Original Paper

Profile of Human β -Defensins 1,2 and Proinflammatory Cytokines (TNF- α , IL-6) in Patients with Chronic Kidney Disease

Dong-Jin Oh^a Hye Ryou Kim^b Mi-Kyung Lee^b Yo Seop Woo^c

^aNephrology Department, Myoung-Ji Hospital; ^bLaboratory Medicine Department, Chung-Ang University College of Medicine, Seoul; ^cInformation & Telecomm. Department, University of Incheon, Incheon, Korea

Key Words

Human β defensins • Innate immunity • Pro-inflammatory cytokines • Diabetic nephropathy • Chronic kidney disease

Abstract

Background/Aim: Our aim was to determine whether altered human β -defensin (HBD), pro-inflammatory cytokines including interleukin (IL)-6 and tumor necrotic factor (TNF)- α could increase the risk of developing and exacerbation of chronic kidney disease (CKD), especially for patients with diabetic nephropathy (DN). **Methods:** Serum samples were obtained from 338 CKD patients and 88 sex, age-matched healthy controls. The concentrations of HBD-1 were assayed using an RIA kit. Serum levels of HBD-2, IL-6 and TNF- α were assayed using an ELISA kit. **Results:** Serum levels of HBD-1, IL-6 and TNF- α were significantly higher in CKD patients compared to healthy controls ($P < 0.05$). HBD-1 levels were inversely related to estimated glomerular filtration rate (eGFR), which was the coefficient factor ($\beta = -0.357$, $P = 0.035$) explaining the variability in HBD-1 in CKD. Diabetic nephropathy (DN) patients at stage 3-5 had significantly higher serum HBD-1 levels than non DN patients ($P = 0.00$). **Conclusion:** Our data support the view that there is increased inflammation in CKD and DN. The inverse correlation between eGFR and serum HBD-1 which we observed is suggestive of a relationship between innate immunity and renal function and should be further investigated.

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Introduction

The innate immunity system includes recognition, phagocytosis and digestion of pathogens, induction of inflammation and presentation of antigens. Human defensins, which

Hye Ryou Kim, MD

Department of Laboratory Medicine, Chung-Ang University College of Medicine,
224-1, Heukseok-Dong, Dongjak-Ku, Seoul 156-755 (South Korea)
Tel. +82-2-6299-2718, Fax +82-2-6298-8630, E-Mail hyekim@cau.ac.kr

are small cationic peptides produced by neutrophils and epithelial cells, are antimicrobial peptides that function in the host innate defense. In humans, β defensins are constitutively expressed in various mucosa and epithelial cells where they can be up-regulated in response to infectious and inflammatory stimuli [1, 2]. The human β -defensin (HBD)-1, which has 36 amino acids, was originally isolated from hemofiltrates of patients with advanced renal failure [3]. The HBD-1 gene is expressed mainly in the urogenital tract and, to a lesser degree, in trachea and lung [4, 5]. HBD-2 was first isolated from psoriatic scales and it is expressed in the skin as well as in urinary, gastrointestinal and respiratory epithelia [6]. Recent reports demonstrated that activated innate immunity and chronic low-grade inflammation appear to be important factors in the pathogenesis of diabetes mellitus (DM) and diabetic nephropathy (DN) [7]. This hypothesis suggests that long-term innate immune system activation, resulting in chronic inflammation, elicits disease instead of repair, leading to the development of type 2 DM [8, 9]. Long-term low-grade inflammation may play a key role in the onset of several disturbances leading to DN, including insulin resistance, hyperglycemia, oxidative stress and endothelial dysfunction, with secondary consequences playing a critical role in perpetuating renal damage and progression. There is now substantial experimental evidence and more recent findings from clinical studies suggesting that high sensitive C-reactive protein (hsCRP), as well as IL-6, are sensitive physiological markers of subclinical systemic inflammation and associated with insulin resistance, metabolic syndrome, hyperglycemia and overt Type 2 DM. Regarding DN, it has been reported that inflammatory parameters in patients with Type 2 DM at early stages of nephropathy are independently associated with clinical biomarkers of glomerular and tubulointerstitial damage [7]. On one hand, hypercytokinemia is a typical feature of uremia, likely due to accumulation of pro-inflammatory cytokines as a consequence of decreased renal elimination and/or increased generation following induction by uremic toxin, oxidative stress, volume overload, or comorbidities [10, 11]. In the current study our aim was to determine whether HBD-1, HBD-2 and pro-inflammatory markers including hsCRP, IL-6 and TNF- α could be relevant factors of the development and aggravation of renal dysfunction in patients with CKD, and especially in patients with DN.

