

Comparative Study of Interleukin-17 and Lipid Levels in the Patients with Moderate to Severe Periodontitis and CHD*

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ABSTRACT Objective: To investigate the relationship between moderate or severe periodontitis and coronary heart disease (CHD) and study the function of serum Interleukin (IL)-17 levels in the two diseases in China. **Methods:** The Serum IL-17, serum lipids levels and clinical periodontal parameters in 155 patients were detected into 4 groups: 40 patients with moderate or severe periodontitis, 28 patients with the CHD and 47 with periodontitis and CHD and 40 healthy numbers as the control group. The serum lipids contains the low density lipoprotein-cholesterol (LDL-C), the high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG). The clinical periodontal parameters contains clinical attachment loss (CAL), probing depth (PD), and bleeding on probing (BOP). **Results:** The serum IL-17 levels in control group, moderate or severe periodontitis patients, CHD patients and patients with both diseases were (13.01 ± 1.23), (24.45 ± 2.13), (59.90 ± 2.23) and (68.87 ± 3.43) ng/L, respectively. The differences among these four groups were statistical significance ($P < 0.05$). The multivariate logistic regression revealed that the possibility of CHD in periodontitis patients was higher than that in the healthy group (OR=2.416 95% CI: 1.126-6.659). The total cholesterol levels were also significantly different among the four groups ($P = 0.018$). **Conclusions:** The severe periodontitis could affect the happening and development of Systemic inflammation and CHD by changing the interleukin 17 levels, which should be a risk factor for CHD.

Key words: Interleukin (IL)-17; Periodontitis; Coronary disease; Risk factors

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Introduction

Coronary heart disease (coronary atherosclerotic heart disease, CHD) is a disease that seriously threaten human health. The disease is a chronic inflammatory disease mediated by T cell subsets, and many vascular wall cells factors contributed to the occurrence and development of the disease. The basic lesion of the disease, which is in essence atherosclerosis (atherosclerosis, AS), is caused by the inflammation: a large number of studies have shown that the chronic responses to infection may be a risk factor for CHD. Chronic periodontitis is a disease caused by microbial infection. A highly correlated with CHD [1,2], but it is still not clear that whether a common risk factor or causal relationship between them and the biological basis, the mechanism of action related to Th17 cells discovered a new subtype of CD4⁺ T cells in recent years, which secrete many cytokines such as IL-17A, and the majority of studies indicate that during the occurrence and development of AS, IL-17A promotes the occurrence of AS [3], and some researchers found that IL-17 was also highly expressed in periodontitis patients [4]. Nowadays there was no report on the changes in levels of IL-17 in the moderate or severe periodontitis patients with the CHD. By studying the differences of IL-17 levels in serum lipid in healthy persons, patients with periodontitis, patients with CHD

and patients suffered CHD and periodontitis, the correlation of the moderate or severe periodontitis and CHD during the Han was analyzed, and the possible role of IL-17 in the correlation was explored.

1 Materials and methods

1.1 Object selecting and grouping

The subjects of this study included 155 cases, including 40 healthy person (healthy group), male 21, female 19, the average age (47.7 ± 5.7) years selected by Green Fuyuan health checkup center; 40 patients with severe periodontitis (periodontitis group), 23 males and 17 females, with the mean age (50.9 ± 5.0) years chosen from patients having been treated in The hospital affiliating the medical college of Qingdao University dental; 28 patients with coronary heart disease (CHD), 16 males and 12 females, mean age (54.6 ± 4.6) years diagnosed by the Cardiology in the hospital affiliating the medical college of Qingdao University; and 47 patients suffering from coronary heart disease and also moderate or severe periodontitis (the CHD + periodontitis group), 29 males and 18 females, mean age (54.4 ± 4.9) years of age. The criteria for patient selection is as follows: (1) the Han nationality; (2) 35-65 years old; (3) treatments without any antibiotics, nonsteroid anti-inflammatory drugs or immunosuppressant within the

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previous 3 months; (4) with no periodontal therapy in the past 6 months; (5) women without pregnancy or lactation; (6) no-smoking during the last 5 years. (7) patients in periodontitis groups without coronary heart disease, diabetes, or other systemic diseases; patients in coronary heart disease group without diabetes or any other systemic diseases. Questionnaires in the study included blood pressure (systolic and diastolic blood pressure), height and body mass index (body mass index, BMI, $BMI = \text{weight} \div \text{height}^2$), and education level. All subjects were confirmed willing to participate in this study.

