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Original Article

Relationship Between Human β-Defensin 2 and the Vaginal Environment

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SUMMARY: As one of the main antimicrobial peptides, human β-defensin 2 (HBD2) plays multiple roles in the lower genital tract. Based on the Nugent score as a diagnostic criterion for bacterial vaginosis, we sought to clarify the correlations among the Nugent score and interleukin-6 (IL-6) and HBD2 levels in vaginal secretions in association with various types of infection. Ninety-eight women were recruited for this study. Levels of HBD2 and IL-6 in vaginal wash were measured by enzyme-linked immunosorbent assays. According to the Nugent method, the number of *Lactobacillus* morphotypes per field of view was well correlated with the HBD2 level. The amount of HBD2 was also well correlated with the presence of *Candida* spp. ($P < 0.01$). In vitro experiments revealed that the expression of HBD2 from the human vaginal epithelial cell line, VK2/E6E7, was induced by the addition of heat-killed *C. albicans* (HKCA). The addition of HKCA induced expression of Dectin-1 mRNA. A luciferase assay for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) responsive elements showed that HKCA activated NF-κB signaling. These results suggested that *C. albicans* induced the activation of Dectin-1 and (NF-κB) signaling, resulting in HBD2 expression. In conclusion, the expression of HBD2 positively correlated with the presence of *Lactobacillus* and *Candida* spp.

INTRODUCTION

Commensal bacteria in the vagina represent one of the first lines of defense against vaginal infection. Some *Lactobacillus* species, in particular, play important roles in this defense (1,2). The bacterial population in the vaginal flora is altered prior to the presentation of symptoms of vaginosis. Therefore, the Nugent score was developed for the diagnosis of bacterial vaginosis (BV) (3). However, some reports indicate that the Nugent score does not always reflect the symptoms or degree of infection (4–7), as the vaginal environment is maintained by lactic acid-producing bacteria, even in the absence of *Lactobacillus* species in most healthy women. In addition, dominant *Lactobacillus* species seem to differ across race and cultural divides (8). Therefore, the diagnosis of BV based on the Nugent score alone remains problematic and is likely to lead to unnecessary medical treatment and disturbance of vaginal flora.

Innate immunity is important for the protection of the lower genital tract against bacterial, fungal and viral infections. Antimicrobial peptides play multiple roles in innate immune mechanisms, including chemotaxis, induction of cytokines and wound healing (9). Antimicrobial peptides are composed of less than 100 amino acids and have several positively charged residues with an amphipathic structure (9). Although the precise mechanism underlying their antimicrobial action remains to be elucidated, antimicrobial peptides are predicted to make a pore in the target membrane through which they exhibit their antimicrobial activity (10).

Humans and other mammals possess two main families of antimicrobial peptides; defensins and cathelicidins (11). Defensins are divided into three main subfamilies; the α-, β- and θ-defensins, which differ in peptide segment length between cysteine residues connected by disulfide bonds (11). Human β-defensins (HBDs) consist of four species; HBD1, HBD2, HBD3, and HBD4. Among them, HBD1–3 are expressed in vaginal epithelium. HBD1 is constitutively expressed in epithelial cells, whereas HBD2 and 3 are induced by inflammation and the infection of various microbes (12–14). These signals were shown to activate transcription factors, NF-κB and AP-1, thereby inducing the expression of HBD2 and HBD3 in in vitro experiments (15,16). However, vaginal HBD2 levels were lower in women with BV than without BV; HBD2 is involved in the innate immunity of the vagina through its antimicrobial activity against *Escherichia*

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coli, *Pseudomonas aureginosa*, and *Candida albicans* (17,18). Alternatively, β-defensin levels increased in vaginal fluid of an animal Candidiasis model (19). In addition, HBD1 and HBD2 show chemotactic activity for T cells and immature dendritic cells through a chemokine receptor, CCR6 (20). In this study, we examined the correlation between the Nugent score and HBD2 levels in association with various infections as a biochemical marker for evaluation of the vaginal environment.