Subjects and Methods

Sample collection

Serum samples were obtained from 338 CKD patients who visited the department of nephrology clinic between February and November, 2011. The control group consisted of 88 sex, age-matched healthy volunteers. The diagnosis of CKD has been classified into stages based principally upon estimated GFR (eGFR) using the IDMS-traceable modified MDRD (Modification of Diet in Renal Disease) and the assessment of proteinuria. The 5 stage classification of CKD based on eGFR as proposed by the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines and modified by National Institute for Health and Clinical Excellence (NICE) [12, 13]. Each stage group of 1, 2, 3, 4, and 5 comprised normal or increased eGFR (n = 10), eGFR 89-60 ml/min per 1.73 m² (n = 39), eGFR 59-30 ml/min per 1.73 m² (n = 102), eGFR 29-15 ml/min per 1.73 m² (n = 31) and eGFR <15 ml/min per 1.73 m² (n = 156) [14]. Inclusion criteria were: a stable clinical state, no intercurrent inflammatory illness, no thrombosis, absence of acute cardiovascular complication (including uncontrolled hypertension, acute coronary syndrome, acute heart failure). But there were presence of past history of coronary artery disease (n = 5), peripheral vascular disease (n = 12) and cerebrovascular disease (n = 6) in HD patients. The cause of renal failure 156 hemodialysis (HD) patients included chronic glomerulonephritis (n = 20), hypertensive nephropathy (n = 20), polycystic kidney (n = 4), diabetic nephropathy (n = 88), others or unknown (n = 24) and all of them was comprised of stage 5. In all HD patients, blood was drawn in the morning before the onset of the midweek dialysis session and all of HD patients who were receiving dialysis on AV fistula. The 88 controls without symptoms of lower genital tract infections who visited Chung-Ang University Hospital Health Center were selected. Institutional Review Board approved this study. All patients were fully informed about the study and gave their consent.

Determination of HBD-1 and HBD-2 concentration

HBD-1 concentration was determined using radioiodinated kits purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA). The incubation buffer for the RIA was 50mM sodium phosphate containing 0.25% bovine serum albumin treated with N-ethylmaleimide. The diluted sample or a standard peptide solution was incubated for 24hrs with 100 μ l of diluted antiserum. The tracer solution was added and the mixture incubated for 24hrs. Solutions of 0.5% normal rabbit serum and 0.5% anti-rabbit IgG goat serum were then added and the whole was stored for 16hrs. Bound and free ligands were separated by centrifugation at 1,700g for 20 min at 4°C. We carefully aspirated all the supernatant immediately following centrifugation, then used a γ -counter to count the cpm of the pellet then HBD-1 amount was calculated using the standard RIA curve.

HBD-2 concentration was determined using the ELISA microplate kits purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA). The protocol described by the manufacturer's instructions was applied. The absorbance was measured at 450 nm on UV microtiter plate reader.

Determination of IL-6 and TNF- α concentration

Samples were aliquotted and stored at -70°C for assay. IL-6 and TNF- α concentrations were determined using the ELISA microplate kits purchased from R&D System. (Minneapolis, MN, USA). The protocol described by the manufacturer's instructions was applied. The absorbance was measured at 490 nm on UV microtiter plate reader.