1.2 The diagnosis of CHD

The diagnosis of CHD was carried by a Cardiologist. The diagnostic criterias: the health condition questionnaire (confirm whether a coronary heart disease history and family history), conventional ECG examination (ecg ST segment, T wave and QT period of change between) and coronary angiography (diagnosis standard: the left anterior descending coronary artery, whirling teams right coronary artery (or at least have a narrow degree more than 50%).

1.3 Diagnosis of moderate or severe periodontitis

It was completed by one trained physician. The test items: attachment loss (AL), probing depth (PD), bleeding on probing (BOP), (0 = no bleeding on probing, 1 = bleeding on probing), each tooth was detected in six sites (distal Surfaces including ,and lingual surface, middle Surfaces including ,and lingual surface and Mesial Surfaces including ,and lingual surface). The mean was supposed as the measurement results of the teeth, and the mean of full mouth teeth was subjected as the measurement results of the patient; At the same time BOP was detected of each tooth on six sites, the percentage of BOP sites of full-mouth teeth sites as the the measurement results of the subjects.

Periodontal health or gingivitis was defined as: the average $AL \leq 0.5\text{mm}$, the number of interproximal site $AL \geq 3\text{ mm}$ was 0, the number of missing teeth ≤ 2 (except for the third molars or the teeth missed because of orthodontic needs, trauma or severe caries and congenital missing); moderate or severe periodontitis is defined as: mean $AL \geq 1.6\text{ mm}$, the sites of interproximal $AL \geq 3\text{ mm}$ located in three different sections, or at least 6 teeth, the number of missing teeth ≤ 14 (ditto)^[5].

1.4 Detection of serum IL-17

Fasting venous blood 5 ml, 4°C 3500 r / min centrifugation for 15 min (centrifuge radius of 8 cm), the separation of serum collected in sterile test tube, sealed shading, stored in refrigerator of -70°C low temperature. Laboratory Test: the sample were assayed by ELISA for IL-17 according to the manufacturer's recommendations (senxiong science and technology industrial Co., LTD, shanghai).

1.5 Detection of serum lipid levels

The determination of low density lipoprotein-cholesterol

(LDL-C), high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG) were carried by the automatic biochemical analyzer (Olympus AU2700, Hitachi, Japan).

1.6 Statistical analysis

Statistical analyses were performed using software SPSS 17.0. Sex between each were compared by using chi-square test (chisquare test); The t-test was used to compare AL and PD of healthy group and periodontitis groups; The comparisons of BOP were performed by using the chi-square test; The age, blood pressure, body mass index and periodontal clinical indices of each test group were compared by using one-way ANOVA; The serum levels of IL-17 and LDL-C, HDL-C, TC, TG levels of each test group were compared by using ANOVA and least significant difference method (least significant difference, LSD) multiple comparisons and analysis of covariance (ANCOVA); The impact of periodontitis disorders on coronary heart disease were analysed by using multivariate Logistic regression. Significance for all statistical tests was predetermined at $P < 0.05$.

2 Results

2.1 The comparative study of the general conditions periodontal linical index and the blood lipid levels in this research

The gender distribution between each group were the same in this research ($p = 0.946$). The mean age of patients with CHD and patients with CHD and CP were older than that in the control and CP groups ($P < 0.05$). The education level of the control group were significantly higher than that in the CP group, CHD group and CHD+ CP group ($P < 0.05$), and there was no significant difference between the three later groups in the education level. The blood pressure and BMI in patient with CHD and CHD+CP groups were more higher than that in the healthy and CP groups ($P < 0.05$, Table 1). The group design t-test, chi-square, and after adjusting for age, education status by ANOVA, PD, AL and BOP of moderate or severe periodontitis subjects (CP group and CHD+CP group) were significantly higher than the periodontal health subjects (healthy group and CHD group), ($P < 0.05$ Table 2). One-way ANOVA showed that it increased significantly in LDL-C and TC levels for patients with CHD, there are statistically significant differences ($P < 0.05$) between the serum levels of LDL-C and TC of each groups; After correlating age, educational status, blood pressure and BMI revealed that for patients with coronary heart disease, TC levels remained significantly elevated, TC levels in serum between the groups remained statistically significant difference ($P = 0.018$, Table 3).

