ISSN: 0067-2904

Allergic Rhinitis and Asthma: A Profile of Beta-defensins in Serum of Iraqi Patients

Marwa B. Ahmed[1], Ali H. Ad'hiah[2,*]
[1]*Biotechnology Department, College of Science, University of Baghdad, Baghdad, Iraq.*
[2]*Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq.*

Received: 4/6/2021 Accepted: 6/7/2021 Published: 30/5/2022

Abstract

Human beta-defenses (hBDs) are antimicrobial peptides involved in innate immune protection, and their association with the risk of respiratory allergy has been proposed. Therefore, this study sought to evaluate this association in allergic rhinitis (AR) and asthma (AS) of Iraqi patients. A case-control study was conducted to investigate serum levels of hBD1, hBD2, hBD3, and hBD4 in 52 AR and 60 AS patients and 61 healthy controls (HC). The hBDs were determined using enzyme-linked immunosorbent assay kits. Results revealed that median levels of hBD1, hBD2, and hBD3 were significantly elevated in the serum of AR and AS patients compared with HC ($p < 0.01$). Levels of hBD4 were also elevated in AR and AS patients but the differences were not significant. The levels of hBDs showed no significant differences between AR and AS patients. Age, gender, symptoms, and disease duration of patients influenced some of these variations. Logistic regression analysis indicated that hBD3 was the most important marker associated with the risk of AR and AS, and the estimated Odds ratios were 25.31 (95% confidence interval: 2.97-215.78; $p = 0.012$) and 32.20 (95% confidence interval: 2.49-415.89; $pc = 0.032$), respectively. Receiver operating characteristic curve analysis revealed that hBD3 occupied a very good area under the curve in AR and AS (0.83 and 0.84, respectively). In conclusion, the role of hBDs in the pathogenesis of AR and AS was indicated. In this context, hBD3 was the most important, and its association with the risk of developing AR and AS was suggested.

Keywords: Allergic rhinitis, Asthma, Human beta-defensins, Logistic regression analysis, Receiver operating characteristic curve.

التهاب الأنف التحسسي والربو: لمحة عن الدفاعات–بيتا في مصل مرضى عراقيين

مروه بكر أحمد[1] ، علي حسين ادحيه[2]*

[1]قسم التقانة الإحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

[2]وحدة البحوث البيولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تعد الدفاعات–بيتا البشرية (hBDs) ببتيدات مضادة للجراثيم وتشارك في الحماية المناعية الذاتية، وقد اقترح بانها مرتبطة بخطر الإصابة بحساسية الجهاز التنفسي. لذلك، سعت هذه الدراسة إلى تقييم هذا الارتباط في التهاب الأنف التحسسي والربو لمرضى عراقيين. أجريت دراسة حالة– ضوابط للبحث في المستويات

*Email: dr.ahadhiah@sc.uobaghdad.edu.iq

المصلية لل hBD1 و hBD2 و hBD3 و hBD4 في 52 مريضا من التهاب الأنف التحسسي و 60 مريضا من الربو و 61 من افراد السيطرة. تم تحديد مستوى hBDs باستخدام مجموعات مقايسة الممتز المناعي المرتبطة بالإنزيم. أظهرت النتائج بأن المستويات المصلية من hBD1 و hBD2 و hBD3 قد ارتفعت معنوياً في مصل مرضى التهاب الأنف التحسسي والربو مقارنة مع السيطرة (الاحتمالية < 0.01). كما ارتفعت ايضا مستويات hBD4 في مرضى التهاب الأنف التحسسي والربو ولكن لم تكن الاختلافات معنوية. ولم تظهر مستويات hBDs فروق ذات دلالة إحصائية بين مرضى التهاب الأنف التحسسي والربو. ولكن كان للعمر والجنس والأعراض ومدة المرض في المرضى بعض التأثير على هذه المستويات. أشار تحليل الانحدار اللوجستي إلى اهمية hBD3 كواسم مرتبط بخطر الاصابة والتهاب الأنف التحسسي والربو، وكانت نسب الأرجحية المقدرة 25.31 (فاصل الثقة 95٪: 2.97–215.78؛ الآحتمالية = 0.012) و 32.20 (95٪ فاصل الثقة: 2.49 –415.89 ؛ الاحتمالية = 0.032)، على التوالي. أظهر تحليل منحنى خاصية التشغيل للمستقبل (ROC) بشغل hBD3 مساحة جيدة جدًا تحت المنحنى في التهاب الأنف التحسسي والربو (0.83 و 0.84، على التولي). وكاستنتاج، فقد اشارة الدراسة الى دور hBDs في التسبب بالتهاب الأنف التحسسي والربو في هذا، كان hBD3 هو الأكثر أهمية وتم اقتراح ارتباطه بخطر تطور التهاب الأنف التحسسي والربو.

1. Introduction

Allergic rhinitis (AR) and asthma (AS) are common chronic inflammatory diseases of the upper and lower respiratory tract, respectively. Both allergies share common pathophysiological mechanisms characterized by heightened bronchial hyperresponsiveness and exaggerated reactivity to a variety of stimuli in genetically susceptible people [1]. The inflammatory response is mediated by the interaction of several innate and adaptive humoral and cellular components and outcomes in disruption of epithelial and parenchymal cells of the airways. The humoral mediators include histamine, leukotrienes, immunoglobulin E (IgE), chemokines, and T helper 2 (Th2) cytokines, while cellular components include eosinophils, basophils and mast cells, neutrophils, and CD4+ T-cells [2], [3][4].

