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Original Article

Correlation between salivary HBD-2 and LL-37 expression levels with blood glucose and periodontal status in patients with type 2 diabetes mellitus

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Abstract: Objective: To examine the correlation between salivary human β defensin-2 (HBD-2) and LL-37 expression levels and blood glucose in relationship to periodontal status in patients with type 2 diabetes mellitus (T2DM). Methods: The trial is available at: https://clinicaltrials.gov/ with ClinicalTrials.gov Identifier: NCT03512675. A total of 89 patients with T2DM with chronic periodontitis (CP) enrolled in our hospital from January 2020 to December 2020 were selected. According to the degree of glycemic control and CP, the patients were randomly divided into four groups, namely the good glycemic control and mild CP group (n=26), good glycemic control with moderate to severe CP group (n=24), poor glycemic control with mild CP group (n=21), and poor glycemic control with moderate to severe CP group (n=18). The periodontal clinical parameters, blood glucose indicators, and saliva HBD-2 and LL-37 expression levels were determined. Results: The expression levels of HBD-2 and LL-37 in the saliva of T2DM patients with moderate to severe CP and poor blood sugar control were significantly increased (P<0.05). Saliva HBD-2 and LL-37 levels were positively correlated with probing depth, clinical attachment loss, plaque index, and glycosylated hemoglobin. There was a synergistic interaction between blood glucose, periodontal status, and saliva HBD-2, LL-37 levels (P<0.05). Conclusion: There is a positively correlated relationship between blood glucose and periodontal status with salivary HBD-2 and LL-37 levels.

Keywords: Type 2 diabetes mellitus, periodontitis, human β defensin-2, antimicrobial peptide-37

Introduction

Diabetes is a chronic non-communicable disease widely seen in China, of which type 2 diabetes mellitus (T2DM) is the main type [1]. Data shows a growing number of diabetic patients in China, which poses an ever-heavier social and economic burden [2]. β-defensin is a cytokine secreted by gingival epithelial cells, which is mainly involved in the immune defense process. Recent research has shown that human β defensin-2 (HBD-2) plays an important role in the innate immune response during chronic periodontitis (CP) [3]. LL-37 is a member of the cathelicidin family discovered in the human body with high antibacterial and immune effects [4]. A previous study found increased levels of HBD-2 and LL-37 in the gingival tissue and gingival crevicular fluid of patients with periodontitis [5]. Moreover, a report pointed out that 12.50% of patients with CP had diabetes [6], while similarly, diabetic patients had an increased risk of CP and severe lesions [7], suggesting the interaction between diabetes and CP. Accordingly, this study explored the correlation of the expression levels of HBD-2 and LL-37 in saliva with blood glucose and periodontal status in T2DM patients, to provide a reference for the prevention and treatment of CP in T2DM patients.

Data and methods

Clinical data

A total of 89 patients admitted to our hospital from January to December of 2020 were selected as research subjects. Inclusion criteria: (1) Patients aged 18-80 years; (2) Patients met the diagnostic criteria for T2DM and CP [8, 9]; (3)

Patients with at least 15 remaining teeth; (4) Patients with signed informed consent. Exclusion criteria: (1) Patients with malignant tumor, severe cardiac, cerebral, hepatic, and renal dysfunction, systemic infectious diseases or immune system diseases; (2) Patients with periodontal treatment within the last six months; (3) Patients with antibiotic or immunosuppressive therapy in the last three months; (4) Patients with oral deformity; (5) Patients who were pregnant or lactating. According to the degree of glycemic control and CP, the patients were randomly divided into four groups: good glycemic control and mild CP group (n=26), good glycemic control with moderate to severe CP group (n=24), poor glycemic control with mild CP group (n=21), and poor glycemic control with moderate to severe CP group (n=18). This study was reviewed and approved by the Hospital Ethics Committee, with the ethics approval number of TH2020DR152.

Methods

General data collection: The gender, age, smoking, alcohol consumption and diabetic course of the patients were recorded. The systolic and diastolic blood pressure, height, and weight were measured, and the Body Mass Index (BMI) was calculated.

Oral examination: The probing depth (PD) and clinical attachment loss (CAL) at the buccal and lingual sides of each tooth were examined with a periodontal probe. The plaque index (PI) was calculated based on the thickness of dental plaque, and the gingival index (GI) was calculated based on gingival color, quality, and bleeding tendency.

Blood and saliva sampling: Fasting cubital venous blood (5 mL) was collected in the early morning and centrifuged at 3000 r/min for 5 min, and the resulting serum and plasma were stored in the freezer at -80°C for later use. Large amounts of food and water were prohibited before saliva sample collection. After rinsing with clear water, all the saliva flowing out of the mouth within 10 min was collected in a sputum cup and centrifuged at 13000 r/min and 4°C for 15 min. The precipitate and supernatant were separated and then stored in a freezer at -80°C for subsequent use.