MATERIALS AND METHODS

Participants and sample collection: Ninety-eight non-pregnant women attending Ehime University Hospital or NTT West Matsuyama Hospital were recruited for this study from July through October, 2012. The background characteristics are summarized in Table 1. Exclusion criteria included malignant diseases, autoimmune diseases, inflammatory bowel diseases, and antibiotics or steroid hormone use. Informed consent in writing was obtained from all participants prior to enrolment in the study. The study was approved by the Ethics Committee of Ehime University Hospital (Permission No: 1206212). Vaginal secretions were collected using cotton swabs for the calculation of the Nugent score. The vagina was subsequently washed with 4 mL of saline, which was collected for bacterial analysis. A portion of vaginal wash was centrifuged at 3,000 rpm for 10 min at 4ºC and the supernatant was stored at –80ºC until required for IL-6 and HBD2 assays. All experimental methods were performed in accordance with the approved guidelines.

Terminal restriction fragment length polymorphism (t-RFLP) and microbial analyses by polymerase chain reaction (PCR): DNA was extracted from the vaginal wash using a QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. The DNA was then amplified by PCR using the following primer set: LYS (5′-FAM-AGAGTTTGATCCTGGCTCAG-3′; the 5′-end was labeled with FAM) and LYSrev [5′-CCGTCAATTC(A/C)TTT(A/G)AGTTT-3′] (Applied Biosystems, Waltham, MA,USA). PCR reactions were carried out using the standard protocol at a volume of 25 μL containing 0.4 mM of each dNTP (TaKaRa Bio, Shiga, Japan), 2.0 mM of $MgCl_2$, 0.15 μM of each primer and 0.5 U KOD FX Polymerase (Toyobo, Osaka, Japan). The reaction profile was as follows: after initial denaturation at 94ºC for 2 min, DNA was amplified with 35 cycles of 98°C for 10 s, 60ºC for 30 s, and 72ºC for 70 s, and subsequently extended at 72ºC for 7 min. PCR products were digested with the *Msp I* restriction enzyme (TaKaRa Bio) and 1 μL of digested product was combined with 9 μL of Hi-Di formamide (Applied Biosystems) and 1 μL of GeneScan 120 LIS Size standard (Applied Biosystems). The samples were analyzed with an ABI PRISM 3100 Genetic Analyzer using GeneScan analysis software version 3.7 (Applied Biosystems). The t-RFLP score was determined by the number of peaks as follows: low = less than 3 peaks, intermediate = 3 to 5 peaks, and high = 6 peaks or more. Gram-stained vaginal fluid smears were examined under an oil immersion objective lens at 1,000 × magnification. In the Nugent scoring method, the presence of large Gram-positive rods (*Lactobacillus* morphotype), small Gram-variable rods (*Gardnerella* morphotype), and curved Gram-negative or -variable rods (*Mobiluncus* morphotype) were quantified. The composite score was categorized into one of the three groups. Scores of 0 to 3 were considered normal (*Lactobacillus* dominant), 4 to 6 were labeled as intermediate (mixed morphotype), and 7 to 10 were BV (absence of *Lactobacilli* and predominance of *Gardnerella* and *Mobiluncus* morphotype). We determined the presence of *Candida* spp. in vaginal flora by microbial cultivation and microscopic analysis.

Table 1. Demographic and clinical information at enrollment

Characteristic	*n* = 98
Age (yr), mean ± SD (min-max)	39 ± 10 (20–65)
20–29	20 (20%)
30–39	34 (35%)
40–49	33 (34%)
50–59	8 (8%)
60–	3 (3%)
Gravidity, mean (min-max)	1.2 (0–4)
Primigravida	41 (42%)
Multigravida	57 (58%)
Parity, mean (min-max)	1.0 (0–3)
Primipara	46 (47%)
Multipara	52 (53%)
Pre-menopausal	90 (91%)
Post-menopausal	8 (8%)
Smoker	19 (19%)
Nugent score	
normal (0 – 3)	59 (60.2%)
intermediate (4 – 6)	22 (22.4%)
BV (7 – 10)	17 (17.3%)

The mean age of them was 39 ± 10 yr and was mean of 1.2 gravida 1.0 multipara. BV, bacterial vaginosis.