Over the past decades, there has been increasing interest in understanding the role of innate immunity in the initiation and regulation of inflammatory response in AR and AS [5], [6]. The innate immune response is mediated by a group of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), a retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and C-type lectin receptors (CLRs), which sense the microbial components pathogen-associated molecular patterns (PAMPs) [7]. These receptors also recognize damage/danger-associated molecular patterns (DAMPs), which are intrinsic components released from damaged cells. The recognition of PAMPs and DAMPs induces robust innate immune responses and initiates inflammatory responses implicated in the pathogenesis of AS and AR [8]. In addition to PRRs, human beta-defensins (hBDs) are other components of innate immunity that have been identified to mediate the pathogenic mechanisms in AR and AS [9-11].

HBDs are described as a class of cationic antimicrobial peptides with influential roles in coordinating innate and adaptive immune responses required to maintain homeostasis. They also participate in various aspects of pathophysiological mechanisms mediated by inflammation [12]. Genome-based analyses have identified 28 HBDs encoding genes, but at the protein level, only a few have been isolated and characterized [13]. Among these are hBD1, hBD2, hBD3, and hBD4, which are primarily found in the epithelial layers of the airways, skin, and urogenital tracts [10]. The hBD1 is constitutively expressed, while the expression of hBD2, hBD3, and hBD4 requires induction. The most important inducers of their expression are microbial pathogens and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) [12]. Functionally, hBDs have the ability to kill various pathogens (viruses, bacteria, and fungi); however, their immunomodulatory potential

has also been recognized [14]. They display chemotactic properties and can bind to chemokine receptors to chemoattract various cells including neutrophils, monocytes, macrophages, mast cells, immature dendritic cells (DCs), and T cells [15]. Moreover, these cells are functionally affected by hBDs. It has been proven that hBDs can activate macrophages and neutrophils and enhance their phagocytic activity, provoke degranulation of mast cells and regulate maturation and differentiation of DCs [16]. Production of cytokines and chemokines can also be induced or suppressed by hBDs [17]. Besides, there has been growing evidence depicting the role of hBDs in controlling the response to danger, but this response has been described as dichotomous; suppressing inflammation on one side and exacerbating the response to a danger on the other side [18].

An association has been proposed between the expression of hBDs and the pathophysiological events of various inflammatory diseases [12]. Their biomarker significance in both infectious and non-infectious diseases has also been indicated [19]. Due to the immunomodulatory functions of hBDs, their role in the pathogenesis of AR and AS has been proposed. It has been shown that epithelial cells of the respiratory system express hBD1, hBD2, hBD3, and hBD4 to inhibit the proliferation of bacteria during respiratory infections [20]. Further, up-regulated expression of hBDs has been indicated to exacerbate the inflammation in the airways and cause damage to the epithelial layer in AS patients [21]. It has been found that hBDs can induce the degranulation of mast cells to release some inflammatory mediators that participate in increasing the vascular permeability and may exacerbate allergic airway inflammation [22]. A link between hBDs and Th2 cell cytokines (IL-4, IL-5, and IL-13) has also been found [10]. Therefore, current evidence points to the role of hBDs in the pathophysiology of AR and AS, and an understanding of their functions and mechanisms may aid in the development of novel therapeutic strategies for both allergic diseases [9].

Although several investigations have indicated significant effects of hBDs in pathogenesis of allergies, the potential role these peptides play in the development of AR and AS has not been well described. Therefore, this study sought to understand the relationship between hBDs (hBD1, hBD2, hBD3, and hBD4) and AR and AS in Iraqi patients at the serum level. Their correlation with some characteristics of patients (age, gender, family history of respiratory allergy, disease duration, symptoms, and allergen type) was also evaluated.

2. Materials and methods

2.1 Populations studied

During December 2019 – February 2020, a case-control study was conducted on 52 AR and 60 AS patients and 61 healthy controls (HC). The patients were recruited from two Allergy Specialized Centers in Baghdad (Al-Karkh and Al-Rusafa administrative districts). The guidelines of Allergic Rhinitis and their impacts on Asthma (ARIA) and the Global Initiative for Asthma (GINA) were followed in the diagnosis of AR and AS, respectively [23], [24]. The patients included are those who followed these guidelines, while patients with other respiratory diseases or pregnant women were excluded. Baseline characteristics of patients included age, gender, family history of respiratory allergy (sibs, parents, and grandparents), disease duration, symptoms (cough, dyspnea, and rhinorrhea), eosinophil percentage, total serum IgE level, allergen type, and atopy. The control sample included healthy children attending health care units (for ages < 16 years) and blood donors (for ages > 16 years), who had no signs or symptoms of allergy. The Ethics Committee at Al-Karkh and Al-Rusafa Health Departments (Iraqi Ministry of Health and Environment) approved the protocol of the study.

2.2 Determination of total and specific IgE and hBDs

Five milliliters of venous blood were collected from each participant and dispensed in a plain tube. After clotting at room temperature (20-25 °C), the blood was centrifuged for 10 minutes (3000 rpm at 4 °C) and serum obtained was frozen at -20 °C until laboratory assessments.

