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Research Article

Role of Human β-Defensin-3 in Rheumatoid Arthritis: An Observational Single-Center Study

Noor Ulhuda Ghazi Mohammed[1]*, Faiq Isho Gorial[2], Fayhaa Magdad Khaleel[1] Baydaa Ahmed Abed[3] Samara Ali Mutar[1], Layla Othman Farhan[1] Isam Noori Salman[3]

[1] *Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq;* [2] *Rheumatology Unit, Department of Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq;* [3] *National Diabetes Center, Mustansiriyah University, Baghdad, Iraq*

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Abstract

Background: It's believed that HBD-3 is involved in the tissue remodeling process of articular cartilage. Also, HBD-3 has anti-inflammatory properties. ***Objectives***: The purpose of this study is to assay human beta-defensine-3 (HBD-3) in serum from rheumatoid arthritis (RA) patients and investigate its correlation with proinflammatory cytokines. ***Methods***: In this case-control study, fifty-eight RA patients were aged 20–65 years, and 29 age-matched healthy subjects (HS) had no inflammatory rheumatic diseases. The disease activity score-28 joint erythrocyte sedimentation rate (DAS28-ESR) was used to measure RA activity. CRP, ACPA, HBD-3, TNF-α, and IL-1β were assessed using the enzyme-linked immunosorbent assay technique (ELISA). ***Results***: There was a significant increase in RF, ACPA, CRP, proinflammatory cytokines, and HBD-3 in the RA group compared with the HS group. There was no significant difference in HBD-3 levels according to the activity of diseases. The results of the correlation between HBD-3 and proinflammatory cytokines showed a significant positive relationship in the RA group. ***Conclusions***: Inflammatory markers and S.HBD-3 demonstrated fair diagnostic performance to differentiate RA from HS. The current study supports the hypothesis that there is a correlation between HBD-3 and the immunoregulatory response.

Keywords: Beta-human defensin-3, DAS28, Defensins, Pro-inflammatory cytokines, Rheumatoid arthritis.

دور β-Defensin-3 البشري في التهاب المفاصل الرثوي: دراسة في مركز واحد قائمة على الملاحظة

الخلاصة

الخلفية: يعتقد أن HBD-3 يشارك في عملية إعادة تشكيل أنسجة الغضروف المفصلي. أيضا، HBD-3 له خصائص مضادة للالتهابات. **الأهداف**: الغرض من هذه الدراسة هو فحص بيتا ديفنسين 3 البشري في مصل الدم لدى مرضى التهاب المفاصل الروثوي والتحقيق في علاقته بالسيتوكينات المسببة للالتهابات. **الطرق**: اجريت الدراسة على ثمانية وخمسون مريضا من التهاب المفاصل الرثوي تتراوح أعمارهم بين 20 و 65 عاما، ومقارنتهم مع 29 شخصا صحيا مطابقا للعمر وبدون أمراض روماتيزمية التهابية. تم استخدام درجة نشاط المرض-28 ومعدل ترسيب كرات الدم الحمراء المشتركة لقياس نشاط التهاب المفاصل الرثوي. تم تقييم CRP و ACPA و HBD-3 و TNF-α و IL-1β باستخدام تقنية مقايسة الممتز المناعي المرتبط بالإنزيم. **النتائج**: كانت هناك زيادة معنوية في RF و ACPA و CRP والسيتوكينات المسببة للالتهابات و HBD-3 في مجموعة RA مقارنة بمجموعة الاصحاء. لم يكن هناك فرق كبير في مستويات HBD-3 وفقا لنشاط الأمراض. أظهرت نتائج الارتباط بين HBD-3 والسيتوكينات المسببة للالتهابات وجود علاقة إيجابية معنوية في مجموعة المرضى. **الاستنتاجات**: أظهرت العلامات الالتهابية و S.HBD-3 أداء تشخيصيا عادلا للتمييز بين مرضى التهاب المفاصل الرثوي والأصحاء. تدعم الدراسة الحالية الفرضية القائلة بوجود علاقة بين HBD-3 والاستجابة المناعية.