Statistical analysis

Data are expressed as mean $\pm$ SD (normally distributed data); geometric mean and range (not normally distributed data). Comparisons between two groups were made by t-test. Analysis of variance (ANOVA) or Kruskal-Wallis ANOVA were used in statistical analysis to compare differences between groups with $p < 0.05$ considered statistically significant, when appropriated. Linear regression analysis employed Pearson or Spearman coefficients as appropriate. We defined that $|r| > 0.7$ represented strong correlation, $0.7 > |r| > 0.4$ represented moderated correlation, $0.4 > |r| > 0.2$ represented weak correlation and $|r| < 0.2$ represented no correlation. Multiple regression analysis was used to determine in dependent factors affecting the dependent variable. The independent relationship between cytokines, HBD concentration and eGFR in CKD patients, were identified by forward stepwise multiple regression analysis adjusting for all potential confounders. Because their distributions were skewed, biochemical parameters were log transformed before the regression analysis. We were log tranformed BUN, Creatinine, eGFR, glucose and triglyceride. Data were expressed as a standardized regression coefficient (β) and p value. Any p value less than 0.05 was considered statistically significant. The software package SPSS 17.0 (statistical package for social sciences, Chicago, IL).

Results

The main clinical and biochemical data of the study population are presented in Table 1 and Figure 1. CKD patients had lower hemoglobin and higher serum HDL cholesterol, hsCRP and glucose when compared with control groups. Serum concentrations of HBD-1, IL-6 and TNF- α were significantly higher in CKD patients over healthy controls (table 1). Biochemical parameters studied in regard to 5 stage classification of CKD patients are based on eGFR (table 2).

Univariate analysis between serum concentrations of IL-6, TNF- α , HBD-1 and HBD-2 and renal function in CKD patients

In CKD patients, serum HBD-1 correlated significantly with eGFR ($r = -0.64$), Hb ($r = -0.33$), quantitation of proteinuria ($r = 0.33$) and fasting glucose level ($r = 0.25$). Serum HBD-2 correlated significantly with only HD vintage ($r = -0.25$) and serum IL-6 correlated significantly with HbA1c ($r = -0.58$), eGFR ($r = -0.31$), serum albumin ($r = -0.31$), Hb ($r = -0.26$) and hsCRP ($r = 0.23$). Serum TNF- α correlated only significantly with HbA1c ($r = 0.59$) (figure 2; figure 3).

Table 1. Main clinical, biochemical and hemodynamic characteristics of CKD patients and the control group

	Control group (n=88)	CKD patients (n=338)	P
Age (years)	57.9±6.0	61.1±14.3	
Male sex n. (%)	43 (48.9%)	206 (60.9%)	
Diabetic n. (%)	-	142 (42.0%)	
Duration of HD (months)	-	38.8±40.1	
Hemoglobin (g/dl)	14.4±1.5	11.9±2.4	0.000
Platelet count (X10 ¹² /l)	-	209.0±67.7	
WBC count (X10 ¹² /l)	-	6.8±2.4	
Creatinine (before HD) (mg/dl)	0.92±0.16	4.86±7.24	0.000
Urea (before HD) (mg/dl)	-	40.8± 23.8	
Total cholesterol (mg/dl)	203.6±39.5	177.3±49.1	0.178
HDL cholesterol (mg/dl)	50.7± 9.4	45.9± 13.4	0.001
LDL cholesterol (mg/dl)	124.6±36.3	91.5±31.4	0.053
Triglycerides (mg/dl)	141.0±80.0	145.6±141.0	0.184
Albumin (g/dl)	-	3.80±0.5	
hsCRP (mg/l)	1.69±3.33	7.94±27.0	0.000
Glucose (mg/dl)	101.5±25.8	136.1±70.2	0.000
eGFR (ml/min/1.73m ²)	79.2±10.1	30.6±25.8	0.000
HBD-1 (pg/ml)	1349.4±775.3	2282.8±792.6	0.000
HBD-2 (pg/ml)	1605.7±331.7	1535.9±420.1	0.099
IL-6 (pg/ml)	2.49±2.45	3.91±3.14	0.000
TNF- α (pg/ml)	1.00±0.62	1.62±2.52	0.022

CKD = chronic kidney disease; LDL = low-density lipoprotein; HDL = high-density lipoprotein; hsCRP = high sensitivity C-reactive protein; eGFR = estimated glomerular filtration rate; IL = interleukin; TNF = tumor necrotic factor; HD = hemodialysis; HBD = human β defensin

Fig. 1. HBD-1 (p=0.000)(a), HBD-2 (p=0.099)(b), IL-6 (p=0.000)(c) and TNF- α (p=0.022)(d) serum levels in CKD patients and control groups.

