ISSN: 0067-2904

Human β-Defensin 2 as a Novel Diagnostic Marker of Iraqi Patients with Rheumatoid Arthritis

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Received: 7/10/2022 Accepted: 16/1/2023 Published: 30/5/2023

Abstract

Rheumatoid arthritis (RA) is an autoimmune disorder of the joints that is characterized by extra-articular involvement in addition to inflammatory arthritis. Joint and periarticular tissue loss brought on by inflammation results in functional impairment. To lessen the significant daily challenges that patients confront and to ensure better outcomes, early detection and treatment are essential. The study's objective was to establish the use of human β-defensin-2 (HBD-2) as a RA diagnostic marker. A total of 60 RA patients and 30 healthy controls participated in the research. The ELISA technique was used to measure serum HBD-2. The following tests were performed: complete blood count (CBC), erythrocyte sedimentation rate (ESR), renal function test, and liver function test. In comparison to the healthy control group, the RA group exhibited a substantially higher blood HBD-2 levels ($p \leq 0.001$). Additionally, there was no significant association between serum HBD-2 and urea, creatinine, AST, ALT, and ESR ($P>0.05$). When RA was distinguished from the group of healthy individuals, the area under the curve (AUC) demonstrated excellent diagnostic accuracy (AUC = 0.990, $p = 0.001$). (0.9667). As a result, serum HBD-2 may be used as a reliable RA diagnostic marker.

Keywords: *Rheumatoid arthritis, Human β-defensin-2, Complete blood count, erythrocyte sedimentation rate.*

Human β−defensin 2 كعلامة تنبؤية جديدة للمرضى العراقيين المصابين بالتهاب المفاصل الرثوي

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الخلاصة :

التهاب المفاصل الروماتويدي (RA) هو مرض مناعي ذاتي للمفاصل يتميز بالتهاب المفاصل الالتهابي بالإضافة إلى إصابة المفاصل الإضافية. يسبب الالتهاب فقدان أنسجة المفصل وحول المفصل ، مما يؤدي إلى إعاقة وظيفية. يعد التعرف والعلاج المبكر أمرًا بالغ الأهمية لتقليل العقبات الكبيرة اليومية التي يواجهها المرضى وضمان نتائج أفضل. كان الهدف من الدراسة هو تحديد دور β−defensin−2 البشري (HBD−2)

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كعلامة تشخيصية لـRA . شملت الدراسة 30 شخصاً يتمتعون بصحة جيدة و 60 مريضاً بالتهاب المفاصل الرثوي ، وتم تقدير مصل HBD−2 بواسطة طريقةELISA . تم إجراء تعداد الدم الكامل (CBC) ، ومعدل ترسيب كرات الدم الحمراء (ESR) ، واختبار وظائف الكلى ، واختبار وظائف الكبد. كان لدى مجموعة RA تركيزات HBD−2 أعلى بكثير من مجموعة الاصحاء (p 0.001) . أيضًا ، لم تظهر اليوريا والكرياتينين و AST و ALT و ESR أي ارتباط معنوي مع HBD−2 (P> 0.05) . كان للمنطقة الواقعة تحت المنحنى (AUC) دقة تشخيصية كبيرة (AUC = 0.990) ، (p = 0.001) في تحديد RA من مجموعة المرضى الأصحاء (0.9667). لتحديد مرضى التهاب المفاصل الروماتويدي من الضوابط الصحية ، يمكن استخدام HBD−2 .

