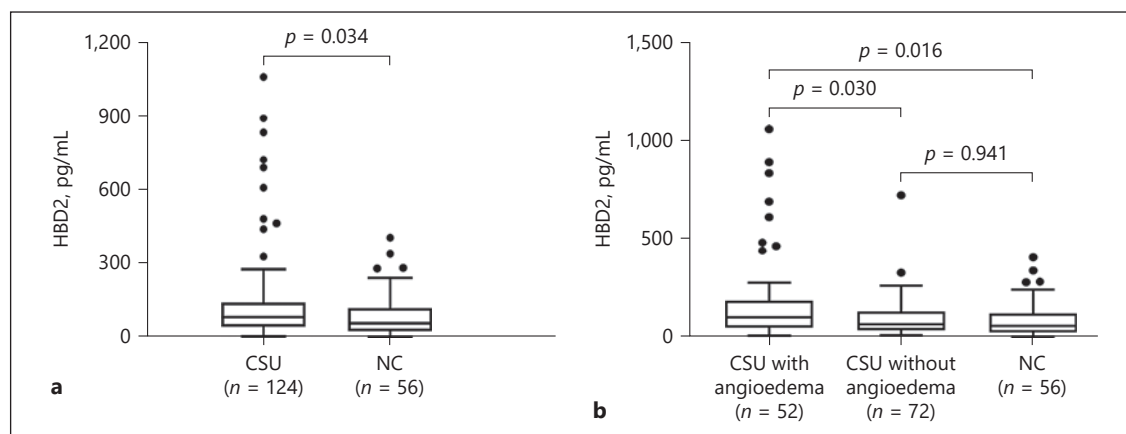


Fig. 1. Comparison of serum levels of HBD2 between CSU and NC subjects (**a**), and by angioedema status (**b**). **a** p value was determined using the Mann-Whitney U-test. **b** One-way ANOVA with post hoc Dunnett T3 test was used for comparisons among the 3 groups. AE, angioedema; CSU, chronic spontaneous urticaria; HBD2, human β -defensin 2; NC, healthy controls.

Table 1. Characteristics of the study subjects

	CSU (n = 124)	NC (n = 56)	p value
Age, years	38.6 $\pm$ 10.3	40.3 $\pm$ 10.9	0.308
Female sex, %	80 (64.5)	32 (57.1)	0.345
Urticaria duration, months	25.5 $\pm$ 42.1	NA	NA
Allergic rhinitis, %	48 (38.7)	0	<0.001
Atopy, %	69 (55.6)	19 (33.9)	0.007
ANA-positivity, %	28 (23.3)	NA	NA
ASST-positivity, %	60/121 (49.6)	NA	NA
UAS7 (range 0–42)	28.4 $\pm$ 3.7	NA	NA
Angioedema, %	52 (41.9)	NA	NA
H1RA responders, %	77 (62.1)	NA	NA
Steroid use, %	40 (21.5)	NA	NA
Total IgE, kU/L*	110.5 (64.5, 275.5)	55.0 (27.5, 130.5)	<0.001
Peripheral basophil, %	0.5 $\pm$ 0.3	NA	NA
Complement 3, mg/dL [†]	112.7 $\pm$ 24.3	NA	NA
Complement 4, mg/dL [†]	26.3 $\pm$ 7.5	NA	NA
25-Hydroxy vitamin D, ng/mL [†]	21.8 $\pm$ 9.2/79	NA	NA
Vitamin D deficiency, n/N %	39/79 (49.4)	NA	NA
TCTP, pg/mL*	29.8 (19.6, 51.3)	27.7 (18.0, 47.7)	0.580

ANA, antinuclear antibody; ASST, autologous serum skin test; CSU, chronic spontaneous urticarial; H1RA, H1-receptor antagonist; IgE, immunoglobulin E; NA, not applicable; NC, healthy controls; TCTP, translationally controlled tumor protein; UAS7, urticaria activity score over 7 days. * Data are presented as medians (with interquartile ranges). [†] Data are presented as means $\pm$ standard deviations.

Elevated HBD2 Levels in CSU Patients

The HBD2 level was significantly elevated in CSU patients (84.1 [43.5, 142.5]) compared to healthy control subjects (59.5 [26.7, 121.5], $p = 0.034$) (Fig. 1a). Dunnett T3 test showed that CSU patients with angioedema had

higher levels of serum HBD2 than in those without angioedema (141.6 [70.8, 211.6] vs. 88.7 [43.5, 159.0], $p = 0.030$, Fig. 1b) and also than in healthy controls ($p = 0.016$). However, no difference in serum levels of HBD2 between CSU patients without angioedema and healthy controls