Total IgE level was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Euroimmun, Germany). A multiplex immunoblot kit (Euroimmun, Germany) was used to profile the sera for specific IgE against 25 inhalation allergens. Due to the low sample size of AR and AS patients, the analysis was limited to four types of allergens (animal dander, grasses, mites, and molds). Serum levels of hBD1, hBD2, hBD3, and hBD4 were assessed using ELISA kits (MyBioSource, Inc., USA). In all cases, the instructions of the manufacturer were followed.

2.3 Statistical analysis

Categorical variables were given as number and percentage frequency, and significant differences between frequencies were assessed using Pearson Chi-square or Fisher exact test. Continuous variables were first tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed variables (parametric) were given as mean and standard deviation (SD) and significant differences between means were assessed using the least significant difference (LSD) test. Non-parametric variables were given as a median and interquartile range (IQR: 25-75%), and significant differences between medians were assessed using Mann-Whitney *U* (to compare two groups) or Kruskal–Wallis (to compare more than two groups) test. Logistic regression analysis (adjusted for age and gender) was used to calculate the odds ratio (OR) and 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was performed to estimate the area under the curve (AUC), cut-off value, sensitivity, and specificity of the tested variable. The correlation coefficient between variables was estimated using Spearman's rank test. A probability (p) value ≤ 0.05 was considered significant. The statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was employed to perform these analyses.

3. Results and Discussion

The mean age was higher in AR patients compared to AS patients or HC, but these differences were not significant (32.4 ± 17.6 *vs*. 27.5 ± 18.2 and 27.1 ± 14.0 years; $p > 0.05$). The participants were divided into three age groups (< 16, 16-40, and > 40 years). The distribution of these age groups showed no significant differences concerning AR patients compared to AS patients or HC, but there were significant differences between AS patients and HC ($p = 0.039$). Females outnumbered males in AR and AS patients and HC, but there were no significant differences between the three groups ($p > 0.05$). Both groups of patients (AR and AS) had a family history of respiratory allergy, but the frequency was higher in AS patients compared to AR patients; however, the difference did not attend a significant level (45.0 *vs*. 38.5%; p = 0.701). Three symptoms were examined in AR and AS patients (cough, dyspnea, and rhinorrhea). Coughing was more frequently observed in both groups of patients with approximated frequencies (84.6 and 85.5%, respectively), but the frequency of dyspnea was significantly higher in AS patients compared to AR patients (80.0 vs. 34.6%; $p < 0.001$). Rhinorrhea was a feature of AR and occurred in 57.7% of patients. Peripheral blood eosinophils accounted for 3.04 ± 2.05 and 4.00 ± 3.56% of total leukocytes in AR and AS patients, respectively, and the difference was not significant ($p = 0.089$). Elevated serum level of total IgE was a significant characteristic of AR and AS patients compared to HC (230.9 ± 48.1 and 229.2 ± 54.7 *vs*. 169.9 ± 16.4 IU/mL, respectively; $p < 0.001$). Of the four types of allergen (animal dander, grasses, mites, and molds), mites were the most common allergen in AR and AS patients (25.0 and 33.3%, respectively), followed by mixed allergens (i.e. seropositive for more than one allergen: 17.3 and 18.3%, respectively). Seronegative patients represented 30.8 and 28.3% in AR and AS, respectively. These patients were considered nonatopic, whereas the patients with seropositive findings for the allergen tested were considered atopic (Table 1).

Table 1-Baseline characteristics of allergic rhinitis and asthma patients and healthy controls.

Characteristic†		**AR N = 52**	**AS N = 60**	**HC N = 61**	***p*-value**		
					AR *vs.* HC	**AS *vs.* HC**	**AR *vs.* AS**
Mean age; year		32.4 ± 17.6	27.5 ± 18.2	27.1 ± 14.0	0.095	0.866	0.126
Age group; year	< 16	16 (30.8)	24 (40.0)	17 (27.9)	0.097	**0.039**	0.596
	16-40	16 (30.8)	16 (26.7)	30 (49.2)			
	> 40	20 (38.4)	20 (33.3)	14 (22.9)			
Gender	Male	20 (38.5)	27 (45.0)	25 (41.0)	0.848	0.715	0.566
	Female	32 (61.5)	33 (55.0)	36 (59.0)			
Family history	Yes	20 (38.5)	26 (43.3)	NA	NA	NA	0.701
	No	32 (61.5)	34 (56.7)	NA	NA	NA	
Disease duration	< 5	38 (73.1)	35(58.3)	NA	NA	NA	0.229
	5-10	8 (15.4)	12 (20.0)				
	> 10	6 (11.5)	13 (21.7)				
Symptoms	Cough	44 (84.6)	51 (85.0)	NA	NA	NA	1.0
	Dyspnea	18 (34.6)	48 (80.0)	NA	NA	NA	< **0.001**
	Rhinorrhea	30 (57.7)	NA	NA	NA	NA	NA
Eosinophils; %		3.04 ± 2.05	4.00 ± 3.56	NA	NA	NA	0.089
Total IgE; IU/mL		230.9 ± 48.1	229.2 ± 54.7	169.9 ± 16.4	< **0.001**	< **0.001**	0.924
Allergen type	Negative	16 (30.8)	17 (28.3)	NA	NA	NA	0.807
	Animal dander	3 (5.8)	4 (6.7)				
	Grasses	5 (9.6)	5 (8.3)				
	Mites	13 (25.0)	20 (33.3)				
	Molds	6 (11.5)	3 (5.0)				
	Mixed	9 (17.3)	11 (18.3)				
Atopy	Atopic	36 (69.2)	43 (71.7)	NA	NA	NA	0.837
	Nonatopic	16 (30.8)	17 (28.3)				

†Values are given as mean ± standard deviation (SD) or number followed by percentage in parentheses.