1. Introduction

Rheumatoid arthritis (RA) is a chronic condition that damages joints and impairs function, particularly in adults. It is a long-term autoimmune inflammatory condition that can induce a variety of clinical symptoms, such as mild to severe joint inflammation that can result in discomfort, dryness, and joint degeneration as well as joint deformities and disability [1- 4]. The most typical kind of chronic inflammatory arthritis, it commonly causes arthritis and physical disability. Because RA is a clinical condition, symptoms that do not only affect the joints can but affect the lungs, heart, pericardium, peripheral nerves, vasculitis, and blood [5]. In the synovial membrane of RA patients, the antimicrobial peptide human beta-defensin (HBD) has been found. An intriguing review found a link between elevated levels of α- and β-defensins in synovial fluid (SF) and serum from RA patients and joint injury. The synthesis of matrix metalloproteinases (MMP) is increased by the HDBs, while the production of tissue inhibitors of metalloproteinases (TIMP)-1 and -2 is decreased. This suggests that the HDBs are involved in the remodeling of articular cartilage tissue. The most frequent types of cells that invade synovial tissue in RA are mononuclear and polymorphonuclear cells. Joint inflammation results from the stimulation of these cells, which produces pro-inflammatory cytokines [6]. Antimicrobial peptides (AMPs)1 known as defensins are small (2-4 kDa), cationic, cysteine-rich proteins that are essential for the host's defense against infections. They combat pulmonary inflammation, urinary tract infections, GI tract infections, acne, irritable bowel syndrome, and bacterial bone infections in humans [7, 8].

Additionally, they control inflammatory responses and signaling pathways, which aids in the immune system's activation. They also contribute to carcinogenesis due to their proliferative or suppressive properties in respect to different cells. Defensins are divided into three categories (R, β, and θ) based on how far apart their cysteine residues are from one another and the structure of their disulfide linkages. Cys1-Cys5, Cys2-Cys4, and Cys3-Cys6 are the disulfide bonds that make up Defensins. The most researched peptides in the human subclass are human - defensins 1, 2, and 3. (HBD1-3). Defensins' amphipathic design, which features spatially separated clusters of hydrophobic and polar residues, is the essential principle behind the antibacterial capabilities of defensins. However, certain bacteria employ the capacity of defensins to bind compounds that are negatively charged as a form of protection [7,8]. One of these is defensin 2 (HBD-2), a cysteine-rich cationic peptide with a low molecular weight [9]. The structures of HBD-2 represent a first step toward understanding the structural basis of antibacterial and chemotactic properties of human – defensin [9-11].

In certain circumstances, they serve to keep the body in a state of homeostasis, but in others, they contribute to malfunctions in the way the body works and the emergence of various disorders. Human-defensin 2 (HBD-2) is an antimicrobial peptide that was discovered

in human skin in 1997. This protein can be released by human epithelial cells in response to interactions with microbes or certain pro-inflammatory cytokines [12]. Some studies suggest that HBDs may contribute to RA by increasing the production of MMP and pro-inflammatory cytokines [13,14]. The first thing the researchers looked into was how HBDs changed the RANKL receptor activator. RANKL has been associated with the immune system as well as bone remodeling and regeneration. The RANKL is a ligand for the RANK, an apoptosis regulator, and a binding partner for osteoprotegerin (OPG). Other research has discovered increased amounts of HBD-2 in the sera of Crohn's disease patients, which may be related to the illness's extra parental inflammations, which cause an excessive production of inflammatory mediators (TNF-, IFN-, and IL-22) [15-18]. The aim of the current study was to determine the function of HBD-2 as a RA diagnostic marker. To the best of our knowledge, no research has been published that examines the connection between RA patients and HBD-

2. Subjects and Methods:

2.1. Patients and control

Patients from Baghdad Teaching Hospital Medical City's Consulting Clinic in Baghdad, Iraq, were employed as test subjects. Ages of the participants in this research ranged from 30 to 50. Participants in the research comprise 60 RA sufferers as well as a control group of 30 healthy persons.

2.2. Exclusion Criteria

There were no smokers or alcoholics among the patients. Furthermore, no one in the patients' families had previously been diagnosed with the disease. Diabetic patients, hypertension, hyperthyroidism, or psoriasis were excluded from the study.