Table 2. Biochemical parameters by 5 stage classification of CKD patients based on eGFR

	Stage 5 (eGFR <15) (n = 156)	Stage 4 (eGFR 15-29) (n = 31)	Stage 3 (eGFR 30-59) (n = 102)	Stage 2 (eGFR 60 -89) (n = 39)	Stage 1 (eGFR >89) (n = 10)	p
Age (years)	61.3±12.9	65.2±13.4	63.4±14.0	55.9±15.4	40.4±17.0	0.000
Male sex n. (%)	89 (57.1%)	19 (61.3%)	64 (62.7%)	28 (71.6%)	6 (60%)	
Diabetic n. (%)	87 (55.8%)	19 (61.3%)	31 (30.4%)	4 (10.3%)	1 (10.0%)	
eGFR (ml/min/1.73m ²)	8.7±7.8	23.6±4.0	44.3±8.6	71.3±7.7	97.5±7.1	0.000
hsCRP (mg/l)	12.63±36.08	8.05±31.77	3.13±5.71	3.33±6.88	0.55±0.92	0.037
IL-6 (pg/ml)	4.98±3.36	3.44±2.56	3.21±2.65	2.10±2.28	2.98±3.11	0.000
TNF- α (pg/ml)	2.03±2.66	1.64±3.13	1.27±2.56	1.05±0.96	0.84±0.11	0.043
HBD-1 (pg/ml)	2806.2±726.8	2276.7±391.7	1866.2±440.0	1588.7±408.5	1627.8±405.5	0.000
HBD-2 (pg/ml)	1479.1±411.2	1666.6±458.8	1570.8±424.6	1567.0±378.8	1540.2±482.7	0.053
Hemoglobin (g/dl)	10.7±1.8	10.6±1.6	13.1±2.2	14.0±1.7	14.1±0.9	0.000
WBC count (X10 ¹² /l)	6.84±2.51	6.74±2.53	6.79±2.19	6.53±2.30	6.05±1.71	0.846
Glucose (mg/dl)	161.4±81.2	116.7±49.1	107.7±28.9	131.7±81.02	109.4±56.5	0.000
Total cholesterol (mg/dl)	161.1±41.0	167.5±45.7	189.5±47.3	202.7±55.1	218.5±70.0	0.000

CKD = chronic kidney disease; hsCRP = high sensitivity C-reactive protein; eGFR = estimated glomerular filtration rate; IL = interleukin; TNF = tumor necrotic factor; HD = hemodialysis; HBD = human β defensin

Fig. 2. Association between eGFR and HBD-1 ($r=-0.64$, $p=0.00$)(a) and eGFR and IL-6 ($r=-0.31$, $p=0.00$)(b) in CKD patients.

Fig. 3. Association between HBD-1 and Hb ($r=-0.33$, $p=0.00$)(a) and HBD-1 and Total cholesterol ($r=-0.23$, $p=0.00$)(b) in CKD patients.

Multivariate analysis between serum concentrations of IL-6, TNF- α , HBD-1 and HBD-2 and renal function in CKD patients

In multiple regression analysis, serum concentration of HBD-1 was negatively associated with eGFR ($\beta=-0.357$, $p=0.035$) and positively associated with triglyceride level ($\beta=0.285$,

Table 3. Independent determinants of serum concentration of IL-6, TNF- α , HBD-1 and HBD-2 in CKD patients

