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·基础研究·

# 中老年2型糖尿病牙周炎患者龈沟液抗菌肽水平及其与牙周临床指标的相关性

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**【摘要】 目的** 探究牙周炎合并2型糖尿病(type 2 diabetes mellitus, T2DM)患者龈沟液中抗菌肽人-β-防御素-2(human beta-defensin-2, HBD-2)和LL-37的水平变化规律及其与牙周临床指标的相关性。**方法** 选取于深圳市慢性病防治中心口腔科和内科就诊的中老年人(45至85岁)为研究对象，分为全身健康并牙周健康对照组22人、全身健康牙周炎组19人、T2DM牙周健康组15人、T2DM合并牙周炎组21人。采用佛罗里达牙周探针进行牙周检查，记录受试者牙周临床指标，包括探诊深度(probing depth, PD)、附着水平(attachment level, CAL)和探诊出血(probing bleeding, BOP)。采用酶联免疫吸附法检测龈沟液中抗菌肽HBD-2、LL-37浓度，比较组间抗菌肽HBD-2、抗菌肽LL-37及牙周临床指标间的差异，并进行相关性分析。**结果** T2DM合并牙周炎组的PD值大于全身健康牙周炎组($P < 0.05$)；全身健康牙周炎组龈沟液中HBD-2和抗菌肽LL-37水平均高于全身健康并牙周健康对照组($P < 0.05$)，全身健康牙周炎组龈沟液中HBD-2水平高于T2DM合并牙周炎组($P < 0.05$)。全身健康牙周炎组龈沟液中抗菌肽HBD-2、LL-37与PD、CAL呈正相关($P < 0.05$)，T2DM合并牙周炎组龈沟液中抗菌肽HBD-2、LL-37与PD、CAL相关性无统计学意义($P > 0.05$)。**结论** 中老年T2DM牙周炎患者龈沟液抗菌肽HBD-2、LL-37水平较低，与牙周临床指标PD、CAL无显著相关性。

**【关键词】** 中老年；2型糖尿病；牙周炎；龈沟液；抗菌肽；LL-37；人-β-防御素-2；探诊深度；附着水平；探诊出血
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**The level of antimicrobial peptides in gingival crevicular fluid and its correlation with periodontal clinical indexes in elderly patients with type 2 diabetic periodontitis**

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**【Abstract】 Objective** To investigate the changes and significance of human beta-defensin-2 (HBD-2) and LL-37 in the gingival crevicular fluid of patients with periodontitis and type 2 diabetes mellitus (T2DM). **Methods** This study was conducted among 45- to 85-year-old patients in the Department of Stomatology and Internal Medicine of Shenzhen Center for Chronic Disease Control, including a healthy control group of 22 people, a systemically healthy control group of 19 people with periodontitis, a T2DM periodontal health group of 15 people, and a T2DM group of 21 people with

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periodontitis. The Florida periodontal probe was used for periodontal examination, and the clinical indexes, including probing depth (PD), clinical attachment level (CAL) and probing on bleeding (BOP), were recorded. The concentrations of HBD-2 and Ll-37 in gingival crevicular fluid were determined by ELISA. The differences in HBD-2, LL-37 and periodontal clinical indexes between the groups were compared, and correlation analysis was conducted. **Results** The PD values in T2DM with the periodontitis group were higher than those of the systemically healthy controls with periodontitis group ($P < 0.05$); the levels of HBD-2 and LL-37 in gingival crevicular fluid in systemically healthy controls with periodontitis group were significantly higher than those in the healthy control group ($P < 0.05$), the level of HBD-2 in gingival crevicular fluid in systemically healthy controls with periodontitis group was significantly higher than that in T2DM with periodontitis group ($P < 0.05$); and the antimicrobial peptides HBD-2 and LL-37 in gingival crevicular fluid were significantly positively correlated with the PD and CAL in systemically healthy controls with periodontitis group ($P < 0.05$), and there was no significant correlation between the antimicrobial peptides HBD-2, LL-37 in gingival crevicular fluid and PD, CAL in T2DM with periodontitis group ($P > 0.05$). **Conclusion** The levels of antimicrobial peptides HBD-2 and LL-37 in gingival crevicular fluid of middle-aged and elderly patients with T2DM periodontitis were lower, and there was no significant correlation with PD and CAL in periodontal clinical indicators.

**【Key words】** middle-aged and elderly; type 2 diabetes mellitus; periodontitis; gingival crevicular fluid; antimicrobial peptides; LL-37; human beta defensins-2; probing depth; attachment level; probing bleeding
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抗菌肽(antimicrobial peptides,AMPs)是一种几乎存在于所有多细胞生物体内的小分子活性多肽。抗菌肽是先天免疫反应的重要组成部分,具有直接的抗菌活性和免疫调节作用[1]。在人类唾液和龈沟液(gingival crevicular fluid, GCF)中发现的抗菌肽主要包括组织蛋白酶抑制素和防御素,抗菌肽LL-37是迄今发现唯一人源性的组织蛋白酶抑制素家族成员[2]。人体内的防御素根据分子结构不同又可分为α防御素和β防御素。牙周炎是一种发生在牙周组织的破坏性炎症疾病[3]。有研究表明抗菌肽人β防御素-2(human beta defensins-2, HBD-2)基因组拷贝数低会增加患严重慢性牙周炎的风险[4],唾液和龈沟液抗菌肽LL-37水平与深袋牙和临床附着丧失呈正相关[5]。抗菌肽HBD-2和抗菌肽LL-37与牙周炎之间存在着密切联系。