2.3. Blood Sample

A venous sample of ten milliliters of blood was taken from each participant. 8 ml of the gel sample and 2 ml of the EDTA sample were collected in separate tubes. The blood samples were divided into two portions; the first portion (2 mL) was collected in an EDTA tube and used the same day to measure the CBC, while the second portion (8 mL) was collected in a gel tube and allowed to clot at room temperature before being centrifuged at (3000 rpm) for five minutes to collect serum. Before usage, serum samples were stored at a temperature of -20 ºC.

2.4 Experimental methods

The ESR was manually measured using the Westergren technique [19]. The HBD-2 ELISA kit was provided by Shanghai - China to measure serum HBD-2. To assess the CBC, a hematological analyzer was employed (Siemens, USA). Urea, creatinine, AST, and ALT levels were measured automatically (ADVIA 1650; Siemens, Tarrytown, NY, USA).

2.5. Statistical analysis

Statistical analysis was accomplished with the statistical program for social sciences (SPSS 24, demo version, IBM). The person correlation test has been utilized to highlight the distinction between the variables. MedClac (19.7.4 soft wear) was used to estimate the HBD-2 cutoff value using receiver operating characteristic curve (ROC) analysis. The data were expressed as mean± standard error (SE).

3. Results

Table 1 displays the mean and standard error of the age distribution for RA patients (48.93 ± 1.503 years) and controls (43.43± 0.88 years), with $p>0.005$ for both groups. The mean and standard error of the BMI in the RA and control groups were, respectively, 30.40±0.777 kg/m2 and 30.74±0.806 kg/m2. There is no discernible difference between them ($P > 0.05$).
As shown in Table1, there was a highly significant decrease in Urea level in RA patients' group (p <0.001) when compare to healthy control. A significant increase was found in creatinine, and ALT in RA patients' group (0.79±0.037) mg/dL, (33.26±1.863) U/L when compared with control group (0.64±0.03) mg/dL, (25.00±2.069) U/L, with p <0.05. There was no significant difference indicated in AST level in RA group than control group, as presented in Table 1.

Table 1: Age, BMI, Urea, Creatinine, AST and ALT for RA patients and control groups.

Parameter (unit)	RA patients mean± SE	control mean± SE	*P-value*
Age (year)	48.93±1.503	43.43±0.88	0.06
BMI kg/m^2	30.40±0.777	30.74 ±0.806	0.963
Urea mg/dL	27.64±1.042	32.40±1.263	<0.001*
Creatinine mg/dL	0.79±0.037	0.64±0.03	<0.05*
AST U/L	23.20±1.980	23.87±1.437	0.825
ALT U/L	33.26±1.863	25.00±2.069	0.05*

BMI: body mass index, AST: Aspartate transaminase, ALT: Alanine transaminase, ESR: Erythrocyte sedimentation rate.

The complete blood count (CBC)

Table 2 displays the comparison of CBC parameters. In contrast to haemoglobin, hematocrit, mean corpuscular volume, and monocytes, which were all considerably lower in RA patients, the mean corpuscular Hb Conc count, red cell distribution width%, PCT, PDW%, and total white blood cell count were all significantly greater in these individuals. RA patients and control groups did not vary significantly in terms of red blood cell count, mean corpuscular hemoglobin, lymphocyte percentage, absolute lymphocytes, granulocyte percentage, mean platelet volume, or absolute monocytes, as indicated in Table 2.

Table 2: Complete blood counts were performed for the control and RA patient groups

Parameter (unit)	RA patients mean± SE	control mean± SE	*P-value*
Total white blood cell counts 10 ^{3}/µL	8.95±0.413	7.37±0.252	<0.05*
Red blood cell counts 10 ^{6}/µL	4.57±0.69	4.64±0.110	0.488
Haemoglobin g/dl	12.28±0.212	13.60±0.287	<0.001*
Hematocrit %	37.72±0.583	39.30±0.029	0.05*
Mean corpuscular volume %	82.55±0.577	84.70±0.657	0.05*
Mean corpuscular hemoglobin pg	27.10±0.244	27.70±0.273	0.048
Mean corpuscular Hb Conc g/dl	32.94±0.075	32.00±0.058	<0.001*
Platelet count 10 ^{3}/µL	331.23±12.735	249.00 ±15.056	<0.001*
Lymphocytes %	30.43±1.266	32.06±0.841	0.354
Absolute lymphocytes 10 ^{3}/µL	2.60±0.117	2.30±0.073	0.079
Monocytes	2.95±0.172	4.10 ±0.201	<0.001*
Absolute monocytes	0.27±0.018	0.30 ±0.006	0.204