AR: Allergic rhinitis; AS: Asthma; HC: Healthy controls; NA: Not applicable; *p*: Least significant difference (LSD), Pearson Chi-square, or two-tailed Fisher exact probability (significant *p*-value is indicated in bold).

Median levels of hBD1, hBD2 and hBD3 were significantly elevated in serum of AR (955.0 [IQR: 683.3-1409.3] pg/mL, 463.2 [IQR: 353.2-602.2] pg/mL and 0.98 [IQR: 0.71-1.25] ng/mL, respectively) and AS (849.7 [729.6-1009.4] pg/mL, 417.9 [286.7-575.0] pg/mL and 1.05 [0.69-1.91] ng/mL, respectively) patients compared to HC (770.9 [566.6-886.1] pg/mL, 247.9 [139.3-407.5] pg/mL and 0.38 [0.21-0.59] ng/mL, respectively; $p < 0.01$). The level of hBD4 was also elevated in AR (4.64 [IQR: 2.62-7.61] pg/mL) and AS (4.02 [3.08-5.53] pg/mL) patients compared to controls (3.49 [2.80-4.82] pg/mL), but the differences were not significant. The levels of hBDs showed no significant differences between AR and AS patients (Figure 1).

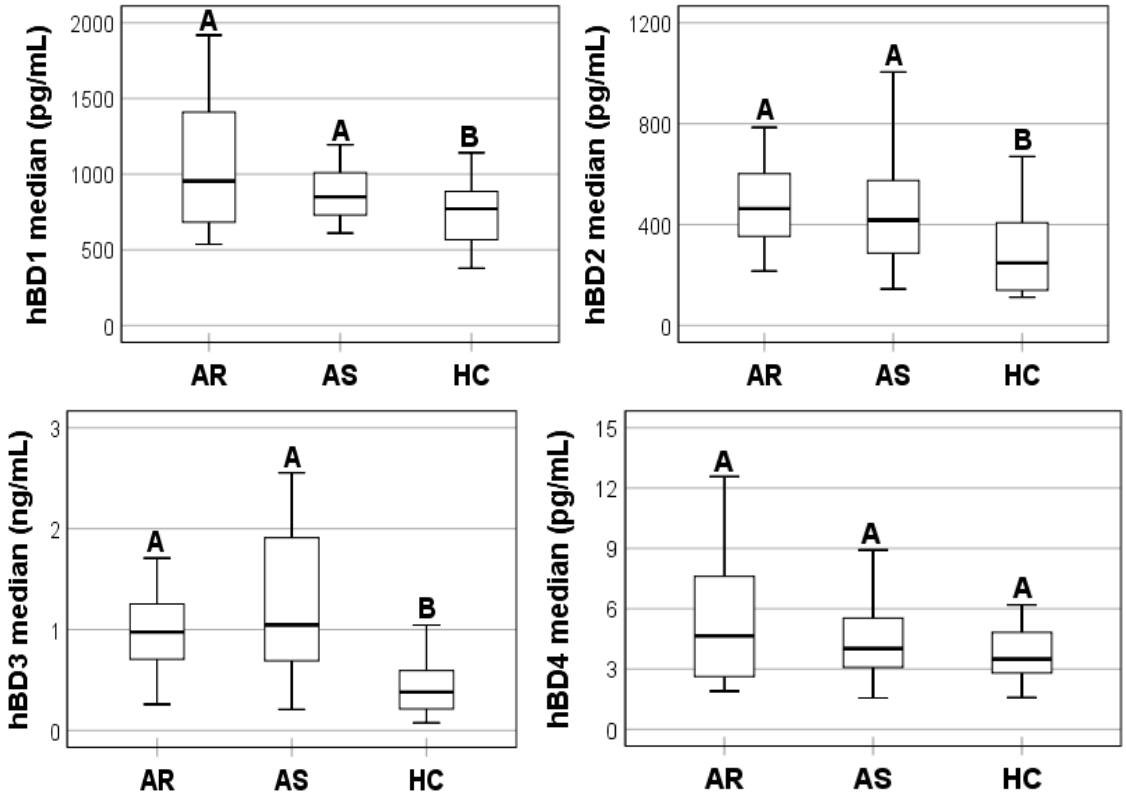

Figure 1- Median levels of human beta-defensins (hBD1, hBD2, hBD3, and hBD4) in serum of allergic rhinitis (AR) and asthma (AS) patients and healthy controls (HC). Boxes represent the interquartile range (IQR) between the first and third quartiles and the line inside the box represents the median. Whiskers indicate the lowest and highest values from the first and third quartiles. Similar uppercase letters over whiskers indicate no significant difference between medians, while different uppercase letters indicate a significant difference (Mann–Whitney *U* test).

Logistic regression analysis indicated that hBD3 is the most important serum marker associated with the risk of AR and AS. The age and gender-adjusted ORs were 25.31 (95% CI: 2.97-215.78; $p = 0.012$) and 32.20 (2.49-415.89; $p = 0.032$), respectively (Table 2).

Table 2-Logistic regression analysis of human beta-defensins in allergic rhinitis and asthma patients.

