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Original Article

Correlation between salivary developmental endothelial locus-1, interleukin 17 expression level and severity of periodontal disease in patients with type 2 diabetes mellitus

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Abstract: Objective: To investigate the correlation of the salivary expression levels of developmental endothelial locus-1 (Del-1) and interleukin 17 (IL-17) with the severity of periodontal disease in patients with type 2 diabetes mellitus (T2DM). Methods: Fifty-seven patients with T2DM and fifty-seven patients with T2DM complicated with chronic periodontitis (CP) admitted to our hospital from March 2020 to March 2021 were enrolled as the research objects and assigned to the T2DM group and T2DM+CP group respectively. Additionally, 57 healthy controls from the hospital physical examination center during the same period were included as the healthy controls. General information of all the enrolled participants was collected to measure the levels of glycated hemoglobin (HbA1c), salivary inflammatory factors, and salivary Del-1 and IL-17, as well as homeostasis model assessment of insulin resistance (HOMA-IR). Results: HbA1c in the T2DM group and T2DM+CP group was significantly higher than that in the healthy control group. Gingival index (GI), plaque index (PI), and probing depth (PD) were significantly higher in case groups than those in the health group. Compared with the healthy control group, salivary IL-17 expression level increased remarkably in the T2DM+CP group and T2DM group, with a higher level observed in the T2DM+CP group (P<0.05). The T2DM+CP group presented significantly higher levels of salivary tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) than the other two groups (P<0.05). Compared with the healthy control group, HOMA-IR and salivary Del-1 declined evidently in the T2DM+CP group and T2DM group, and the lowest values were observed in the T2DM+CP group (P<0.05). Salivary Del-1 and HbA1c were independent risk factors for CP in T2DM patients (P<0.05). Conclusion: Salivary Del-1 was downregulated and IL-17 was up-regulated in T2DM patients complicated with CP, indicating their correlation with the occurrence of CP in T2DM patients.

Keywords: T2DM, periodontal disease, Del-1, IL-17, correlation

Introduction

Type 2 diabetes mellitus (T2DM) is one of the main diseases threatening people's life and health [1]. Hyperglycemia is the main clinical manifestation of T2DM patients, which can cause varying degrees of pathological changes in human organs if left unattended for a long time [2]. Chronic periodontitis (CP) leads to infectious diseases of periodontal support tissues such as gingiva. In recent years, research has found a mutual promotion effect between T2DM and CP; that is, diabetes increases the risk of CP and aggravates the degree of periodontal disease [3], and periodontitis, as a high-risk factor for diabetes, elevates the risk of diabetes-related complications [4]. It has also been shown that effective periodontal treatment contributes to the control of blood glucose in diabetic patients [5]. Developmental endothelial locus-1 (Del-1) can inhibit the initiation of endothelial adhesion and inflammation. Folwaczny M [6] revealed that Del-1, with a significantly downregulated expression profile in gingival tissues of CP patients, was closely related to the periodontal lesion [7]. In addition, interleukin-17 (IL-17), a pro-inflammatory cytokine secreted by activated CD4+ T cells, is

reported to be an important cytokine involved in infectious inflammatory diseases such as periodontitis. Evidence has shown that the blood glucose and inflammation levels in T2DM patients complicated with CP are closely correlated with periodontal lesions [8]. Accordingly, this study analyzed the correlation between the expression of Del-1 and IL-17 in saliva and the severity of periodontal disease in T2DM patients, so as to provide some reference for clinical treatment.

Materials and methods

Clinical data

This study included 57 T2DM patients (T2DM group) and 57 T2DM complicated with CP patients (T2DM+CP group) admitted to the hospital from March 2020 to March 2021. Inclusion criteria of the T2DM group: (1) Patients were aged from 18 to 60 years old; (2) Patients met the diagnostic criteria for T2DM [9] with a confirmed diagnosis for more than 2 years; (3) Patients with healthy periodontal status. Exclusion criteria: (1) Patients with other systemic diseases, severe hepatic and renal insufficiency, infectious diseases, congenital heart diseases, hypertension, hematopoietic dysfunction, or mental illness; (2) Patients with poor treatment compliance; (3) Patients with prior antibiotics or periodontal therapy in the past 3 months; (4) Severe malocclusion. Additionally, 57 healthy controls from the hospital physical examination center during the same period were selected as the health group. Simultaneously, patients with T2DM complicated with CP, that is, probing pocket depth (PPD) ≥4 mm and CAL≥4 mm, presence of bleeding on probing (BOP) and a minimum of five teeth with at least one site with CAL and PPD≥5 mm, were enrolled into T2DM+CP group. The study was approved by the hospital ethics committee, with an ethics approval number of 2019-12-23, and the participants all signed the informed consent form after being fully informed of the research process.