Furthermore, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Chlamydia trachomatis* were analyzed using PCR. Oligonucleotide primers for the PCR reactions were designed as shown in Table 2 and the reactions were carried out using EmeraldAmp PCR Master Mix (TaKaRa Bio) with a Thermal Cycler (Chromo4 System, Bio-Rad Laboratories, Hercules, CA, USA). The reaction profile was as follows: initial denaturation at 94°C for 2 min, 35 cycles of 98°C for 10 s, 54°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 7 min.

HBD2 and IL-6 ELISA: The HBD2 and IL-6 levels in the vaginal wash were measured by enzyme-linked immunosorbent assays (ELISA; HBD2 ELISA

Table 2. Primers used in this study

Primer	Sequence (5′→3′)
Ureaplasma ulealyticum U-5F	CAATCTGCTCGTGAAGTATTAC
Ureaplasma ulealyticum U-4R	ACGACGTCCATAAGCAACT
Mycoplasma genitalium MgPaW1F	AAGTGGAGCGATCATTACTAAC
Mycoplasma genitalium MgPaR1R	CCGTTGTTATCATACCTTCTGA
Mycoplasma hominis F-P	GACACTAGCAAACTAGAGTTAG
Mycoplasma hominis R	CACCATCTGTCACTCTGTTAACCTC
1st PCR *Chlamydia trachomatis*	TTGCGA TCCTTGCACCACTT
Chlamydia trachomatis	GCTCGAGACCATTTAACTCC
Nested PCR *Chlamydia trachomatis*	GGTGACTTTGTTTTCGACCG
Chlamydia trachomatis	CTCCAATGTAGGGAGTGAAC
Dectin-1 forward	TGGGAGGATGGATCAACATT
Dectin-1 reverse	TGGGTTTTCTTGGGTAGCT
Dectin-2 forward	TTCAGTGAAGGGACAAAGGTG
Dectin-2 reverse	AAGTAGCAACTGGAACCAAATGA
Mincle forward	CTGGGATCCCCATCCTATTT
Mincle reverse	TTTGAAAGATGCGAAATGTCA
Mannose Receptor forward	CACCATCGAGGAATTGGACT
Mannose Receptor reverse	ACAATTCGTCATTTGGCTCA

kit: Phoenix Pharmaceuticals, Inc., Mannheim, Germany; IL-6 Quantikine ELISA kit: R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturers' instructions. Light absorbance was measured at 450 nm on a microplate reader (iMark; Bio-Rad Laboratories). All assays were performed in triplicate.

Cell culture: A human vaginal epithelial cell line (VK2/E6E7; CRL-2616, American Type Culture Collection, Manassas, VA, USA) was cultivated in keratinocyte serum-free medium (KSFM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 50 μg/mL of bovine pituitary extract, 0.1 ng/mL of recombinant epidermal growth factor, 0.4 mM $CaCl_2$ and antibiotics including penicillin, streptomycin and amphotericin (Sigma-Aldrich, St. Louis, MO, USA). The VK2/E6E7 cells were seeded in a 24-well plate at 1×10^5 cells per well and the culture incubated with a final concentration of 10 μg/mL lipopolysaccharides (LPS) (Sigma-Aldrich) or a multiplicity of infection (MOI) of 10 cfu per cell of a heat-killed preparation of C. albicans (HKCA; Thermo Fisher Scientific; final concentration) in KSFM for 24 h at 37ºC. The supernatant was retrieved and centrifuged at 600 rpm for 5 min. The aliquot was stored at –80ºC until measurement of HBD2.