Group†	hBD	OR	95% CI	*p*-value
AR	hBD1	1.01	1.00-1.01	**0.008**
	hBD2	1.00	0.99-1.00	0.599
	hBD3	25.31	2.97-215.78	**0.012**
	hBD4	1.33	1.04-1.71	0.1
AS	hBD1	1.01	1.00-1.01	0.12
	hBD2	1.00	0.99-1.01	0.78
	hBD3	32.20	2.49-415.89	**0.032**
	hBD4	0.76	0.50-1.14	0.74

†The reference category is healthy controls. AR: Allergic rhinitis; AS: Asthma; hBD: Human beta-defensin; OR: Odds ratio; CI: Confidence interval; *p*: Logistic regression analysis probability adjusted for age and gender (significant *p*-value is indicated in bold).

ROC curve analysis confirmed the significance of hBD3 in the evolution of AR and AS. The hBD3 occupied a very good AUC in AR and AS (0.83 and 0.84, respectively). Lower AUCs were recorded for hBD1 and hBD2, and their range was between 0.65 (hBD1 in AS) and 0.78

(hBD2 in AR). For hBD4, a much lower AUC was found in AR and AS (0.61 and 0.55, respectively) (Table 3 and Figure 2).

Table 3-Receiver operating characteristic curve analysis of human beta-defensins in allergic rhinitis and asthma patients.

Group	hBD	AUC	95% CI	*p*-value	Cut-off value	Sensitivity; %	Specificity; %
AR	hBD1	0.71	0.62-0.81	< **0.001**	852.3 pg/mL	61.5	62.3
	hBD2	0.78	0.69-0.86	< **0.001**	341.6 pg/mL	76.9	72.1
	hBD3	0.83	0.75-0.90	< **0.001**	0.65 ng/mL	76.9	75.4
	hBD4	0.61	0.50-0.72	0.20	4.44 pg/mL	53.8	54.1
AS	hBD1	0.65	0.55-0.75	**0.02**	806.2 pg/mL	60.0	59.0
	hBD2	0.71	0.62-0.80	< **0.001**	329.0 pg/mL	66.7	68.9
	hBD3	0.84	0.77-0.91	< **0.001**	0.64 ng/mL	76.4	75.4
	hBD4	0.55	0.44-0.66	0.378	3.56 pg/mL	52.7	50.8

AR: Allergic rhinitis; AS: Asthma; hBD: Human beta-defensin; AUC: Area under the curve; CI: Confidence interval; *p*: Probability (significant *p*-value is indicated in bold).

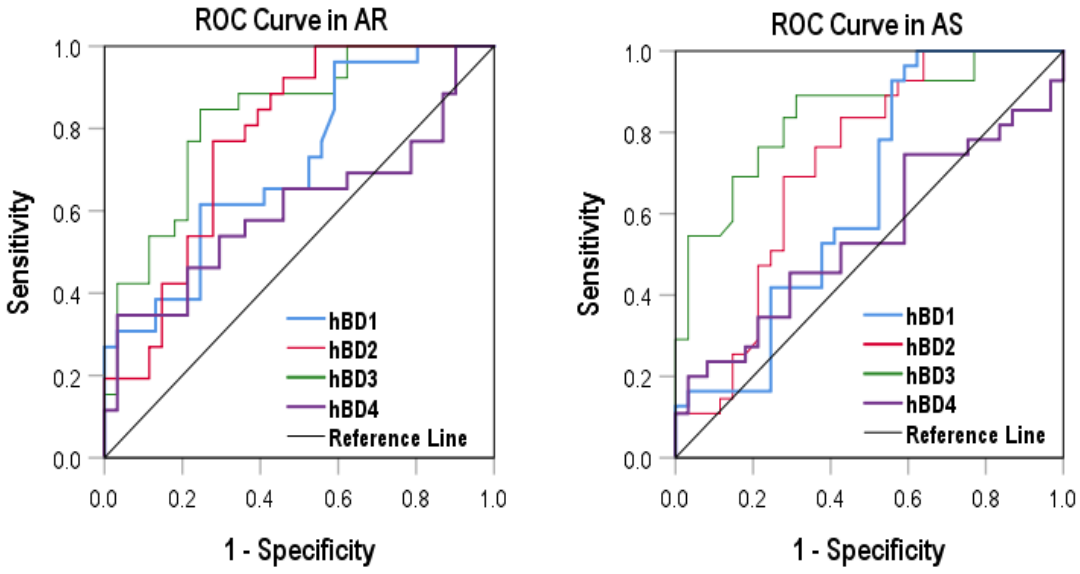

Figure 2-ROC curve analysis of hBD1, hBD2, hBD3, and hBD4 in allergic rhinitis (AR) and asthma (AS) (data of the figure are given in Table 3).

In AR, the levels of the four hBDs showed variations between age groups of patients but the differences were not significant. However, gender may be effective in determining hBD1and hBD4 levels, which were significantly elevated in females compared to males (p = 0.05 and 0.02, respectively). AR patients with a family history of respiratory allergy had a significantly higher level of hBD4 compared to patients without a family history (p = 0.04). The level of hBD1 was influenced by disease duration, and AR patients with < 5 years duration showed a significantly higher level of hBD1 compared to patients with 5-10 and > 10 years (p = 0.005). The hBD1 was also associated with AR symptoms, and significantly elevated levels were found in patients with coughing, dyspnea, and rhinorrhea compared to patients without these symptoms (p = 0.005, < 0.001 and < 0.001, respectively). Dyspnea was also associated with elevated levels of hBD2 (p = 0.05). The type of allergen had no significant influence on hBD

levels, and a similar observation was made when patients were classified to atopic and nonatopic AR (Table 4).