Clinical trial registration: https://clinicaltrials.gov/, ClinicalTrials.gov Identifier: NCT043176-43.

Methods

Hb1Ac and HOMA-IR: Fasting cubital venous blood (5 mL) was collected from all the participants for analysis. The whole blood glycated hemoglobin (Hb1Ac) level was determined with an automatic biochemical analyzer (Mindray, model b0005). The serum fasting blood glucose (FPG) level was determined by enzyme-linked immunosorbent assay (ELISA) with a kit purchased from Shanghai Fusheng Industrial Co., Ltd. (Cat. No. fs13987). ELISA was also adopted to determine serum fasting insulin (FINS) level using a kit purchased from Shanghai Sig Biotechnology Co., Ltd. The formula for calculating homeostasis model assessment of insulin resistance (HOMA-IR) is as follows: HOMA-IR = [FPG (mmol/L) * FINS (μU/mL)]/22.5.

Periodontal examination: Probing depth (PD) refers to the distance from the gingival margin of the upper and lower molars to the bottom of the periodontal pocket measured by the periodontal probe. The mean value of PD was obtained by measuring the values at 6 sites of mesial-buccal, central-buccal, distal-buccal, mesial-lingual, central-lingual, and distal-lingual. Gingival color, texture changes, and bleeding tendency were observed with the probe. The average score of each tooth was the mean of mesiobuccal, distal buccal, mid buccal, and lingual scores. The gingival index (GI) is the average score of all teeth. According to the number and thickness of plaque, each tooth was scored as the mean of mesial-, distal- and central-buccal and lingual surfaces and the plaque index (PI) was the mean of all examined teeth.

Salivary inflammatory factors, Del-1 and IL-17: From 8:00 to 10:00 a.m. before the examination, the participants were instructed to gargle the oral cavity with water. Then, the whole saliva from each participant within 10 minutes was collected into a sterile centrifuge tube for centrifugation at 13000 r/min and stored at 4°C for 15 minutes. The resulting supernatant was used for ELISA to determine the levels of the tumor necrosis factor-α (TNF-α), IL-1β, Del-1, and IL-17 in the whole saliva, with kits purchased from Shanghai Fusheng Industrial Co., Ltd. (Cat. No. fs-e1826), Shanghai Yiyan Biotechnology Co., Ltd. (Cat. No. ey-3770), Shanghai Meixuan Biotechnology Co., Ltd. (Cat. No. mexn-h2928) and Nanjing SenBeiJia Biological Technology Co., Ltd. (Cat. No. sbj-h0416), respectively. All examinations above were completed in our hospital laboratory.

Table 1. General information of participants in three groups

Group	n	Gender (male/female)	Age	Course of diabetes (year)	BMI (kg/m^2)
Health	57	28/29	58.31±10.23	-	23.11±2.14
T2DM	57	26/31	59.12±10.25	7.12±2.14	23.23±2.25
T2DM+CP	57	29/28	60.11±10.28	7.15±2.16	23.67±2.18
F/χ^2			0.441	0.075	1.033
P			0.644	0.941	0.358

Table 2. HbA1c and HOMA-IR of participants in three groups ($\bar{x}\pm s$)

Group	n	HbA1c (%)	HOMA-IR
Health	57	4.98±0.76	1.63±0.34
T2DM	57	6.23±0.74①	1.21±0.28①
T2DM+CP	57	8.79±0.73①,②	0.75±0.31①,②
F		389.010	114.196
P		<0.01	<0.01

Note: HbA1c: glycated hemoglobin; HOMA-IR: homeostasis model assessment of insulin resistance; ①compared with the healthy group, P<0.01; ②compared with the T2DM group, P<0.01.

Table 3. Comparison of periodontal clinical indicators in participants among three groups ($\bar{x}\pm s$)

Group	n	GI	PI	PD (mm)
Health	57	0.42±0.11	0.54±0.15	1.63±0.28
T2DM	57	0.79±0.12①	0.98±0.12①	1.96±0.25①
T2DM+CP	57	2.31±0.08①,②	2.26±0.14①,②	2.34±0.27①,②
F		5198.573	2420.681	100.916
P		<0.01	<0.01	<0.01

Note: GI: gingival index; PI: plaque index; PD: probing depth; ①compared with the healthy group, P<0.01; ②compared with the T2DM group, P<0.01.