Determination of blood index levels: The level of fasting plasma glucose (FPG) was determined by Enzyme-Linked Immunosorbent Assay (ELISA), the kit was purchased from Shanghai Yiyan Biotechnology Co., Ltd. (Cat. No. EY-H97903). The level of serum Glycosylated hemoglobin (HbA1c) was also detected by ELISA, the kit was purchased from Shanghai Yanhui Biological Technology Co., Ltd. (Cat. No. E90190).

Determination of salivary HBD-2 and LL-37 levels: The ELISA kits for the determination of HBD-2 and LL-37 in saliva supernatant were purchased from Jiangxi Albain Biotechnology Co., Ltd. (Cat. No. IB-E20311) and Shanghai Yimu Industry Co., Ltd. (Cat. No. FK-vx1049), respectively.

Observational indexes

(1) Glycemic control: HbA1c <7% meant good glycemic control, HbA1c ≥7% meant poor glycemic control. (2) Periodontal status: Mild CP: 2 adjacent sites with CAL ≥3 mm and two or more different teeth with PD ≥4 mm or 1 site with PD ≥5 mm; Moderate CP: CAL ≥4 mm at 2 or more adjacent surfaces of different teeth, or PD ≥5 mm at 2 or more adjacent surfaces of different teeth; Severe CP: 2 or more proximal sites of different teeth with CAL ≥6 mm and 1 or more proximal sites with PD ≥5 mm.

Statistical analysis

SPSS 20.0 software was used for the statistical analysis of the data. The measurement data conforming to a normal distribution were represented by ($\bar{x}$±s), one-way analysis of variance was adopted for the comparison of multiple groups, and the Snk-q test was used for pairwise comparison. Counting data were expressed by frequency or composition ratio, and analyzed by the chi-square non-correction method. The correlation between saliva HBD-2 and LL-37 levels and each index was analyzed by Pearson correlation analysis. The interaction between blood glucose, periodontal status, and saliva HBD-2, LL-37 was analyzed by 2×2 factorial analysis. If the difference was statistically significant, a separate effect analysis was performed. P<0.05 indicated that the difference was statistically significant.

Results

Comparison of general data among the four groups

There was no significant difference in male/female composition ratio, smoking ratio, alco-

Table 1. Comparison of general data among the four groups

Groups	Good Glycemic control with mild CP group	Poor Glycemic control with mild CP group	Good Glycemic control with moderate to severe CP group	Poor Glycemic control with moderate to severe CP group	F/χ^2	*P*
n	26	24	21	18		
Male (n/%)	13/50.00	13/54.17	11/52.38	10/55.56	13.293	0.829
Smoking (n/%)	13/50.00	12/50.00	11/52.38	10/55.56	35.928	0.912
Drinking (n/%)	14/53.85	13/54.17	12/57.14	11/61.11	26.825	0.917
Age (year)	58.93±10.42	59.43±10.56	60.12±11.14	61.47±11.21	0.211	0.888
Diabetes course (years)	7.95±1.45	8.11±1.38	8.23±1.42	8.28±1.41	0.244	0.866
BMI (kg/m^2)	22.14±2.55	22.36±2.53	22.69±2.51	22.78±2.48	0.307	0.820
SBP (mmHg)	123.36±12.48	125.45±12.56	127.28±12.52	128.31±12.54	0.674	0.571
DBP (mmHg)	69.97±5.36	70.15±5.39	71.52±5.41	71.69±5.44	0.604	0.614

Note: BMI, Body Mass Index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure.

Table 2. Comparison of periodontal clinical parameters among the four groups ($\overline{x}\pm s$)

Groups	n	PD (mm)	CAL (mm)	PI	GI
Good Glycemic control with mild CP group	26	2.34±0.48	2.46±0.45	0.52±0.41	0.27±0.04
Poor Glycemic control with mild CP group	24	2.49±0.47	2.57±0.53	0.54±0.38	0.29±0.03
Good Glycemic control with moderate to severe CP group	21	3.96±0.51①,②	4.83±0.49①,②	2.13±0.35①,②	1.27±0.05①,②
Poor Glycemic control with moderate to severe CP group	18	4.47±0.45①,②	6.12±0.51①,②	2.64±0.43①,②	1.62±0.06①,②
F		92.980	247.910	143.678	3826.700
P		<0.01	<0.01	<0.01	<0.01

Note: Compared with the group with good glycemic control and mild CP, ①P<0.05; compared with the group with poor glycemic control and mild CP, ②P<0.05.

hol consumption ratio, age, duration of diabetes, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) among the four groups (P>0.05). See **Table 1**.