Determination of gene-expressions by reverse transcription PCR (RT-PCR): After 24 h of stimulation by HKCA, the expression of mRNAs from Dectin-1, Dectin-2, Mincle and Mannose receptor genes in VK2/E6E7 cells were analyzed by RT-PCR. Total RNA was extracted from cells by TRIzol reagent (Thermo Fisher Scientific) and RNeasy Mini Kit (Qiagen), and reverse transcribed into complementary DNA (cDNA) using an iScript cDNA synthesis kit (Bio-Rad Laboratories) in accordance with the manufacturer's instructions. Oligonucleotide primers were designed as shown in Table 2. RT-PCR was performed with iQSYBR Green Supermix (Roche Diagnostics, Basel, Switzerland) using a Chromo4 Thermal Cycler (Bio-Rad Laboratories). PCR consisted of an initial denaturation step of 2 min at 95°C, followed by 40 cycles of 5 s at 95°C and 10 s at 60°C, and a final extension of 15 min at 72°C. Semi-quantitative analysis was performed by correction against Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The mRNA of Toll-like receptors (TLRs) were detected by TLR RT-Primer set (InvivoGen, San Diego, CA, USA).

Luciferase assay: The VK2/E6E7 cells were transfected with a reporter plasmid (pGL4.32) containing NF-κB responsive elements at the luciferase promoter region using the Gene Pulser II electroporation system (Bio-Rad Laboratories). Briefly, 40 μg of the plasmid was added to a trypsinized cell suspension (1×10^7 cells) in a 0.4 mm cuvette. After electroporation (260 V, 975 μF, ∞ Ω), Dulbecco's Modified Eagle Medium containing 20% fetal calf serum without antibiotics was added to the cuvette, and the suspension was transferred to 35 mm dishes. After culturing for 24 h, culture medium was changed to KSFM containing 1×10^8 cells/mL of HKCA. After culturing for a further 24 h, cells were harvested and analyzed with a luciferase detection kit (TOYOBO). The assay was repeated in triplicate.

Table 3. Concentrations of HBD2 and IL-6 in the vaginal fluid from women with and without BV[1)]

	n	Age(yr)	HBD2 (ng/mL)	IL-6 (pg/mL)
Nugent score				
normal (0–3)	59	38 ± 8	28.8 [9.7-87.2]	30.5 [5.5–81.4]
intermediate (4–6)	22	41 ± 11	39.6 [16.7-79.8]	20.0 [9.0–84.8]
BV (7–10)	17	36 ± 12	32.9 [6.2-56.8]	23.1 [7.3–47.9]
Lactobacillus morphotype point				
0–2 (≥ 1 /oil immersion field)	66	38 ± 8	39.2[14.9–97.4][2)]	31.6 [6.1–99.3]
3–4 (< 1/ oil immersion field)	32	40 ± 12	19.0 [5.1–48.1][2)]	18.8 [4.6–63.9]

[1)]: Data were expressed as the median [inter-quartile range].
[2)]: The *Lactobacillus* morphotype point was well correlated with HBD2 ($P < 0.01$).
HBD2, human beta defensin 2; IL-6, interleukin 6; BV, bacterial vaginosis.

Immunofluorescence assay: The VK2/E6E7 cells were grown on coverslips. Cells were fixed with 4% paraformaldehyde, permeabilized with or without 0.05% Triton X-100, and subjected to an immunofluorescence assay using 4′, 6-diamidino-2-phenylindole (DAPI), anti-Dectin1 rat mAb (2A11; Abcam, Cambridge, England) and anti-Rat IgG-Alexa 488 antibody (Thermofisher Scientific). Samples were analyzed with Carl Zeiss LSM 700 confocal microscope system (Carl Zeiss, Oberkochen, Germany).

Statistical analysis: All statistical analyses were performed using EZR software (21) (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Data are expressed as the average [range] or mean ± SD. Other experiments on human specimens were analyzed by Mann-Whitney U test. RT-PCR results were analyzed using the Mann-Whitney U test. Differences were considered significant at $P < 0.05$.