Granulocyte%	64.37±2.113	63.80 ±0.969	0.753
Red cell distribution width %	13.96±0.152	12.20±0.234	<0.001*
PCT %	0.25±0.009	0.19±0.011	<0.001*
Mean platelet volume fL	7.91±0.082	7.8±0.038	0.364
PDW %	17.69±0.102	17.00±0.059	<0.001*

The HBD-2 and ESR levels

Table 3 illustrated the HBD-2 and ESR levels in serum RA patients and control groups

Table 3: The HBD-2 and ESR for patient with RA and control

Variable	RA patients mean± SE	control mean± SE	*P-value*
HBD-2 ng/ml	42.65±1.010	21.28±0.313	<0.001*
ESR mm/hr	44.69±3.182	13.53±1.587	<0.001*

ESR: Erythrocyte sedimentation rate

The HBD-2 and ESR levels in the RA group were highly significant increased (42.65±1.010) ng/ml, (44.69±3.182) mm/hr, when compared with the control group (21.28±0.313) ng/ml, (13.53±1.587) mm/hr, respectively as shown as in Table 3.

The correlation study between HBD-2 and other parameters

There was no significant correlation between serum HBD-2 and other parameters including urea, Creatinine, AST, ALT and ESR as shown in Table 4.

Table 4: Correlation between HBD-2 with Urea, Creatinine ,AST, ALT ,and ESR in RA group.

Parameters	r value	*p-value*
Urea	0.12	(0.926)
Creatinine	0.059	(0.654)
AST	0.071	(0.587)
ALP	0.037	(0.776)
ESR	-0.046	(0.627)

AST: Aspartate transaminase, ALT: Alanine transaminase, ESR: Erythrocyte sedimentation rate

Receiver operating characteristic (ROC) curve analysis

The capacity of serum HBD-2 levels to distinguish RA patients from healthy persons was examined using the ROC curve analysis (Table 5; Figure1). The ROC curve for RA demonstrated excellent sensitivity (96.7%) and specificity (100%), and was much higher than the disease diagnostic test. The AUC of the ROC curve, which was 0.990 ($p< 0.001$) for the existence of a RA diagnosis, was used to determine the amount of RA prediction accuracy that was optimal.

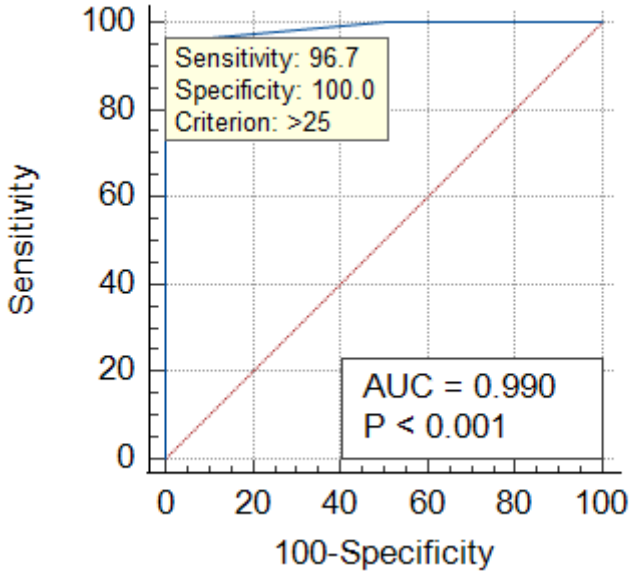

Figure 1: ROC curve analysis of serum HBD-2 concentrations in RA against healthy participants

Table 5: The HBD-2 AUC and validity in distinguishing between RA patients and healthy subjects

Variable	AUC	*P-Value*	Cut off value	Sensitivity	Specificity	Accuracy	PPV	NPV
HBD-2	0.990	0.001	25	98.7	100	0.9667	100	**93.8**

The term AUC refers to the area under the curve. The terms negative predictive value (NPV) and positive predictive value (PPV) are used interchangeably.