Table 4: Median serum levels of human beta-defensins stratified according to characteristics of allergic rhinitis patients.

Characteristic	Median (IQR: 25-75%)			
	hBD1; pg/mL	**hBD2; pg/mL**	**hBD3; ng/mL**	**hBD4; pg/mL**
Age group; year				
< 16	1010.9 (808.4-1502.6)	408.2 (284.4-509.7)	0.81 (0.44-1.04)	2.52 (2.31-5.21)
16-40	1076.9 (870.1-1456.9)	429.6 (370.7-660.5)	0.91 (0.61-1.19)	5.88 (2.69-8.83)
> 40	784.1 (648.2-993.6)	561.1 (318.8-693.7)	1.11 (0.78-1.54)	4.85 (4.35-7.61)
p-value	0.095	0.29	0.13	0.19
Gender				
Male	808.4 (665.8-1087.1)	467.0 (355.2-556.1)	0.99 (0.73-1.09)	2.62 (2.41-5.02)
Female	1003.7 (784.1-1512.8)	384.7 (336.0-651.1)	0.81 (0.66-1.49)	5.03 (4.04-7.79)
p-value	**0.05**	0.763	0.451	**0.02**
Family history				
Yes	984.5 (766.3-1823.5)	370.7 (353.2-564.4)	0.90 (0.62-1.44)	7.35 (4.35-7.79)
No	934.8 (682.7-1248.2)	467.0 (287.5-651.1)	0.99 (0.72-1.11)	3.34 (2.41-5.04)
p-value	0.292	0.88	0.547	**0.04**
Disease duration; year				
< 5	1040.4 (801.8-1471.6)	425.8 (318.8-564.4)	0.83 (0.62-1.09)	3.74 (2.41-7.79)
5-10	664.4 (591.9-1013.7)	368.2 (318.3-535.9)	0.94 (0.76-1.12)	4.83 (3.40-6.34)
> 10	665.8 (648.2-913.0)	602.2 (557.7-785.2)	1.25 (0.96-1.71)	5.02 (4.35-7.61)
p-value	**0.005**	0.22	0.505	0.691
Cough				
Present	1003.7 (764.8-1442.1)	463.2 (353.2-564.4)	0.98 (0.62-1.12)	6.32 (5.01-8.30)
Absent	707.3 (647.4-784.1)	570.2 (337.0-2042.3)	0.99 (0.72-1.35)	4.04 (2.41-7.10)
p-value	**0.005**	0.276	0.7	0.12
Dyspnea				
Present	1409.3 (1087.1-1823.5)	556.1 (388.3-602.2)	0.99 (0.77-1.09)	4.69 (2.41-7.62)
Absent	801.8 (682.1-975.3)	353.2 (259.0-381.2)	0.73 (0.62-1.54)	4.59 (2.62-6.70)
p-value	**< 0.001**	**0.05**	0.729	0.969
Rhinorrhea				
Present	1442.1 (1013.7-1823.5)	463.2 (360.2-602.2)	0.99 (0.73-1.26)	5.01 (2.62-7.62)

Absent	766.3 (682.1-934.8)	353.2 (281.3-608.5)	0.78 (0.33-1.12)	4.59 (2.41-7.10)
p-value	**< 0.001**	0.74	0.405	0.683
Allergen type				
Negative	1040.4 (857.4-1456.9)	556.1 (336.0-703.1)	1.02 (0.74-1.49)	4.04 (2.62-6.06)
Animal dander	1013.7 (764.8-1918.1)	608.5 (353.2-712.5)	0.77 (0.62-1.12)	7.79 (5.06-10.56)
Grasses	801.8 (683.3-934.8)	556.1 (388.3-564.4)	0.78 (0.73-0.83)	4.59 (3.74-6.70)
Mites	913.0 (682.1-1013.7)	360.2 (353.2-463.2)	0.73 (0.49-1.12)	5.02 (2.41-7.62)
Molds	1045.7 (665.8-1471.6)	354.2 (293.7-463.2)	0.85 (0.62-1.09)	5.65 (2.39-7.62)
Mixed	1087.1 (682.1-1442.1)	470.8 (381.2-602.2)	1.06 (0.96-1.26)	2.98 (2.41-4.69)
p-value	0.442	0.505	0.575	0.339
Atopy				
Atopic	934.8 (682.1-1113.6)	425.8 (353.2-583.3)	0.90 (0.66-1.11)	4.85 (2.52-7.61)
Nonatopic	1040.4 (857.4-1456.9)	556.1 (336.0-703.1)	1.02 (0.74-1.49)	4.04 (2.62-6.06)
p-value	0.71	0.361	0.25	0.606

IQR: Interquartile range; hBD: Human beta-defensin; *p*: Mann-Whitney *U* (to compare two groups) or Kruskal-Wallis (to compare more than two groups) test probability (significant *p*-value is indicated in bold).

In AS, hBD3 showed significantly elevated levels as a function of age (i.e. the lowest level in the age group < 16 years and the highest in the age group > 40 years) ($p = 0.05$). Gender subgroups (males and females) did not show significant differences in the levels of hBDs. A positive family history of respiratory allergy was significantly associated with elevated levels of hBD1 ($p < 0.001$). Disease duration significantly affected hBD2, which showed the lowest level in AS patients with disease duration > 10 years ($p = 0.005$). With regard to symptoms of AS, coughing was significantly associated with elevated levels of hBD3 ($p = 0.005$), while elevated levels of hBD2 and hBD3 were observed in patients with dyspnea ($p = 0.03$ and 0.005, respectively). Levels of hBDs showed some differences between AS patients stratified according to allergen type or atopy, but the differences were not significant (Table 5).