RESULTS

Correlations among Nugent Score and t-RFLP, HBD2 and IL-6 levels: Vaginal swab specimens were analyzed by Gram-staining according to the Nugent criteria. The prevalence of normal, intermediate, and BV was 60.2%, 22.4%, and 17.3%, respectively (Table 1). The t-RFLP profiles were classified into three groups based on the number of bands. According to our analysis, t-RFLP profiles highly correlated with the Nugent score with a *kappa value* of 0.76. There was no statistically correlation between the Nugent Score and HBD2. However, the Nugent score for *Lactobacillus* morphotype point was well correlated with HBD2. The higher *Lactobacillus* morphotype group was 0–2 points and the lower group was 3–4 points (Table 3). IL-6, a marker of inflammation, was not correlated with the Nugent Score or the *Lactobacillus* morphotype point.

In addition, we determined the relationships between the presence of *Candida* spp., *M. hominis*, *M. genitalium*, *U. urealyticum*, and *C. trachomatis* in the vaginal flora and the results of the ELISA analysis. As shown in Table 4, the HBD2 level was only well correlated with the presence of *Candida* spp. Vaginal candidiasis was treated with an oxiconazole nitrate vaginal tablet (600 mg). After anti-fungal treatment, the HBD2 level decreased in Candidiasis patients (Fig. 1).

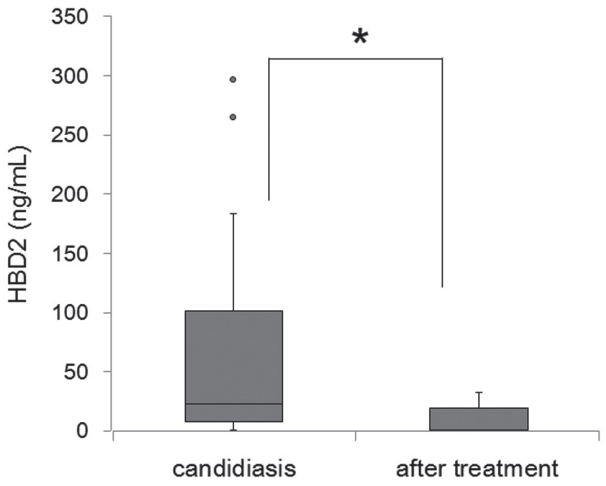

Fig. 1. Concentration of HBD2 before and after treatment for vaginal candidiasis. After anti-fungal treatment, the HBD2 level was significantly decreased in Candidiasis patients. Data were expressed as the median [inter-quartile range].
* $P < 0.05$.

The induction of HBD2 via NF-κB signaling in vaginal epithelial cells: HBD2 is known to be produced by epithelial cells. We evaluated the production of HBD2 from the vaginal epithelial cell line VK2/E6E7 cells. After stimulation by HKCA or LPS for 24 h, the supernatant of the VK2/E6E7 cell culture was analyzed using ELISA. As shown in Fig. 2A, the production of HBD2 was stimulated by HKCA and not by LPS. TLR4 mRNA was not detected in VK2/E6E7 cells (data not shown). Since β-glucan from *C. albicans* was previously reported to be recognized by the Dectin-1 receptor (22,23), we analyzed the expression of Dectin-1 mRNA in comparison with Dectin-2, Mincle, and Mannose receptor mRNAs in VK2/E6E7 cells exposed to HKCA. Results show that only Dectin-1 mRNA expression was enhanced by HKCA (Fig. 2B). The protein expression of Dectin-1 in VK2/E6E7 cells was confirmed using the immunofluorescence assay (Fig. 2C). In addition, the luciferase assay demonstrated the induction of NF-κB signaling activity in the presence of 10^8 cells/mL of HKCA (Fig. 2D).