Statistic analysis

SPSS 20.0 software was used to analyze the data. Measurement data conforming to normal distribution were expressed as ($\bar{x}\pm s$). Independent samples t-test was used for comparisons between two groups, one-way analysis of variance for comparison among three groups, and SNK q test for pairwise comparisons. Counting data were expressed by frequency or percentage. If the total number of cases was less than 40 or the minimum theoretical frequency was less than 1, the chi exact test was adopted. If the minimum total number of cases was 40 and the minimum theoretical frequency ranged from 1 to 5, the chi-square correction was used. If the total number of cases was greater than or equal to 40 and the minimum theoretical frequency exceeded 5, the chi-square non-correction was employed. Pearson analysis was used to analyze the bivariate correlation which fitted the normal distribution. Logistic regression analysis was for risk factors of CP in T2DM patients. P<0.05 represented a significant difference. GraphPad Prism 7 (GraphPad Software, San Diego, USA) was used to plot the graphics.

Results

General information of participants in three groups

Gender, age, and BMI were not significantly different among the three groups (P>0.05). T2DM group and T2DM+CP group were similar in the course of diabetes (P>0.05; **Table 1**).

HbA1c and HOMA-IR of participants in three groups

T2DM+CP group and T2DM group presented higher HbA1c levels and lower HOMA-IR than the health group; moreover, both values in the T2DM+CP group exceeded those in the T2DM group (P<0.05; **Table 2**).

Comparison of periodontal clinical indicators among three groups

GI, PI, and PD were significantly higher in the T2DM group and T2DM+CP group compared with the health group, and the highest values were found in the T2DM+CP group (**Table 3**).

Comparison of salivary inflammatory factors expression among three groups

Salivary TNF-α and IL-1β levels were higher in the T2DM group than in the health group, without a significant difference (P>0.05). The

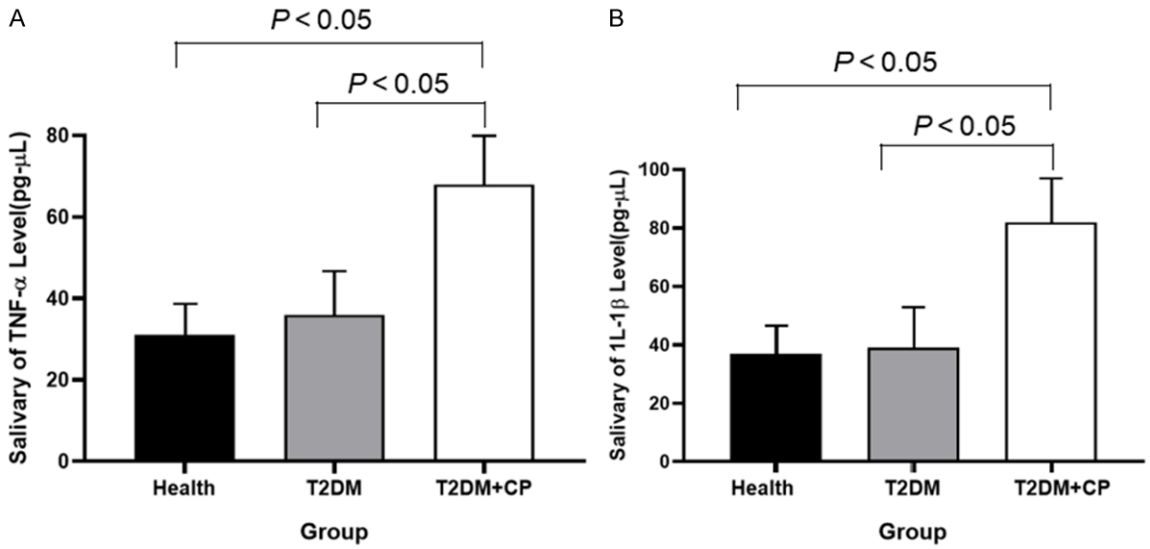

Figure 1. Comparison of the expression levels of inflammatory factors in the saliva of the three groups.