	HBD-1		HBD-2		IL-6		TNF- α	
	β value	p	β value	p	β value	p	β value	p
Urea	0.166	0.152	0.069	0.567	0.097	0.411	0.065	0.619
Creatinine	-0.065	0.672	0.195	0.230	0.011	0.943	0.094	0.591
eGFR	-0.357	0.035	0.207	0.238	0.073	0.669	-0.029	0.877
Glucose	0.044	0.617	-0.182	0.050	0.050	0.579	0.211	0.037
WBC count	-0.033	0.755	0.104	0.350	0.344	0.002	0.055	0.643
Hemoglobin	0.059	0.515	0.092	0.336	-0.002	0.986	0.079	0.445
HsCRP	0.107	0.322	0.009	0.938	-0.014	0.900	-0.022	0.856
Albumin	0.001	0.987	-0.181	0.066	-0.292	0.003	-0.017	0.875
HD vintage (months)	-0.102	0.287	-0.282	0.005	0.028	0.777	0.085	0.429
Total cholesterol	-0.081	0.719	0.172	0.469	0.026	0.912	-0.086	0.738
Triglycerides	0.285	0.044	-0.193	0.189	-0.146	0.307	0.013	0.933
HDL cholesterol	-0.079	0.570	-0.393	0.008	-0.049	0.728	0.013	0.932
LDL cholesterol	0.046	0.823	-0.141	0.516	0.165	0.435	0.088	0.706

CKD = chronic kidney disease; LDL = low-density lipoprotein; HDL = high-density lipoprotein; hsCRP = high sensitivity C-reactive protein; eGFR = estimated glomerular filtration rate; IL = interleukin; TNF = tumor necrotic factor; HD = hemodialysis; HBD = human β defensin

Table 4. Main biochemical characteristics of DN and non-DN in CKD patients (including only stage 3-5)

	Diabetic Nephropathy (n=137)	Non-diabetic Nephropathy (n=152)	p
Age (years)	64.6 $\pm$ 11.6	60.6 $\pm$ 14.7	0.012
Hemoglobin (g/dl)	11.1 $\pm$ 1.7	11.9 $\pm$ 2.6	0.001
Platelet count (X10 ¹² /l)	213.1 $\pm$ 50.8	229.5 $\pm$ 14.8	0.671
WBC count (X10 ¹² /l)	7.0 $\pm$ 2.4	6.6 $\pm$ 2.4	0.252
Creatinine (before HD) (mg/dl)	5.67 $\pm$ 3.47	5.33 $\pm$ 10.03	0.709
Urea (before HD) (mg/dl)	52.1 $\pm$ 21.4	38.7 $\pm$ 22.8	0.000
Total Cholesterol (mg/dl)	161.9 $\pm$ 39.1	180.9 $\pm$ 49.2	0.000
HDL cholesterol (mg/dl)	40.6 $\pm$ 10.0	48.6 $\pm$ 13.1	0.000
LDL cholesterol (mg/dl)	82.9 $\pm$ 27.4	93.2 $\pm$ 31.3	0.031
Triglycerides (mg/dl)	147.4 $\pm$ 107.2	143.1 $\pm$ 123.8	0.817
Albumin (g/dl)	3.73 $\pm$ 0.5	3.83 $\pm$ 0.5	0.077
hsCRP (mg/l)	7.00 $\pm$ 21.03	10.40 $\pm$ 34.53	0.319
Glucose (mg/dl)	171.6 $\pm$ 84.8	107.0 $\pm$ 24.9	0.000
eGFR (ml/min/1.73m ²)	17.3 $\pm$ 14.1	27.9 $\pm$ 20.0	0.000
HBD-1 (pg/ml)	2599.6 $\pm$ 769.6	2261.2 $\pm$ 693.2	0.000
HBD-2 (pg/ml)	1558.6 $\pm$ 417.7	1507.3 $\pm$ 430.4	0.305
IL-6 (pg/ml)	4.58 $\pm$ 3.22	3.84 $\pm$ 3.06	0.045
TNF- α (pg/ml)	1.93 $\pm$ 2.86	1.53 $\pm$ 2.53	0.213
Protein (mg/dl),urine	142.3 $\pm$ 166.4	55.8 $\pm$ 89.2	0.053
HbA1c (%)	7.18 $\pm$ 1.20	6.27 $\pm$ 0.95	0.023