Table 5-Median serum levels of human beta-defensins stratified according to characteristics of asthma patients.

Characteristic	**Median (IQR: 25-75%)**			
	hBD1; pg/mL	**hBD2; pg/mL**	**hBD3; ng/mL**	**hBD4; pg/mL**
Age group; year				
< 16	993.2 (785.9-1105.8)	404.5 (158.3-462.8)	0.69 (0.53-1.05)	3.17 (2.80-3.67)
16-40	833.5 (684.8-990.6)	358.3 (307.6-664.5)	0.85 (0.51-1.91)	4.95 (3.20-5.64)
> 40	795.7 (729.6-1001.2)	433.4 (367.1-592.9)	1.30 (1.05-1.94)	4.45 (3.99-8.91)
p-value	0.935	0.575	**0.05**	0.095
Gender				
Male	741.6 (719.8-849.8)	457.2 (158.3-575.0)	1.16 (0.69-1.96)	3.23 (3.08-4.46)

Female	946.5 (831.0-1081.1)	367.1 (286.7-516.7)	1.05 (0.69-1.40)	4.44 (2.77-7.07)
p-value (*pc*)	**0.016** (0.08)	0.982 (1.0)	0.563 (1.0)	0.214 (1.0)
Family history				
Yes	995.9 (849.8-1130.5)	457.2 (351.6-575.0)	1.22 (0.85-1.91)	4.05 (3.17-4.81)
No	729.6 (684.8-741.6)	404.8 (206.8-462.8)	1.05 (0.51-2.02)	3.23 (3.08-6.03)
p-value	< **0.001**	0.269	0.532	0.531
Disease duration; year				
< 5	852.0 (684.8-1017.6)	404.8 (215.5-462.8)	0.89 (0.58-1.40)	3.17 (2.77-7.07)
5-10	849.8 (831.0-1130.5)	584.0 (575.0-1005.3)	1.91 (1.05-1.91)	3.60 (3.23-4.46)
> 10	741.6 (719.8-849.6)	286.7 (274.3-435.8)	1.05 (0.85-1.31)	4.11 (3.99-5.09)
p-value	0.224	**0.005**	0.528	0.769
Cough				
Present	849.8 (849.8-1001.2)	431.1 (286.7-575.0)	2.55 (1.28-2.55)	3.99 (2.77-5.82)
Absent	836.0 (729.6-1039.8)	311.0 (144.8-462.8)	0.89 (0.58-1.40)	4.11 (3.17-4.11)
p-value	0.975	0.69	**0.005**	0.671
Dyspnea				
Present	849.8 (733.6-1039.8)	446.5 (331.3-584.0)	1.28 (0.85-1.94)	4.08 (3.17-6.03)
Absent	760.4 (684.8-852.0)	267.6 (158.3-365.0)	0.63 (0.21-0.89)	2.61 (1.66-5.09
p-value	0.13	**0.03**	**0.005**	**0.026**
Allergen type				
Negative	741.6 (684.8-849.8)	457.2 (367.1-575.0)	1.05 (0.85-1.94)	3.99 (3.08-5.09)
Animal dander	1001.2 (995.9-1001.2)	462.8 (433.8-462.8)	2.55 (1.80-2.55)	3.01 (3.17-3.61)
Grasses	1193.5 (1177.0-1193.5)	215.5 (215.5-431.1)	0.69 (0.51-1.40)	4.81 (4.44-7.07)
Mites	840.3 (684.8-1049.4)	340.1 (211.1-457.2)	0.85 (0.35-1.22)	3.96 (2.05-5.17)
Molds	719.8 (610.5-929.6)	351.6 (311.0-351.9)	1.22 (0.53-1.28)	6.03 (2.52-8.91)
Mixed	849.8 (741.6-1017.6)	462.8 (274.3-627.2)	1.31 (0.77-1.91)	4.11 (3.23-6.03)
p-value	0.065	0.325	0.135	0.59
Atopy				
Atopic	852.0 (729.6-1039.8)	351.9 (215.5-462.8)	1.05 (0.53-1.40)	4.05 (3.08-5.82)
Nonatopic	741.6 (684.8-849.8)	457.2 (367.1-575.0)	1.05 (0.85-1.94)	3.99 (3.08-5.09)
p-value	0.325	0.445	0.228	0.876

IQR: Interquartile range; hBD: Human beta-defensin; *p*: Mann-Whitney *U* (to compare two groups) or Kruskal-Wallis (to compare more than two groups) test probability (significant *p*-value is indicated in bold).

In AR, Spearman's rank correlation analysis revealed that hBD2 was positively correlated with hBD3 and hBD4 (correlation coefficient = 0.448 [$p < 0.01$] and 0.330 [$p < 0.05$], respectively). In AS, hBD2 was positively correlated with hBD3 (correlation coefficient =

0.627; $p < 0.01$). Finally, hBD2 was positively correlated with hBD3 and hBD4 in HC (correlation coefficient = 0.532 [$p < 0.01$] and 0.281 [$p < 0.05$], respectively) (Table 6).