* ***Corresponding author***: Noor Ulhuda G. Mohammed, Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq; Email: nour.ghazi1005a@csw.uobaghdad.edu.iq

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INTRODUCTION

Defensins are a subfamily of antimicrobial peptides (AMPs) that are tiny cysteine-rich cationic proteins found in immune system cells and have a molecular weight of 3.5–4.5 kDa [1,2]. The discovery of HBDs in mucosa has led to the realization that they play an important function in innate immune defense, protecting mucosal surfaces from microbial attacks [3]. The three HBDs, HBD1, 2, and 3, are cationic, β-sheeted peptides that are predominantly expressed by epithelial cells and range in length from 33 to 47 amino acid residues [4]. HBD-3 is the most positively charged of the three defensins (+11), followed by HBD-2 (+6) and HBD-1 (+4) [5,6]. HBD-1 and HBD-2 are monomers in solution, but HBD-3 is a dimer. The three HBDs have anti-microbial action across a broad spectrum, with HBD-3 typically being the most effective. Cells in the skin, salivary gland, and bone marrow release the antibacterial and immunomodulatory protein known as HBD-3. HBD-3 was first discovered in human psoriatic scales, but it has since been discovered in tonsils, trachea, cervix, esophagus, and colon [3,7]. Furthermore, HBDs have also been linked to autoimmune disorders like RA. HBD-3, an antimicrobial peptide, has been found in the synovial membrane of RA patients. Increased levels of α-defensins (HNP) in SF and serum from RA patients were linked to joint destruction, according to an interesting review [8]. Also, HBD-3 has been shown to play a role in the remodeling of articular cartilage tissue by increasing the production of MMP and decreasing the levels of tissue inhibitors of metalloproteinases 1 and 2. Mononuclear and polymorphonuclear cells mostly penetrate synovial tissue in RA. When these cells are activated, proinflammatory cytokines are released, which lead to joint inflammation. The synthesis of MMP from chondrocytes is also a factor in the articular cartilage degeneration seen in RA patients [8–11]. Some HBDs are found in large amounts in the SF of RA patients with joint damage. This suggests that they may make more molecules that break down bone and cartilage during the disease [1]. An interesting study indicates that HBD-3 might play a role in RA by promoting the secretion of proinflammatory cytokines and MMP. In this respect, it's worth noting that HBDs are produced by a diversity of cell types, each of which can influence different phenomena [1,12]. Furthermore, HBD effects may vary when proinflammatory triggers like cytokines and LPS are present. As a result, it would be interesting to research the role of HBD in the progression of inflammatory autoimmune diseases, taking into consideration their potential antagonistic and synergistic interactions with other agents, particularly proinflammatory cytokines [13,14]. In another study, researchers looked into the role of human-defensin-3-C15 (part of HBD-3) in preventing bone resorption by reducing osteoclast activity [15]. The authors initially looked at how HBD-3 affected the receptor activator of RANKL. RANKL has been found to have an impact on the immune system as well as bone remodeling and regeneration. The researchers discovered that, as expected, RANKL increases the number of multinucleated osteoclast-like cells. Nevertheless, HBD-3 inhibited the RANKL-induced increase in tartrate-resistant acid phosphate (TRAP+) multinucleated cell formation. Furthermore, HBD-3 also prevented the creation of the RANKL-induced podosome belt, which is a characteristic of mature osteoclasts with bone-resorbing potential [15–17]. It is interesting to note that osteoblasts in healthy bones can produce the protein HBD-3. In a previous investigation, HBD-3 expression was measured in samples of healthy and osteomyelitis-infected human bones. Bacteria were found to promote osteoblastic HBD-3 synthesis quickly and effectively in this study [18–20]. Taken all together, the purpose of this study is to determine the HBD-3 levels in the serum of RA patients and to investigate their relationship with routinely evaluated RA biomarkers and proinflammatory biomarkers.