Comparison of periodontal clinical parameters among the four groups

The PD, CAL, PI, and GI were significantly higher in T2DM patients with moderate to severe CP than in those with mild CP (P<0.05). See **Table 2**.

Comparison of blood glucose indicators among the four groups

The FPG and HbA1c levels were significantly higher in T2DM patients with CP and with poor glycemic control than in those with good glycemic control (P<0.05). See **Table 3**.

Comparison of the expression levels of HBD-2 and LL-37 in the saliva of the four groups of patients

T2DM patients with moderate to severe CP and those with poor glycemic control had higher expression levels of HBD-2 and LL-37 in saliva than those with mild CP and good glycemic control, respectively (P<0.05), as shown in **Figure 1**.

Correlation between saliva HBD-2 and LL-37 levels and various indicators

Saliva HBD-2 and LL-37 levels were positively correlated with PD, CAL, PI, and HbA1c (P<0.05). See **Table 4**.

Main effect analysis

There was a significant interaction between blood glucose and periodontal status, and levels of saliva HBD-2 and LL-37 expression (P<0.05). See **Table 5**.

Analysis of individual effects

In patients with mild CP or good glycemic control, the individual effects of blood sugar and periodontal status on saliva HBD-2 and LL-37 levels were weak (P>0.05). In patients with moderate to severe CP, glycemic control had a significant individual effect on saliva HBD-2 and LL-37 levels (P<0.05). In patients with poor glycemic control, periodontal status also had a

Table 3. Comparison of blood glucose indicators among the four groups ($\bar{x}\pm s$)

Groups	n	FPG (mmol/L)	HbA1c (%)
Good Glycemic control with mild CP group	26	7.58±1.24	5.56±0.41
Poor Glycemic control with mild CP group	24	9.13±1.46①,③	8.43±0.37①,③
Good Glycemic control with moderate to severe CP group	21	7.62±1.21	5.51±0.38
Poor Glycemic control with moderate to severe CP group	18	10.79±1.44①,③	8.78±0.43①,③
F		38.948	437.487
P		<0.01	<0.01

Note: Compared with the group with good glycemic control and mild CP, ①P<0.05; compared with the group with good glycemic control and moderate to severe CP, ③P<0.05.

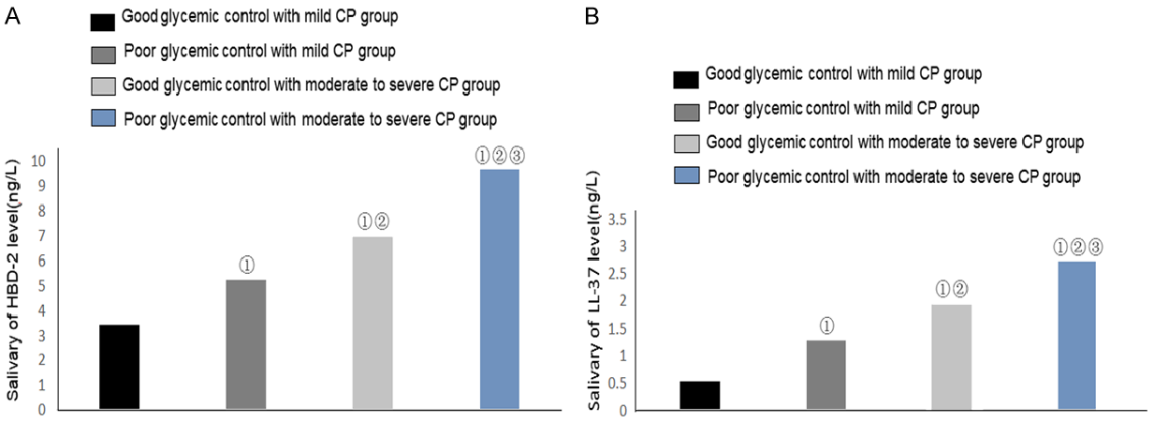

Figure 1. Comparison of the expression levels of HBD-2 and LL-37 in the saliva of four groups of patients. A. Comparison of HBD-2 expression levels in saliva of four groups of patients. B. Comparison of LL-37 expression levels in saliva of four groups of patients. Note: Compared with the group with good glycemic control and mild CP, ①P<0.05; compared with the group with poor glycemic control and mild CP, ②P<0.05; compared with the group with good glycemic control and moderate to severe CP, ③P<0.05.