CKD = chronic kidney disease; LDL = low-density lipoprotein; HDL = high-density lipoprotein; hsCRP = high sensitivity C-reactive protein; eGFR = estimated glomerular filtration rate; IL = interleukin; TNF = tumor necrotic factor; HD = hemodialysis; HBD = human β defensin

p=0.000) in patients with CKD. Serum concentration of HBD-2 was significantly negatively associated with duration of hemodialysis period (β =-0.282, p=0.005), fasting blood glucose (β =-0.182, p=0.050) and HDL cholesterol (β =-0.393, p=0.008) in patients with CKD. WBC count (β =0.344, p=0.002) and albumin (β =-0.292, p=0.003) were statistically significant predictors of serum IL-6 and fasting blood glucose (β =-0.211, p=0.037) was only statistically significant predictors of serum TNF- α concentration in CKD patients (table 3).

Biochemical parameters in CKD (stage 3-5) patients in regard to diabetic nephropathy

We divided into two classes in according with diabetic nephropathy. Main biochemical data of the two study population are presented in Table 4. No difference was observed in serum concentrations of HBD-2 and TNF- α , but serum concentrations of HBD-1 and IL-6 were higher in DN patients than non-DN patients (p=0.000, p=0.045).

Discussion

Our study revealed that CKD patients had higher serum levels of IL-6, TNF- α and hsCRP compared to control groups. Circulating levels of serum IL-6 and TNF- α increased according to reduced eGFR and reached the highest levels in patients with severe CKD. Study of Bolton et al. have had similar results [15]. But Spoto et al. demonstrated that plasma IL-6 underwent an early increase in chronic nephropathies and did not increase further in moderate and severe CKD [16]. However, TNF- α was inversely associated with eGFR indicating a substantial difference in the dynamics of the relationship between these cytokines and renal function [16]. More research is needed to answer this discrepancy whether the raised IL-6 in CKD represents an early response to renal disease or not.

The results also showed that IL-6 was better than TNF- α for distinction among the five different stage of CKD patients ($p=0.00$ vs $p=0.04$). That was already known as IL-6 was better serum marker for renal function than TNF- α because IL-6 was a systemic effective inflammatory marker but TNF- α was a local producing marker in CKD patients [11]. Additionally, IL-6 was associated HbA1c, eGFR, Hb, hsCRP and albumin in CKD patients. These associations might suggest IL-6 link to glycated hemoglobin, iron metabolism, inflammation and nutritional status. Although decreased elimination might be a major cause of elevated IL-6 levels in CKD patients increased cytokine generation also might play a role. Even before the initiation of dialysis therapy, patients with decreased renal function demonstrated signs of inflammation [11].

Further, antimicrobial peptides are important components of the innate host defense system [17]. HBDs are produced directly by epithelial cells and neutrophils [17]. Among the different HBDs, HBD-2 responded very well and could be easily altered under both infections and inflammatory conditions [18]. CKD was now considered a prototypical situation of chronic inflammation state, but our data did not corresponded with HBD-2 as it exhibited a stronger potential in antimicrobial activity under inflammatory conditions, especially in CKD patients. There could be three reasons for no rise of HBD-2 levels in CKD patients. First, because most CKD patients had a low grade inflammation, they expressed only the more sensitive inflammatory serum markers (hsCRP, IL-6 and TNF- α) and not higher levels of HBD-2. Secondly, HBD-2 usually exhibits a stronger potential antimicrobial activity against gram-negative organisms. However, the precise mechanisms employed were not understood and not defined to one organism under a common inflammatory status in CKD patients. Third, Because HBD-2 was also expressed elsewhere as well as in urinary, gastrointestinal and respiratory epithelia, its relatively low influence on the total serum level [6].