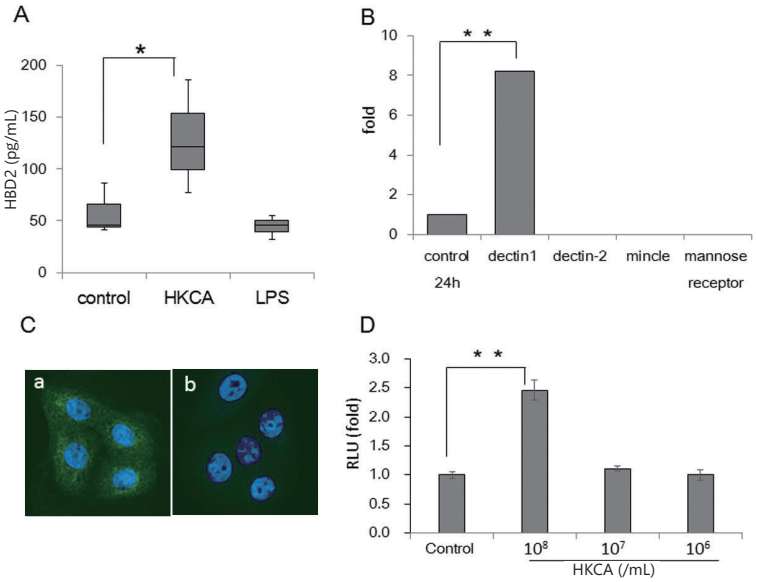

Fig. 2. (Color online: C) Production of HBD2 through Dectin-1. A: Production of HBD2 by VK2/E6E7 cells was measured by ELISA. HBD2 level was significantly higher after stimulation by HKCA but not by LPS. Data were expressed as the median [inter-quartile range]. B: After 24 h from stimulation by HKCA, the expression of receptor mRNAs in VK2/E6E7 were analyzed by RT-PCR. HKCA induced the expression of Dectin-1 but not the Dectin-2, Mincle or Mannose receptor. Data are expressed as the mean ± SD. C: The expression of Dectin-1 was analyzed with immunofluorescent assay. After treated with (a) or without (b) 0.05% Triton-X100, VK2/E6E7 cells were stained with anti-Dectin1 antibody (Green) and DAPI (Blue) as described in Materials and Methods. D: Luciferase assay of VK2/E6E7 cells transfected with pGL4.32 containing NF-kB responsive elements. It demonstrated the induction of NF-kB signaling activity in the presence of 10^8 cells/ml of HKCA. Data are expressed in relative luminescence units (RLU) as the mean ± SD. * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

In the present study, we compared the Nugent score and HBD2 level in vaginal fluid. Although the HBD2 level did not correlate with Nugent score or IL-6, it correlated with the presence of *Lactobacillus* (*Lactobacillus* morphotype point). *Lactobacillus* species in general and *L. crispatus* in particular, which is known as a "good bacteria," were previously reported to induce the expression of HBD2 in cultured cells (24). The high level of HBD2 induced by *L. crispatus* is thought to prevent the growth of some Gram-negative bacteria and the pathogenesis of BV (9). Also, it has been reported that HBD2 production is stimulated in the vaginal epithelium cells by the presence of *Lactobacillus jensenii* (25). Nugent score is not only based on the presence or absence of *Lactobacillus*, but also dependent on the presence of bacteria such as *Mobiluncus* and *Gardnerella*. According to our analysis, Nugent score was highly correlated with the t-RFLP score. Therefore, it is clear that Nugent score reflects actual bacteria. We showed that HBD2 reflected an increase of *Lactobacillus* than actual bacteria.

Although IL-6 is a marker of inflammation, our results indicated that there were no statistically association between IL-6 and clinical criteria of BV, as shown in Table 3. The relationship between BV and IL-6 has been reported in many studies with varying results. Mitchell et al. reported a relationship between BV and decreased HBD2 expression but no relation to IL-6 (26). Alternatively, in *C. trachomatis* infections, IL-6 is reportedly increased with BV patients but not with *Lactobacillus* dominant flora (27). Our results suggested that IL-6 might not be strongly involved in the pathogenesis of BV and Candidiasis in some vaginal environments (Tables 3 and 4).