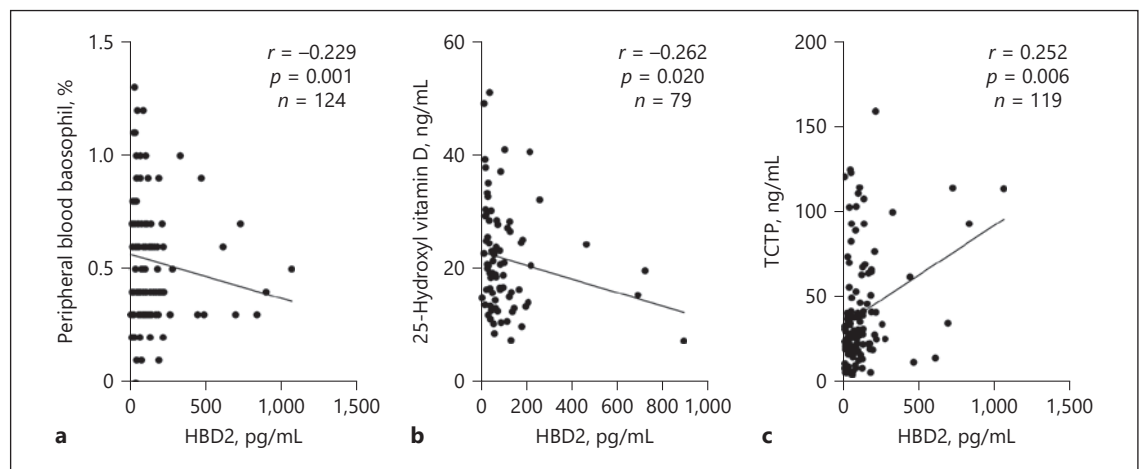


Fig. 2. Correlations between HBD2 levels, and peripheral basophil percentages (a), serum vitamin D levels (b), and serum TCTP levels (c), in CSU patients. *p* values were determined using the Spearman correlation. HBD2, human β -defensin 2; TCTP, translationally controlled tumor protein; CSU, chronic spontaneous urticaria.

(Fig. 1b). The levels did not differ by ASST result (99.9 [45.7, 152.2] vs. 69.7 [38.7, 111.1], $p = 0.117$), aspirin hypersensitivity (88.7 [43.8, 181.5] vs. 82.3 [42.6, 133.9], $p = 0.147$), atopy (84.9 [43.5, 139.7] vs. 83.2 [43.5, 166.7], $p = 0.850$), and the response to H1-antihistamines (97.7 [50.1, 166.7] vs. 74.0 [37.1, 132.0], $p = 0.133$).

Serum level of HBD2 in CSU patients correlated negatively with peripheral basophil percentages (Spearman rho = -0.229 , $p = 0.010$) and 25-hydroxy vitamin D levels (-0.262 , $p = 0.020$), but positively with TCTP levels (0.252 , $p = 0.006$, Fig. 2). We found no significant correlations between HBD2 levels and UAS7 (Spearman rho = 0.052 , $p = 0.567$, urticaria duration (-0.168 , $p = 0.062$), total IgE (-0.025 , $p = 0.779$), complement 4 (0.114 , $p = 0.213$) levels, and peripheral or the peripheral eosinophil counts (-0.143 , $p = 0.114$).

Multiple logistic regression showed that both an HBD2 level >72 pg/mL (odds ratio 5.55, 95% confidence interval [CI] 1.83–16.85, $p = 0.003$) and UAS7 ≥ 28 (odds ratio 4.94, 95% CI 1.55–15.73, $p = 0.007$) were significantly associated with angioedema in CSU patients, while age, sex, and vitamin deficiency did not affect the occurrence of angioedema (Table 2).

Discussion/Conclusion

Mast-cell activation by Fc ϵ RI regulators, IgE-independent pathways (e.g., Mas-related G protein-coupled receptor-X2 [MRGPRX2]), tetraspanins, and the CD300

Table 2. Factors related to accompanying angioedema in patients with CSU

	OR	95% confidence interval	<i>p</i> value
Age	0.987	0.950, 1.025	0.486
Sex	2.026	0.886, 4.630	0.094
HBD2 ≥ 72 pg/mL	2.766	1.266, 6.044	0.011
UAS7 ≥ 28	2.934	1.298, 6.632	0.010

CSU, chronic spontaneous urticaria; HBD2, human β -defensin 2; UAS7, urticaria activity score over 7 days; OR, odds ratio.

protein family has been well-studied [18]. Thyroid proteins, nuclear antigens (double-stranded DNA), and IL-24 induce mast-cell activation via Fc ϵ RI cross-linking in CSU patients [19]. Both CSU endotypes, types I and IIb autoimmunity are associated with Fc ϵ RI receptor; however, a significant elevation in the serum level of substance P, triggering mast-cell activation via MRGPRX2, is also evident in CSU patients [20], suggesting a potential contribution of an IgE- Fc ϵ RI receptor-independent mechanism in CSU pathogenesis. Notably, viral and parasitic infections are important causes of CSU [1]. HBDs not only combat infections by exerting antimicrobial properties, but also act as mast-cell secretagogues, inducing neurogenic inflammation, pain, and itching via a non-Fc ϵ RI-cross-linking mechanism [10, 12, 21]. We found that serum HBD2 level was significantly elevated

in the sera from CSU patients with significant correlations with percentage of peripheral basophil, serum TCTP, and vitamin D levels, as compared with those in healthy control in the present study.