Table 6: Spearman's rank correlation coefficients between hBD1, hBD2, hBD3, and hBD4 in allergic rhinitis and asthma patients and healthy controls.

Group	hBD	hBD1	hBD2	hBD3	hBD4
AR	hBD1	1.000	-0.156	-0.037	-0.063
	hBD2		1.000	**0.448****	**0.330***
	hBD3			1.000	-0.185
	hBD4				1.000
AS	hBD1	1.000	0.232	0.134	0.098
	hBD2		1.000	**0.627****	0.068
	hBD3			1.000	0.217
	hBD4				1.000
HC	hBD1	1.000	-0.014	0.052	0.183
	hBD2		1.000	**0.532****	**0.281***
	hBD3			1.000	0.135
	hBD4				1.000

AR: Allergic rhinitis; AS: Asthma; HC: Healthy controls; hBD: Human beta-defensin; *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed); Significant correlation is indicated in bold.

Three types of hBDs (hBD1, hBD2, and hBD3) showed up-regulated levels in serum of AR and AS patients. This may indicate that both types of respiratory allergy share common pathophysiologic pathways involving the hBDs. Although most investigations into the function of hBDs have focused on their antimicrobial effects, other biological potentials have been identified, more specifically the immunomodulatory functions of these peptides in inflammatory conditions [12], [17]. AR and AS are both chronic inflammatory diseases of the respiratory airways; therefore, it is reasonable to propose a role for hBDs in the pathogenesis of the two allergic diseases. However, there is no direct evidence supporting or refuting our results. Further, current literature has not shown well-detailed information regarding the role of hBDs in the pathogenesis of AR and AS, and this study was probably the first that simultaneously investigated the four types of hBDs. As early as 2003, Claeys and colleagues measured the immunohistochemical expression of hBD2 and hBD3 in surgical tissues obtained from patients with the tonsillar disease, hypertrophic adenoids, and sinonasal disease. Quantification of hBD2 and hBD3 mRNA revealed ten-fold higher expression in biopsies of tonsillar disease compared to adenoids, while negligible expression was found in nasal tissues [25]. In a subsequent study, the expression of hBD1, hBD2, and hBD3 in tonsillar tissues obtained from AR patients were examined, and contrasting results were presented. The expression was reduced in AR patients compared to HC. It was also found that stimulation of airway epithelial cells with IL-4, IL-5, and histamine was associated with down-regulation of hBDs, and this effect was not observed in cultured tonsils or lymphocytes [11]. Choi et al. investigated the effect of AR on hBD2 expression in tonsils. Immunofluorescent staining showed HBD2 expression in the surface epithelial tissues; however, the levels of hBD2 mRNA and protein were significantly lower in the tonsils of AR patients compared to the non-AR group or adenoids [26]. A determination of hBD2 levels in nasal secretions collected from patients with perennial AR (PAR) and recurrent sinusitis (RS), PAR alone, or HC showed no significant differences between the three groups [27]. It was also found that patients with seasonal AR and who achieved three years of allergen-specific immunotherapy showed elevated levels of hBD1 and hBD2 in nasal fluid compared to pre-treatment, whereas hBD3 levels were not affected by the treatment [28]. In AS, it has been

found that hBD1 was significantly elevated in sputum of patients with severe AS compared to patients with controlled or uncontrolled AS [29]. Further, variants of the *DEFB1* gene (the gene encoding hBD1) have been associated with susceptibility to AS in Caucasian and Chinese patients [30], [31]. Additionally, Wang et al. demonstrated elevated levels of hBD1, hBD2, and hBD3 in plasma of AS patients compared to HC [32].

Of the four hBDs investigated, logistic regression analysis revealed that hBD3 was the most significant peptide associated with susceptibility to AR and AS, with ORs of 25.31 and 32.12, respectively. ROC curve analysis confirmed the significance of hBD3 in both types of respiratory allergy, with estimated AUCs of 0.83 and 0.84, respectively. Both analyses indicated the pathophysiological impact of hBD3 on AR and AS. In line with our findings, hBD3 plasma level was significantly increased in AS patients, and MBD-14 expression (the mouse orthologue for hBD3) was also elevated in mice with induced AS. It has also been found that IL-8 synthesis was induced in human airway smooth muscle cells by hBD3. These cells were found to express CCR6 and blocking this receptor significantly decreased the enhanced synthesis of IL-8. These findings may provide clear evidence of hBD3 involvement in the promotion of airway inflammation and remodeling of airway smooth muscles in AS patients [32].

Serum levels of hBDs were also influenced by some characteristics of AR and AS. For instance, hBD3 tended to have elevated levels in patients older than 40 years. It was also markedly associated with coughing and dyspnea in AS patients. The small number of patients may limit these findings; therefore, future studies have to consider these characteristics in evaluating the role of hBDs in the immunopathogenesis of AR and AS.

Conclusions

The role of hBDs in the pathogenesis of AR and AS was indicated. In this context, hBD3 showed up-regulated levels and was associated with an increased risk of developing AR and AS. These variations may also be influenced by age, gender, symptoms, and disease duration. However, the study was limited by a low sample size of patients and controls, and larger samples may contribute to a better understanding of the role of these serum markers in the pathogenesis of AR and AS.

Acknowledgments

The authors appreciate the cooperation of the medical staff at the Allergy Specialized Centers in Baghdad (Al-Karkh and Al-Rusafa administrative districts).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that there were no conflicts of interest.

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