Table 4. Correlation between saliva HBD-2 and LL-37 levels and various indicators

Indicators	HBD-2		LL-37	
	r	*P*	*R*	*P*
PD	0.612	0.041	0.624	0.036
CAL	0.634	0.027	0.659	0.021
PI	0.659	0.015	0.636	0.018
GI	0.436	0.182	0.462	0.249
FPG	0.469	0.241	0.521	0.262
HbA1c	0.741	0.023	0.659	0.021
TC	0.432	0.469	0.528	0.595
TG	0.364	0.587	0.587	0.612
HDL-C	0.388	0.571	0.482	0.434
LDL-C	0.369	0.145	0.344	0.221
TNF-α	0.484	0.693	0.396	0.711
IL-1β	0.411	0.742	0.352	0.736

significant individual effect on saliva HBD-2 and LL-37 levels (P<0.05). See **Table 6**.

Discussion

Diabetes is currently one of the major chronic non-communicable diseases threatening people's life and health [10]. Data show that the number of diabetic patients in China grows by 5%-10% annually [11]. T2DM accounts for more than 95% of the total number of diabetes mellitus [12]. Different from type 1 diabetes, T2DM features insulin resistance and is more common in the middle-aged and elderly [13]. Periodontitis is an inflammatory disease caused by bacteria that weakens the supporting tissues of teeth [14]. It was found that the prevalence of periodontitis was higher in T2DM patients than in the healthy adults, and blood glucose and TC were the main risk factors for periodontal health in T2DM patients [15]. The study conducted by Huang et al. [16] revealed a significant positive correlation between the incidence and severity of periodontitis and

Table 5. The main effects of blood glucose and periodontal status on saliva HBD-2 and LL-37 levels

Indicators	*F (P)*		
	Blood control	Periodontal status	Interaction
HBD-2	8.542 (0.000)	7.542 (0.008)	4.345 (0.038)
LL-37	8.265 (0.000)	7.113 (0.012)	4.213 (0.041)

Table 6. Individual effects of blood glucose and periodontal status on saliva HBD-2 and LL-37 levels

	F (P)	
	HBD-2	LL-37
Mild CP	0.535 (0.146)	0.541 (0.321)
Moderate to severe CP	12.982 (0.001)	12.254 (0.001)
Good glycemic control	0.348 (0.347)	0.126 (0.482)
Poor glycemic control	11.231 (0.001)	9.868 (0.001)

HbA1c levels in T2DM patients, with more severe damage when HbA1c ≥7.5%. Evidence has shown a close relationship between the occurrence and development of periodontitis and the cascade events regulated by the innate and adaptive immune systems [17]. HBD-2 and LL-37 are important factors that regulate host defense and immune response [18]. Previous research has found elevated antimicrobial peptide contents in the gingival epithelium of patients with T2DM and increased HBD-2 and LL-37 levels in patients with periodontitis compared with periodontal healthy controls [19].

This study included 89 patients with T2DM with CP. The comparison of periodontal clinical parameters showed that the PD, CAL, PI, and GI were significantly higher in T2DM patients with moderate to severe CP than in those with mild CP, which was in line with the results of previous studies. The comparison of blood glucose indicators shows that the fasting blood glucose (FPG) and glycated hemoglobin (HbA1c) levels of T2 DM patients with poor blood sugar control and CP were significantly higher than those of T2DM patients with good blood sugar control. In the study of Lin et al., it was found that FPG and HbA1c in patients with severe periodontal disease were higher than those in mild patients, which was similar to the results of our study, and they further determined through regression analysis that FPG and HbA1c are the main risk factors of type 2 diabetes complicated with chronic periarthritis. This is further cross-validated with our results [20]. The detection of HBD-2 and LL-37 levels in the saliva of the four groups of patients revealed that T2DM patients with moderate to severe CP and those with poor glycemic control were observed with higher expression levels of HBD-2 and LL-37 in saliva than those with mild CP and good glycemic control, respectively, which indicates that the increase in saliva HBD-2 and LL-37 levels may be a contributing factor to the occurrence of periodontitis in T2DM patients and the pathological process of CP [21]. The main effect analysis showed that there was a significant interaction between blood glucose and periodontal status, and saliva HBD-2 and LL-37 levels. Further individual effect analysis results demonstrated a positive synergistic effect of blood sugar and periodontal status with saliva HBD-2 and LL-37 levels, which demonstrates that the worse the glycemic control, the more severe the CP, and the higher the saliva HBD-2 and LL-37 levels, suggesting the essential role of saliva HBD-2 and LL-37 in the deterioration of periodontal inflammation in T2DM patients.

Conclusion

In summary, there is a positively correlated synergistic effect of blood glucose and periodontal status with salivary HBD-2 and LL-37 levels.

Disclosure of conflict of interest

None.

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