TCTP is a well-studied histamine release factor active in patients with allergic diseases including asthma and CSU [22]. As we suggested previously, the oxidative cytokine-rich tissue environment induced by activation of various inflammatory cells in CSU skin, and the autoimmunity reflected by the presence of IgG antibody to FcεRIα in CSU patients, may lead to a TCTP conformational change to the active dimeric form [23]. We found that, in CSU patients, the level of dimerized form of TCTP was increased and caused mast-cell degranulation and basophil activation with or without IgE sensitization [23]. The peripheral basopenia in CSU patients suggests that skin basophil activation is in play [24]. The correlations between the HBD2 levels, and fewer basophils and higher TCTP levels, in CSU patients, indicate that an increased HBD2 level serves as a marker of basophil and mast-cell activation. Kanda and Watanabe [25] suggested that a positive-feedback control loop existed between histamine and HBD2 levels in patients with inflammatory skin diseases. Histamine increased HBD2 production in synergy with TNF-α or IFN-γ expression in human keratinocytes. As HBD2 can induce histamine release from mast cells and chemotaxis of TNF-α-activated neutrophils, a paracrine loop between the HBD2 and histamine levels may amplify the interactions among keratinocytes, and mast cells or other inflammatory cells, in CSU skin [25, 26]. Skin inflammation that is triggered by infections, and that persists because of activation of mast cells, basophils, macrophages, and/or T cells, may increase HBD2 production in the dermis of CSU patients.

Most angioedema in CSU patients is histaminergic, mast cell-mediated, and associated with pruritus [27]. The histopathological findings of angioedema associated with CSU include vascular leakage and perivascular infiltration of monocytes, mast cells, basophils, eosinophils, and activated CD4+ T cells [28]. Clinically, CSU patients with angioedema exhibit greater CSU activity and a longer disease duration than those lacking angioedema [2, 29]. However, as the UAS7 is based exclusively on assessment of wheals and pruritus, angioedema presence/severity in CSU patients is missed. To precisely predict the quality of life of CSU patients, an objective indicator that reflects angioedema status is needed. In the present study, we found that higher UAS7 and HBD2 levels were associated with angioedema, although the HBD2 level was not significant associated with the UAS7

score. We are the first to show that HBD2 is potentially involved in the angioedema pathogenesis of CSU patients, although HBD2 does not strongly enhance pruritus and wheal formation.

Vitamin D plays important role in the immune system; a low vitamin D level is associated with various allergic diseases including asthma, allergic rhinitis, and atopic dermatitis [30–32]. In recent years, low vitamin D levels have been reported in adult CSU patients, but not children, compared to healthy controls [33], and vitamin D deficiency is associated with urticaria activity and ASST-positivity [34]. A negative correlation was apparent between the HBD2 and 25-OH vitamin D levels in CSU patients, but neither level was directly correlated with the UAS7 score, although vitamin D enhances HBD2 production by keratinocytes [35]. We found that CSU patients with low 25-OH vitamin D levels had higher HBD2 levels, indicating that activated macrophages and monocytes in the dermis may contribute more to HBD2 production than do keratinocytes. However, as we did not measure skin HBD2 expression in CSU patients, further studies are needed to elucidate how HBD2 levels increase in CSU patients, particularly those with angioedema. It is necessary to compare HBD2 protein and gene expression levels between CSU patients and healthy controls. We plan to study how the complex interactions between HBD2 and the major inflammatory cells and mediators of CSU patients differ from those of other inflammatory skin diseases such as psoriasis and atopic dermatitis.

In conclusion, we are the first to report that an elevated HBD2 can be involved in the pathogenic mechanism of accompanying angioedema in CSU patients. Studies to elucidate which pathways among histaminergic or kininergic or others are related to increased HBD2 levels in CSU patients are needed in future.

Statement of Ethics

Written informed consent was obtained from all subjects. The biospecimens of, and data on, healthy subjects were provided by the Biobank of Ajou University Hospital, a member of the Korea Biobank Network. The study was approved by the Institutional Review Board of Ajou University Hospital (No. AJIRB-BMR-SMP-18-74).

Conflict of Interest Statement

There are no financial or other issues that might create a conflict of interest.

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Author Contributions

T.B.T.C. wrote the first draft of this paper and performed experiments. H.Y.C. designed and performed experiments. E.M.Y. performed experiments and interpreted data. B.Y.C. performed experiments and the statistical analyses. H.S.P. collected samples and clinical data. Y.M.Y. designed experiments, interpreted data, and revised this paper.

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