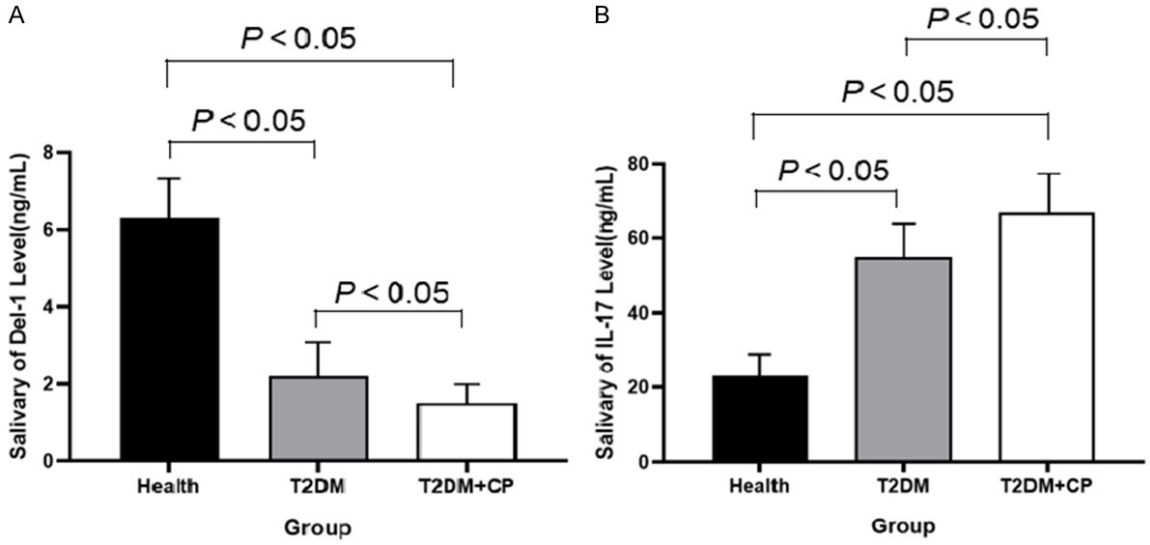

Figure 2. Comparison of the expression levels of Del-1 and IL-17 in the saliva of the three groups.

T2DM+CP group presented significantly higher salivary TNF-α and IL-1β than the other two groups (P<0.05; **Figure 1**).

Comparison of salivary Del-1 and IL-17 expression levels among three groups

Different from the health group, the salivary Del-1 expression levels in the other two groups dropped greatly, with a lower value in the T2DM+CP group (P<0.05). T2DM group and T2DM+CP group showed a higher salivary IL-17 expression level than the health group, with a higher level in the T2DM+CP group than that in the T2DM group (P<0.05; **Figure 2**).

Correlation of salivary Del-1 and IL-17 with HbA1c and HOMA-IR

Saliva Del-1 was negatively correlated with the HbA1c levels (r=-0.647) and positively correlated with the HOMA-IR index (r=0.633). Saliva IL-17 was positively correlated with the HbA1c levels (r=0.629) and negatively correlated with the HOMA-IR index (r=-0.639) (P<0.05) (**Table 4**).

Table 4. Correlation of salivary Del-1 and IL-17 with HbA1c and HOMA-IR

Indicator	Statistics	HbA1c	HOMA-IR
Del-1	*r*	-0.647	0.633
	P	0.032	0.041
IL-17	*r*	0.629	-0.639
	P	0.045	0.035

Note: Del-1: developmental endothelial locus-1; IL-17: interleukin 17; HbA1c: glycated hemoglobin; HOMA-IR: homeostasis model assessment of insulin resistance.

Table 5. Correlation of salivary Del-1 and IL-17 with periodontal clinical indicators

Indicator	Statistics	GI	PI	PD
Del-1	*r*	-0.581	-0.575	-0.569
	P	0.033	0.031	0.018
IL-17	*r*	0.633	0.621	0.645
	P	0.006	0.012	0.024

Note: Del-1: developmental endothelial locus-1; IL-17: interleukin 17; GI: gingival index; PI: plaque index; PD: probing depth.

Correlation of salivary Del-1 and IL-17 with periodontal clinical indicators

GI, PI, and PD were negatively correlated with salivary Del-1 (r=-0.581, r=-0.575, r=-0.569, P<0.05) and positively correlated with salivary IL-17 (r=0.633, r=0.621, r=0.645, P<0.05) (**Table 5**).

Correlation between salivary Del-1 and IL-17 and inflammatory factors

Similarly, a negative correlation was observed between Del-1 and TNF-α, IL-1β (r=-0.711, r=-0.698, P<0.05), and salivary IL-17 was positively correlated with TNF-α and IL-1β (r=0.702, r=0.706, P<0.05) (**Table 6**).