METHODS

In this case-control study, fifty-eight RA patients aged 25–65 were consecutively chosen based on ACR/EULAR criteria 2010 [21] from the outpatient rheumatology clinic of Baghdad Teaching Hospital in Iraq between November 2022 and May 2023. An arthritis specialist performed blood tests (ESR, RF, and ACPA) and the DAS28 evaluation of RA activity on each participant. All patients were on medication. As DAS28 of recruited RA patients was from remission to severe, subjects were divided into 3 groups; "since the number of remissions and low disease activity is very low, they were added together and considered as one group". A twenty-nine healthy control group aged (25–65 years) was apparently well with no rheumatic diseases. All subjects in this study underwent routine biochemical blood analysis. The National Centre for Training and Human Development of the Iraqi Ministry of Health, the Medical City Committee of Ethics, and the University of Baghdad Ethical Committee all gave their approval for this study. All subjects gave informed written consent before being enrolled in the study. Samples of venous blood were taken from each participant. The CRP, ACPA, TNF-α, IL-1β, and HBD-3 were determined by the enzyme-linked immune-sorbent assay (ELISA) technique.

Statistical analysis

It was done with SPSS version 25, MedClac (19.7.4 soft wear), and graphic illustration under GraphPad Prism (version 9). Quantitative variables were shown as mean±standard deviation, or median (IQR: 25th–75th percentile), while categorical variables were shown as frequency and percentage. The Shapiro-Wilk test was used to look into the normal distribution of the variables. It was decided to use Spearman's rank coefficient. The cut-off value for the serum HBD-3 level was assessed using the receiver operating characteristic (ROC) curve method. Additionally, the

specificity, sensitivity, and negative and positive predictive values were calculated. Significant results were those with a *p*-value less than 0.05.

RESULTS

Table 1 shows the demographic characteristics and clinical features of the fifty-eight patients with RA and the twenty-nine with HS. The mean age of the RA group was 44.52±10.73 years, with the comparable age of the HS group (44.61±4.74 years) showing no significant difference ($p>0.05$). Regarding gender, the number of females in the RA group was 53 (91.4%) compared to 16 (55.21%) in the HS group. A percentage of females was significantly higher in RA compared to HS ($p<0.05$) (the female-to-male ratio in RA is 10.6:1 and 1.2:1 in HS).

Table 1: The demographic characteristics of patients and healthy subjects (HS) group

Parameters	RA (*n*=58)	HS (*n*=29)	*p*
Age (year)	44.52±10.73	44.61±4.74	0.958
Male	5(8.6)	13(44.79)	0.0001
Female	53(91.4)	16(55.21)	
Body mass index (kg/m^2)	28.69±3.76	29.2±3.7	0.636
Duration of disease	6.89±6.21	-	-
DAS-28	4.15±1.10	-	-
CDAI	16.3±7.5	-	-
ESR (mm/hour)	20.49(13-38)	9.10 (4.0-15.7)	0.0001
RF (+ve)	55(94.9)	-	0.0001
RF (-ve)	3(5.10)	29(100)	
C-RP (µg/ml)	12.2(4.1-62.0)	5.0(2.0-8.9)	0.0059
AntiCPA (u/ml)	212.20(47.0-640.35)	24.0(14.9-31.1)	0.000

The results were presented as mean±SD, *n*(%), and Median(IQR).