2.2 The comparison of IL-17 levels in serum of each groups

One -way ANOVA showed that IL-17 levels of the healthy group, CP group, CHD group, and CHD + CP group were (13.01 ± 1.23), (24.45 ± 2.13), (59.90 ± 2.23) and (68.87 ± 3.43) ng /

L. The serum levels of IL-17 was statistically significant difference blood pressure and BMI ,there are still significant differences in (P <0.05) by ANOVA after correlating age, educational status, serum levels of IL-17 (P <0.05, Table 4).

Table 1 The general situation of the 4 groups of subjects

Group	N	age	M	FEM	EDU(year)	SBP(mmHg)	DBP(mmHg)	BMI(kg / m ²)
H	40	47.7 ± 5.7	21	19	10.0± 3.2	107.2± 7.2	78.3± 5.5	23.2± 0.4
CP	40	50.9 ± 6.0	23	17	7.8 ± 2.8	104.8± 6.5	77.1± 5.2	25.1 ± 0.5
CHD	28	54.6 ± 4.6	16	12	6.2± 2.5	141.0± 7.0	105.1± 7.2	26.3 ± 1.3
CHD+CP	47	54.4 ± 4.9	29	18	6.3± 2.7	140.1 ± 5.5	104.2± 7.3	26.0 ± 1.2
P	-	<0.05	0.946		<0.05	<0.05	<0.05	<0.05

Table 2 The comparison of Periodontal clinical indicators($\bar{x} \pm s$)

Group	N	PD(mm)	AL(mm)	BOP
H	40	0.80± 0.11	0.41± 0.03	36± 7
CP	40	3.23± 0.13	2.34 ± 0.34	82± 7
CHD	28	1.24 ± 0.23	0.78± 0.33	37± 16
CHD+CP	47	3.14 ± 0.25	2.44± 0.34	77± 14
P	-	<0.05	<0.05	<0.05
P-	-	<0.05	<0.05	<0.05

After adjustment for age and the education condition

Table 3 Comparison of blood biochemical indices (mmol / L, $\bar{x} \pm s$)

Group	N	TG	TC	LDL	HDL
H	40	1.07± 0.11	3.24± 0.23	1.43± 0.34	1.84± 0.66
CP	40	1.20± 0.13	3.43± 0.30	1.45± 0.54	1.55 ± 0.45
CHD	28	1.42 ± 0.23	4.35± 1.11	2.56± 0.45	1.34 ± 0.32
CHD+CP	47	1.82 ± 0.43	4.45± 0.89	2.35± 0.46	1.25± 0.24
P	-	0.252	0.001	<0.001	0.104
P-	-	0.760	0.018	0.145	0.345

After adjustment for age, education condition, by blood pressure and body mass index

Table 4 Comparison of IL-17 level in serum (ng / L, $\bar{x} \pm s$)

Group	N	IL-17
H	40	13.01 ± 1.03
CP	40	24.45± 2.13
CHD	28	59.90 ± 2.24
CHD+CP	40	68.87 ± 3.43
P	-	<0.05
P-	-	<0.05

After adjustment for age, education condition, by blood pressure and body mass index

2.3 Correlation analysis and regression analysis results

Table 5 showed that only periodontal clinical parameters and serum IL-17 levels were significantly correlated (P<0.05). Multivariate Logistic regression analysis showed that excluding the effect of blood pressure and BMI, moderate or severe periodontitis patients had higher likelihood of suffering from coronary heart disease than the periodontal healthy subjects , their relative risk index

OR = 2.416 (P = 0.039; 95% CI: 1.126 ~ 6.659), that was, the risk of moderate or severe periodontitis patients suffering from coronary heart disease was 2.416 times higher than those of periodontal health subjects. Blood pressure and BMI also had significant effect (P=0.017 and P=0.012) on the incidence of coronary heart disease, the OR value (2.627) is similar to that of periodontal disease (2.219)(Table 6).