Table 6. Correlation between salivary Del-1 and IL-17 and inflammatory factors

Indicator	Statistics	TNF-α	IL-1β
Del-1	*r*	-0.711	-0.698
	P	<0.001	0.005
IL-17	*r*	0.702	0.706
	P	<0.001	<0.001

Note: Del-1: developmental endothelial locus-1; IL-17: interleukin 17; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β.

Logistic regression analysis on risk factors of CP in T2DM patients

Decreased saliva Del-1 level [0.369 (0.036~0.931)] and increased IL-17 level [1.654 (1.119~6.782)] were independent risk factors for CP in patients with T2DM (**Table 7**).

Discussion

A meta-analysis on diabetes prevalence in Chinese adults has indicated a rising trend of diabetes in China year by year with an annual growth rate of approximately 12%, which brings a heavy disease burden to society [11]. Studies have confirmed a close relationship between diabetes and periodontal disease [12]. Some data demonstrated that the prevalence of periodontal diseases in elderly patients with T2DM is 90.1%, which is closely related to FPG and TG [13]. Inflammation is one of the important pathological bases of CP [14]. In addition, hyperglycemia is reported to induce the generation of oxygen free radicals, activate oxidative stress signaling pathways, and promote the release of inflammatory cytokines [15]. Moreover, the release of inflammatory factors can activate osteoclast collagenase and damage periodontal and bone tissue, further aggravating periodontal lesions [16]. Therefore, elevated glucose metabolism can evoke inflammatory reactions and affect periodontal health, and periodontal injury can undermine the treatment effect of diabetic patients.

This study included healthy individuals, T2DM patients, and T2DM patients complicated with CP. The detection of blood glucose indexes showed that HbA1c and HOMA-IR in the T2DM group and the T2DM+CP group were significantly higher than those in the health group, conforming to previous research results [12, 13]. The higher levels of HbA1c and HOMA-IR in the T2DM+CP group than in the T2DM group further indicated the involvement of blood glucose control in the prevention and treatment of CP in T2DM patients. As to salivary inflammatory factors, there was a rise in salivary TNF-α and IL-1β in the T2DM group, as compared to the health group. However, the difference was not statistically significant, which was inconsistent with the results of previous study [17]. It may be related to the small sample size of this study, which needs further analysis in future

Table 7. Logistic regression analysis on risk factors of CP in T2DM patients

Variable	β	*SE*	*Wlad* χ^2	*OR*	*P*	95% CI
Del-1	-1.163	0.632	5.824	0.369	<0.001	0.036~0.931
IL-17	1.236	0.541	7.136	1.654	<0.001	1.119~6.782
Constant	-2.112	0.715	8.943	0.576		

Note: Del-1: developmental endothelial locus-1; IL-17: interleukin 17.

studies. The critical role of Del-1 in the initial stage of inflammatory reaction has been recently reported [18]. Hikaru Tamura [19], by establishing CP models, proposed that Del-1 could immensely inhibit the activity of neutrophils to prevent the occurrence of destructive inflammation and gingival bone loss. IL-17, mainly produced by Th17 cells, is involved in the pathogenesis of various infectious diseases. Husniah B [20] put forward that the level of salivary IL-17 was significantly elevated in CP patients complicated with dental calculus and increased with the progression of CP. Saliva sample collection is simple and noninvasive. In this study, the whole saliva of participants was collected to determine the levels of Del-1 and IL-17. The results showed that compared with the healthy group, the salivary Del-1 level decreased significantly in the T2DM group and T2DM+CP group, while salivary IL-17 increased dramatically, indicating the association of the down-regulation of Del-1 expression and up-regulation of IL-17 expression in saliva with the CP occurrence in T2DM patients, which is consistent with results of prior studies [16, 17]. Furthermore, by analyzing the correlation between salivary Del-1 and IL-17 and related indicators, it was found in this study that salivary Del-1 was negatively correlated with blood glucose control, inflammation level, and periodontal damage degree, with which IL-17 was positively correlated, indicating that the involvement of Del-1 and IL-17 in the process of periodontal disease in T2DM patients may be related to the promotion of inflammatory reaction and the interference of glucose metabolism. *Logistic* regression analysis revealed that the decline of salivary Del-1 and the increase of IL-17 were risk factors of CP in patients with T2DM, further confirming the close correlation of salivary Del-1 and IL-17 with CP in patients with T2DM, which provides a new clinical reference for the prevention and treatment of T2DM and CP.

In conclusion, Del-1 is downregulated and IL-7 is upregulated in the saliva of patients with T2DM complicated with CP, suggesting the correlation of the two with CP in T2DM patients.

Disclosure of conflict of interest

None.

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