4. Discussion

Although they are less frequent now that medicinal therapies for the illness have advanced, renal symptoms can arise in RA. The main cause of renal insufficiency in people with RA in the past was nephrotoxicity of RA treatments, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs), such as penicillamine, bucillamine, gold, and cyclosporine [20].

A common side effect of disease-modifying anti-rheumatic drugs is abnormal liver function tests, which are often seen in patients with inflammatory arthritis (DMARDs). A patient is more likely to suffer abnormal LFTs when they have a history of underlying liver disease or regularly use alcohol (up to 22%) of methotrexate patients [20, 21].

In contrast to a prior research by Koiwa et al., who discovered that the neutrophil count in all patients declined dramatically [22,23], the parameters of CBC demonstrate a significant increase in Total White Blood Cell Count with (p 0.05) in the RA patient group. The current study examines many biomarkers, including ESR, a crucial diagnostic indicator for RA, in the assessment of RA patients. These results are consistent with earlier research by Vanichapuntu *et al.*[25], and Shen *et al* [24, 25]. ESR is an intriguing prospective marker for the diagnosis of RA even if it is too non-specific for that purpose.

HBD-2 expression is extremely low during normal physiological function, but it can be increased by a number of stimuli, including host inflammatory triggers such (IL-1, TNF). In

this regard, the mechanism connecting HPD-2 to the existence of bone erosions may be as straightforward as their capacity to promote the production of IL-6 and TNF-, inflammatory cytokines that are known to contribute to bone degradation and swollen joints seen in RA [6, 25]. It is an intriguing result that immunosuppressive drugs like dexamethasone and methotrexate do not activate osteoblast-derived HBD-2. These medications were frequently prescribed for chronic inflammatory diseases like RA, and by reducing ABD-2 expression in the bone and articular joints, they may be to blame for the patients' increased vulnerability to bone and joint infection [27, 28]. The HBDs have a broad spectrum of action against gram-positive and gram-negative bacteria, viruses, and fungi. Neutrophils are drawn to defensins and cathelicidins, which also activate T and B cells, encourage angiogenesis and wound healing. [27, 28].

In vitro, Kraus and colleagues were able to show that HBD-1, -2, and -3 were expressed in osteoblast-like MG63 cells [29 , 30]. Furthermore, they could show that HBD-2 boosts their proliferation and that HBD-2 and -3 positively affect their differentiation processes, and earlier research has shown that HBD-2 and HBD-3 are expressed by cells and microorganisms in response to pro-inflammatory cytokines [35]. The ROC analysis was described as having the following "area under the curve" (AUC): 0.90 stands for "perfect," 0.80 for "good," 0.70 for "fair," 0.60 for "poor," and 0.60 for "failure" [36]. Therefore, the separation power obtained by patients and healthy controls is perfect, with an AUC of 0.99 ($p < 0.001$). As shown in Table 5 Figure 1, this means that situations where the test result is less than (25 ng / ml) are seen to be healthy, whereas cases where the test value is more than (25 ng / ml) are thought to be abnormal.

Conclusion

In conclusion, our results demonstrate that serum HBD-2 concentrations are higher in RA patients than in control participants and that these levels are correlated with disease activity. Blood levels of HBD-2 can be a helpful biomarker in the RA diagnosis.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental, health, higher education, and scientific research ministries in Iraq.

Conflict of interest:

The authors have no conflicts of interest regarding this investigation

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