At the time of blood collection, 43.1% of RA patients were receiving MTX therapy, 20.6% were receiving etanercept (ETC) therapy, and 36.2% were receiving MTX combined with ETC therapy. Table 1 also shows disease duration and other feature distributions. Serum levels of HBD-3 were significantly higher ($p<0.05$) in RA patients (0.475, 0.365–0.659 ng/ml) compared to HS (0.307 (0.215-0.50) ng/ml), as shown in Figure 1A. While there was no significant difference ($p>0.05$) in HBD-3 levels according to the activity of diseases (Figure 1B), there is no significant correlation between HBD-3 and BMI in RA patients $r=0.240$, $p=0.069$. In addition, there is no significant correlation between HBD-3 and DAS28 in RA patients $r=0.038$, $p=0.454$. While there is a highly positive correlation between HBD-3 and YKL-40, TNF-α, and IL-1β in RA, $p=0.0001$, $r=0.668$, $p=0.0001$, and $r=0.617$, $p=0.0001$, respectively. Table 2 shows the validity parameters for the studied parameters that show the specificity, sensitivity, PPV, and NPV for each marker. The optimum cut-off value was derived from the ROC curve based on the Youden index for all biomarkers in the study. HBD-3 protein was set to 0.386 ng/ml, which had a sensitivity of 82.7% and a specificity of 55.17%. The PPV and NPV were 78.7 and 61.5, respectively, as shown in Figure 2.

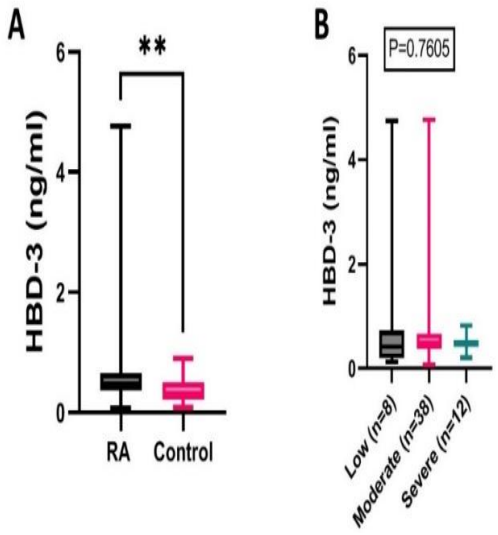

Figure 1: HBD-3 levels in RA patients and HS groups (A). HBD-3 levels in RA patients based on DAS28 score (B).

Concerning proinflammatory biomarkers, the IL-1β showed the cut-off value, which had sensitivity of 72.4% and specificity of 96.5%, and the TNF-α showed the cut-off value, which had sensitivity of 75.8% and 100% specificity.

Table 2: Validity parameters of test variables to differentiate RA patients from HS

Variables	Cut-off value	SN	SP	Accuracy (Youden index)	+PV	-PV
HBD-3 (ng/ml) Optimum cut off value	0.386	82.7	55.17	0.379	78.7	61.5
TNF-α (ng/ml) Optimum cut off value	95.31	75.86	100.00	0.7586	100	67.4
IL-1B (pg/ml) Optimum cut off value	1567.169	72.41	96.55	0.6897	97.7	63.6

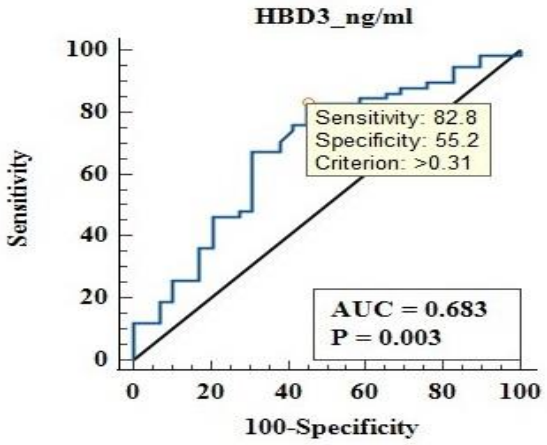

Figure 2: The ROC curve of HBD-3.