Table 5 Serum IL-17 and lipid levels and periodontal clinical index correlation analysis(r-value)

	AL	PD	BOP	IL-17
TC	0.150	0.125	0.109	0.286
HDL-C	-0.112	-0.080	-0.156	-0.287
TG	0.112	0.108	0.098	0.273
IL-17	0.403 ^a	0.346 ^b	0.377 ^c	1

Table 6 Periodontal inflammatory, blood pressure and body mass index to the regression analysis of coronary artery disease

	PRC	SE	Wald	P	OR	95% CL
CP	0.938	0.386	1.564	0.039	2.416	1.126-6.659
BP	1.127	0.372	1.705	0.017	2.627	1.168-6.713
BMI	1.096	0.383	1.361	0.012	2.219	1.207-6.998

3 Discussions

A large number of epidemiological and clinical studies had shown that moderate or severe periodontitis are highly related to the CHD, and it might be a risk factor for the CHD^[12,6]. Arbes^[7] studied the relationship between periodontal status and coronary heart disease of 5564 patients aged over 40, had found that, after eliminating the effect of age, gender, race and other factors, the OR were 1.38, 2.28 and 3.77 of the patients whose AL \geq 3 mm teeth accounting for the remaining teeth in the mouth 0 ~ 33%, 33% to 67% and 67% and had got coronary heart disease. This study ruled out the role of smoking, diabetes, and gender, and established a multiple Logistic regression model, correlating for some unrelated factors such as age, educational status, and blood pressure and BMI, and finally found: there was higher possibility to suffer from CHD for the moderate or severe periodontitis than the healthy group, OR = 2.416 (P = 0.039; 95% CI: 1.126 ~ 6.659), that was, the moderate or severe periodontitis patients take 2.417 times higher risk at CHD than the healthy group, the same as the results of Genco^[8], etc. and Morrison,^[9] etc. (OR = 2.68 and 2.15, respectively).

The study also found that the patients' blood pressure and BMI had obvious effect on the occurrence of CHD (P = 0.017, P = 0.012), and OR value of blood pressure and the BMI were similar to that of periodontal inflammation. Offenbacher's^[10] studies had showed that the periodontitis had a similar OR value with classic risk factors such as serum lipid, blood pressure, BMI, and smoking, which is similar to our results. Although a large number of studies had showed that the periodontitis was associated with the occurrence of CHD, it was not very clear about the biological basis and mechanism between the two factors. Serum levels of IL-17 can affect the occurrence and development of the CHD. Biological activity of IL-17 can be summarized as (1)-induced the production of cytokines, such as IL-6, IL-8, TNF- α , etc. IL-17 is not only a powerful pro-inflammatory cytokines, but also a fine-tune the inflammatory response factor^[11]. (2) IL-17 is able to have synergetic

function with other cytokines to enlarge the inflammatory response^[12].

(3) IL-17 has a powerful role in attracting neutrophils. (4) Recently IL-17 is found to be able to cause bone absorption^[13,14]. Patel^[15] found that IL-17 could promote smooth muscle cells to express CRP and the development of atherosclerosis^[7]; Intensify myocardial inflammation and injury by up-regulating the level of IL-6 and other pro-inflammatory pro-apoptotic factors; Promote atherosclerotic plaque formation and rupture through activating NF-KB channels to accelerate the production of atherosclerotic factors (such as tumor necrosis factor) and chemical factors such as matrix metalloproteinases.

Differences in age, educational status, blood pressure and BMI of the study among the groups was statistically significant (P < 0.05), while blood pressure and BMI were all-known risk factors for the CHD. Therefore, it was justified to correlate age, educational status, blood pressure and BMI before analyzing the relationship of periodontitis and the CHD. The analysis of covariance after rectifying age, educational status, blood pressure and BMI showed that the differences of serum levels of IL-17 had statistical significance (P < 0.05). The results suggest that, IL-17 may act as an intermediary role in increasing the risk of coronary heart disease for those suffering from periodontitis; Zhou-yan^[16] found that IL-17 levels were significantly higher in patients with periodontitis, Takahashi^[17] researched IL-17 production and expression patterns on gene and protein levels, the results showed: IL-17 had appeared in periodontal damage specimens, accompanying with some Th1 cytokines such as IL-2 and IFN- γ expression^[8]; IL-17 induced gingival fibroblast cells to produce IL-6 by dose- time-dependent manner in vitro. IL-17 and cells- produced RANKL and RANK jointly act to directly stimulate the differentiation of the osteoplast cell, to promote secretion of TNF- α , IL-2, IL-6, and indirectly to increase expression of RANKL in fibroblast^[18], thus activate the NF-kB, adjust the differentiation and maturation of osteoplast cell, finally cause bone destruction^[19,20]. This research showed that, the more IL-17 concentration in serum was, the more serious clinically relevant periodontal damage was, (the two has a signifi-