HBD-1 is probably the most important antimicrobial peptide in urinary and respiratory epithelial tissues. It is unique in its constitutive expression, but is still capable of upregulation upon inflammation or microbial stimuli. It was not clear whether HBD-1 responded to infection and if this response reflects more secretion from normal epithelium or release from damaged cells [19]. Hiratsuka et al. demonstrated that the plasma and urine concentrations of HBD-1 increased in pyelonephritis, but not in chronic glomerulonephritis. Because the lesion of pyelonephritis involves tubules and interstitium of the kidney, but the major lesion of chronic glomerulonephritis affects glomeruli. Conclusively, HBD-1 was a significant factor pathophysiologically in infectious renal diseases [19]. In the present study, we found that serum HBD-1 levels were higher in CKD patients than in control groups, but we did not found direct correlation between serum HBD-1 concentration and hsCRP. Our results were not consistent with Hiratsuka's findings. The results of this study suggest that serum HBD-1 concentration may be a potential marker for renal functions regardless of infectious renal diseases. Because the CKD patients had already severe damage in tubules, interstitium and glomeruli of the kidney, serum HBD-1 concentrations were markedly more increased than infectious renal diseases. However we didn't measure the HBD-1 concentrations of urine and localized renal tissues, and we have yet to clarify the HBD-1 performed a pathophysiological signification in CKD. In multiple regression analysis eGFR was the determinant of serum HBD-1 levels ($p=0.035$). In a univariate analysis, HBD-1 was related to Hb, quantitation of proteinuria and fasting glucose levels as well as related to kidney function (creatinine,

BUN and eGFR). Significant increment in serum HBD-1 levels in CKD patients according to varying stages of renal failure was observed. These predictors might suggest HBD-1 link to renal function, hemoglobin, severity of inflammation and glucose status. The activation of the innate immune system and low-grade chronic inflammation were very deeply related to the pathogenesis of Type 2 DM [7]. This concept suggested that long-term activation of the innate immune system and subsequent development of a low grade chronic inflammatory reaction (an ongoing cytokine-induced acute phase response) elicited disease instead of repair, leading to the development of Type 2 DM [20]. In addition, long-term low-grade inflammation might play a key role in the onset of several disturbances leading to DN and atherosclerosis [10, 11]. DN is one of the most important concerns in nephrology, as well as in medicine at large. Rapidly increasing rates of diabetes mellitus (DM) throughout the world represents an emerging epidemic with profound consequences. Our study showed that 137 patients at stage 3-5 DN had significantly higher serum HBD-1 levels than 152 patients at stage 3-5 non DN, but did not differ in regards to HBD-2, IL-6, hsCRP and TNF- α . These results suggested that the role of innate immunity, especially in regards to HBD-1, was a primary process in the development of DM. Malik et al. found that the increased expression of HBD-1 mRNA in high glucose suggested a role for HBD-1 in the molecular pathways induced during hyperglycemia [21]. Further, the high serum HBD-1 levels in DM patients might contribute to the development of DN. Our data demonstrated HBD-1 was significantly increased in DN patients regardless of the levels of inflammatory markers. But our study has limitations. First, we could not compare serum HBD-1 levels between DM without nephropathy and with nephropathy. The second, owing to the relationships between this innate immunity state and the development and progression of DN involved with very complex network processes, it is difficult for HBD-1 to be proposed as a significant marker. The third, because there is the problem of the cross-sectional nature of the study design we cannot prove direct cause and effect relationship for nephropathy development. It was well known that low-grade elevation of circulating markers of inflammation (hsCRP and pro-inflammatory cytokines) is associated with future development of DN and myocardial infarction [22, 23]. Despite these limitations, it would be worth considering the potential of serum HBD-1 level as a renal function marker in DN.

Conclusion

Our data suggested that HBD-1 was not only unique in its constitutive expression, but also capable of upregulation upon inflammation in CKD patients. Serum concentration of HBD-1 was inversely associated with the eGFR indicating a substantial difference in the dynamics of the relationship between innate immunity and renal function. In addition, DN patients had significantly higher serum HBD-1 levels than the non DN patients, but there was no difference in HBD-2, IL-6, hsCRP and TNF- α . Therefore HBD-1 can be proposed as one of the potential factors involved in the development of CKD and development of complications. Further studies are needed to determine the mRNA expression of HBD-1 in human kidney cells and to establish the mechanistic role of HBD-1 in renal failure and in development of complications of CKD patients.

Conflict of Interests

The authors declare that none of them has any conflict of interest.

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