DISCUSSION

In the current study, the HBD-3 levels in RA patients were significantly higher than those in HS. This result is in agreement with Santos *et al.*, who reported in an *in vitro* study that HBD-3 plays a role in RA by inducing the production of proinflammatory cytokines and MMP by chondrocytes, degrading the extracellular matrix of cartilage [1]. The promotion of the secretion of proinflammatory cytokines by HBD could play a role in the cytokine imbalance that leads to synovium destruction in RA [8]. Also, another study shows that the presence of proinflammatory cytokines in the diseased joints may likely explain the increased expressions of HBD-3 and other AMPs. Increased expression of HBD-3 may also contribute to recruiting immune cells and amplifying the inflammatory response in the joint [8]. HBD-3 and other AMPs are only expressed at very low levels in normal physiological conditions but are inducible by a range of stimulants, such as host inflammatory triggers such as IL-1β and TNF-α. In this regard, the mechanism by which HPD-3 is related to the existence of bone erosion may consist only of their capacity to enhance the production of IL-6 and TNF-α, thus causing the inflammatory cytokines to contribute to bone destruction and the swollen joints seen in RA [1]. In an interesting study, HBD-3 was highly expressed in all samples of inflamed synovial membrane [22]. The inducibility of HBD-3 and the fact that it is more potent than other HBDs make HBD-3 a stronger candidate for antimicrobial defense in articular joints [23]. HBD-3 has a crucial role in the pathogenesis of inflammatory autoimmune conditions. In an interesting study, HBD-3 was also observed in OA cartilage in the absence of a bacterial challenge. In vivo and in vitro studies have suggested that HBD-3 plays a role in articular cartilage extracellular matrix disruption in OA (24). In other studies, HBD-3 has been investigated for use in the diagnosis of peri-prosthetic joint infections [25]. Numerous investigations are concentrating on the therapeutic usage of AMPs as a novel class of antibiotics due to their potent antibacterial action, low Mwt, and immunogenicity [26]. HBD-3, one of the discovered human B-defensins, is particularly intriguing for structural and functional research as well as potential therapeutic uses. In general, AMPs, including HBD-3, are secreted into mucosal tissues in order to support the host's innate immunity by protecting against infection. Remarkably, mounting evidence indicates that these AMPs are frequently present in bone tissues and are generated by bone cells [15]. Most of the above-mentioned studies were in vivo investigations. The only current clinical study to investigate serum HBD-3 in RA Given that dysregulation of HBD-3 is frequently linked to disease, it makes sense that these molecules could act as biomarkers for particular diseases. This has proven to be especially helpful for diagnosing individuals who have bacterial infections at the root of their symptoms, which would normally require a lot of time. The current study identified that S.HBD-3 has a fair diagnostic performance to differentiate RA from HS, and a positive correlation between HBD-3 and proinflammatory cytokines (TNF-α and IL-1β) was detected. This is the first clinical study that may confirm the inflammatory connection of the protein. According to the results obtained in this study, HBD-3, together with IL-1β and TNF-α, could be used as additional useful biomarkers of inflammation, helpful in the diagnosis of RA. Since there have been many in vivo studies that have linked HBD-3 with cytokine production, this suggests that this HBD-3 link is related to innate and adaptive immunity. Results supported the hypothesis that there is a correlation between HBD-3 and the immunoregulatory response [27,28]. The strengths of the study are the age compatibility between the study groups and the fact that the patients take one type of treatment and do not have any other inflammation. The patients were divided into groups based on DAS28. There are also some limitations in the study, including the small size of the sample, and it is also preferable to measure the protein with SPF to compare it with the serum, as well as follow-up protein measurement for groups of newly diagnosed patients and after taking treatment for standard periods, which can give a full picture of the role of therapeutic protein for patients with RA.

Conclusion

The study proves that HBD-3 protein cannot be used as a diagnostic marker for RA, and because it has a relationship with proinflammatory cytokines, it can be used as a marker for treatment follow-up.

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Conflict of interests

No conflict of interest was declared by the authors

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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