cant positive correlation) especially closely related with AL. This showed that the severity of periodontitis has correlation with the levels of IL-17. Hashmi^[21] found that, compared with healthy controls, patients with unstable angina (UA) or acute myocardial infarction (A MI) had higher levels of pro-inflammatory cytokine IL-17, IL-6, IL-8 and CRP^[12]; Those with complex coronary artery disease higher levels in IL-17 than simple lesions; The same as patients with or without traditional risk factors of the CHD in contrary to the healthy. The results showed that, the periodontitis alone or together with coronary heart disease significantly influenced IL-17 level, and the periodontitis with coronary heart disease have more obvious effect on the level of IL-17 in serum. This study also suggested that severe periodontal infection could affect the level of inflammatory mediators in the system, and these changes in levels of inflammatory mediators could also affect the incidence and development of the CHD.

The study showed that, without correlating age, educational status, blood pressure and BMI in the case, there were significantly differences between ($P < 0.05$) the serum levels of LDL-C and TC each group, while there were no significant differences between ($P = 0.103$ and $P = 0.257$) HDL-C and TG levels. The analysis of covariance after correlating age, educational status, blood pressure and BMI showed that the difference of serum TC level each group was still statistically significant ($P = 0.017$); There was no statistical significance ($P = 0.146$, $P = 0.344$ and $P = 0.761$) for the differences of LDL-C, HDL-C and TG levels. LDL-C, HDL-C, TC and TG and other known risk factors for the CHD and blood pressure and BMI may have synergetic effects on the incidence of the CHD, periodontal tissue inflammation may affect lipid metabolism and thus alter lipid levels. Taking into account that the LDL-C, HDL-C, TC and TG levels themselves may be closely associated with periodontitis, so if they are to be corrected, over-correction may appear, that we uncorrected LDL-C, HDL-C, TC and TG in the analysis of IL-17 in correlation of the moderate or severe periodontitis and coronary heart disease in this study.

In this study, by contrasting and analyzing the levels of IL-17, LDL-C, HDL-C, TG, TC and periodontal clinical indices in serum of four groups of subjects, it was found that patients suffering with from moderate or severe periodontitis had higher risk of suffering from CHD than periodontally healthy subjects (OR = 2.416; 95% CI: 1.126 ~ 6.659); Severe periodontal infection may be in turn affect the systemic inflammatory response and the occurrence and development of the CHD by changing the levels of IL-17 in serum, which may be one of risk factors of CHD. It need to increase the sample size and completing datas such as reasons for tooth loss and family history of CHD for further study.

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中、重度牙周炎与冠心病患者白细胞介素 17 及血脂水平的比较 *

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摘要 目的 研究汉族人群中、重度牙周炎与冠心病的相关性并初步探讨白细胞介素 17 在二者相关性中的可能作用。方法 检测和分析 40 名健康者(健康组)、40 例中、重度牙周炎患者(牙周炎组)、28 例冠状动脉粥样硬化性心脏病患者(冠心病组)及 47 例患冠心病伴中、重度牙周炎的患者(冠心病 + 牙周炎组)血清白细胞介素 17 水平、血脂水平(血清低密度脂蛋白、高密度脂蛋白、胆固醇、总胆固醇和甘油三酯)和牙周临床指数(附着丧失、探诊深度和探诊出血)。结果 单因素方差分析结果显示,健康组、牙周炎组、冠心病组及牙周炎 + 冠心病组的血清白细胞介素 17 水平分别为(13.01 \pm 1.23)、(24.45 \pm 2.13)、(59.90 \pm 2.23)和(68.87 \pm 3.43)ng/L,各组血清白细胞介素 17 间的差异具有统计学意义($P < 0.05$),且经协方差分析校正年龄、受教育状况、血压和体重指数后显示,各组血清白细胞介素 17 水平间的差异仍具有统计学意义($P < 0.05$)。多元 Logistic 回归分析结果显示,中、重度牙周炎患者发生冠心病的可能性高于牙周健康者,其发生冠心病的相对风险率比值为 2.416($P = 0.039$, 95%CI :1.126-6.659)。经协方差分析校正年龄、受教育状况、血压和体重指数后,各组血清总胆固醇水平间差异仍具有统计学意义($P = 0.018$)。结论 严重的牙周感染可能通过改变白细胞介素 17 水平,影响全身炎症反应和冠心病的发生及发展,可能是冠心病事件的危险因素之一。

关键词 白细胞介素 17;牙周炎;冠状动脉疾病;危险因素

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