We also showed that vaginal HBD2 level was increased in Candidiasis but not in other microbes. HBD2 would be increased by the overgrowth of *Candida* spp., because the HBD2 level of patients was reduced by the anti-fungal treatment (Fig. 1). Most Candidiasis patients presented normal Nugent score and higher *Lactobacillus* morphotype point, since *Candida* are able to cohabitate with *Lactobacillus*. In this study, 69% of *Candida* patients had normal Nugent scores and normal amounts of *Lactobacillus* morphotype point. It is reported that *Lactobacillus* could inhibit the overgrowth of *C. albicans* by producing a wide variety of secondary metabolites with antimicrobial activity such as lactic acid, hydrogen peroxide, bacteriocin-like compounds, and biosurfactants (28). In our study, HBD2 was increased in vaginal fluid of Candidiasis patients who had high amounts of *Lactobacillus* morphotypes. The cohabitation of *Candida* and *Lactobacillus* could induce the expression of HBD2.

We showed that HBD2 increases in the presence of *Candida* spp. and responds to *Candida* sterilization by anti-fungal treatment (Fig. 1). Therefore, examination carried out in vitro showed production of HBD2 was significantly increased by the HKCA stimulation in the vaginal epithelial cell line VK2/E6E7 cells (Fig. 2A) ($P < 0.05$). Fungal species are recognized by immune system via pattern recognition receptors such as the TLR family and C-type lectin receptors (CLRs) (29). The CLR signaling pathway is critical for immune responses to fungal species (30). Previous studies showed that CLRs, mainly Dectin-1 and Dectin-2 play important

Table 4. Concentrations of HBD2 and IL-6 in the vaginal fluid from women with and without microbes[1)]

Group		*n*	HBD2 (ng/mL)	IL-6 (pg/mL)
Candida spp.	(+)	13	92.7 [48.4–215.8][2)]	52.3 [16.1–79.9]
	(–)	85	28.9 [9.5–61.7][2)]	23.1 [3.5–81.7]
Mycoplasma hominis	(+)	7	37.1 [8.2–108.4]	31.7 [14.6–52.2]
	(–)	91	30.7 [9.7–77.6]	23.3 [5.6–81.7]
Mycoplasma genitalium	(+)	4	28.3 [8.3–48.6]	36.8 [11.1–76.0]
	(–)	94	31.8 [10.1–82.9]	25.0 [5.0–81.6]
Ureaplasma ulealyticum	(+)	40	35.0 [8.7–101.4]	30.3 [12.0–81.3]
	(–)	58	30.0 [10.1–61.3]	15.4 [3.4–85.6]
Chlamydia trachomatis	(+)	5	23.7 [16.0–32.9]	25.5 [23.3–29.1]
	(–)	93	33.1 [9.5–81.8]	24.5[4.6–81.7]

1): Data were expressed as the median [inter-quartile range].
2): The HBD2 levels were only correlated with the presence of *Candida* spp. ($P < 0.05$).
HBD2, human beta defensin 2; IL-6, interleukin 6; PCR, polymerase chain reaction; t-RFLP, terminal restriction fragment length polymorphism.

roles in the host immune response to *C. albicans* (31,32). Some studies indicated Dectin-1 and Dectin-2 are expressed on other epithelial cell lines, pulmonary epithelial cells (33), corneal epithelial cells (34), and intestinal epithelial cells (35). We demonstrated the expression of Dectin-1 in VK2/E6E7 cells and the activation of NF-κB signaling by the addition of HKCA (Fig. 2). Although other receptors might be involved, Dectin-1 is the candidate receptor for this signaling, because Dectin-1 is able to recognize β-1,3-glucan which is derived from the cell walls of several fungi such as *Candida*, *Aspergillus* and many other fungal species (36). The production of HBD2 is stimulated by microbial materials through NF-κB signaling activation (16,37). Therefore, activation of NF-κB through Dectin-1 would lead to induced expression of HBD2.

In conclusion, HBD2 is an important factor for the immune systems defense against microbial infection. This study also showed that HBD2 expression was positively correlated with the presence of *Lactobacillus* and *Candida* spp.

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Conflict of Interest None to declare.

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