与牙周炎相关的一些全身危险因素(如吸烟、糖尿病、肥胖等)也会引起抗菌肽的表达失调[6]。糖尿病是一种以血糖水平升高,糖耐量降低为主要症状的代谢性疾病,其中具有胰岛素抵抗的2型糖尿病(type 2 diabetes mellitus,T2DM)发病机制与慢性炎症反应密切相关,是牙周炎的重要危险因素之一[3]。体外实验显示高糖环境下角质形成细胞分泌HBD-2显著减少[7]。Arampatzioglou等[8]发现糖尿病患者抗菌肽LL-37表达降低与创面难以愈合和继发感染相关。但是关于2型糖尿病人群龈沟液中抗菌肽的变化规律研究尚不充分。本试验研究牙周炎合并T2DM患者龈沟液中抗菌肽LL-37、HBD-2水平的变化及其与牙周临床指标的相关性,旨在探讨龈沟液中抗菌肽与T2DM和牙周炎之间的关系及其互相影响机制,为阐明糖尿病患者牙周破坏的致病机理、早期诊断提供基础。

## 1 材料和方法

### 1.1 主要试剂和仪器

弗罗里达探针(Florida Probe,美国);滤纸条(Whatman,英国);酶联免疫吸附法(ELISA)试剂盒Human BD-2/beta Defensin-2 ELISA Kit(Arigobio,中国),Human LL-37 ELISA Kit(HycultBiotech,荷兰);Multiskan FC型酶标仪(Thermo,美国)。

### 1.2 研究对象

本研究选择2019年11月至2020年6月到深圳市慢性病防治中心口腔科和内科就诊的患者77人,平均年龄为(60.35 ± 8.87)岁。该研究获得了深圳市慢性病防治中心伦理委员会批准(批准号:20181202),所有志愿者均在取样和收集信息前签署了书面知情同意书。

纳入标准:年龄45~85岁;受试者口内至少有

20颗牙齿。排除标准：患有T2DM之外的任何全身性疾病；3个月内使用过抗生素的患者；6个月内接受牙周治疗的患者。

分组标准：根据1999年世界卫生组织提出的糖尿病诊断标准进行T2DM诊断，按照美国牙周学会和欧洲牙周联盟提出的2018年牙周病和植体周病国际新分类[9]进行牙周炎诊断。根据参与者的全身和牙周健康状况分为4个研究组：全身健康并牙周健康对照组（健康对照组）22人、全身健康牙周炎组19人、T2DM牙周健康组15人、T2DM合并牙周炎组21人。

1.3　*方法*

1.3.1　牙周临床指标收集　采用弗罗里达探针对每颗牙齿的颊（唇）、舌侧分别在近中、中央、远中6个位点进行探诊检查（除了第三磨牙和被牙龈覆盖的残根）。检查内容包括探诊深度（probing depth，PD）、临床附着力丧失（attachment level，CAL）；根据探测30 s内是否存在出血将探诊出血（probing bleeding，BOP）分为阳性和阴性，记录并计算探诊出血阳性位点所占百分比（BOP%）。每位患者的PD、BOP%值为上述各部位的平均值，CAL值为上述各部位的最大值。牙周检查均由一位已进行培训的口腔科医生完成，并做标准一致性检验，Kappa > 0.75。

1.3.2　龈沟液的收集　牙科三用枪清洁牙面上的软垢后，棉卷隔湿患者的待检测牙位，在四颗第一磨牙的近中、远中及颊侧龈沟或牙周袋内分别插入3条2 mm × 10 mm滤纸条，并放置30 s。若第一磨牙缺失，则检测该区第二磨牙。将同一受试者的12条滤纸条一并放入1.5 mL离心管中，并加入500 μL的PBS液并保存在-80 ℃冰箱中备用。

1.3.3　抗菌肽的检测　保存待测的龈沟液样本与试剂盒常温放置30 min，加入缓释液离心后，保留上清液，使用ELISA试剂盒Human BD-2/beta Defensin-2 ELISA Kit和Human LL-37 ELISA Kit检测龈沟液中HBD-2和LL-37浓度。

1.4　*统计学分析*

采用SPSS 23进行统计学分析。Shapiro-Wilk检验显示数据均不服从正态分布，数据用中位数（第一四分位数，第三四分位数）表示，多组间比较使用Kruskal-Wallis $H$检验，两组间比较使用Mann-Whitney $U$检验；Spearman相关检验分析抗菌肽与临床牙周参数之间的相关性。$P < 0.05$为差异有统计学意义。

## 2　结　果

2.1　*全身健康组与T2DM组牙周临床指标比较*

如表1所示：T2DM牙周健康组与健康对照组的PD、CAL、BOP牙周临床指标值差异不明显（$P > 0.05$）；T2DM合并牙周炎组的PD值大于全身健康牙周炎组（$P < 0.05$）。

**表1**　全身健康组与T2DM组牙周临床指标比较

Table 1　Comparison of the periodontal clinical indexes between the healthy group and T2DM group　Median（IQR）

| Groups | PD（mm） | *P* | CAL（mm） | *P* | BOP % | *P* |
|---|---|---|---|---|---|---|
| Healthy control group | 1.63（1.49，1.75） | 0.191 | 1.48（1.30，1.53） | 0.636 | 8.00（3.50，14.25） | 0.963 |
| T2DM with periodontal health group | 1.69（1.57，1.83） | | 1.42（1.24，1.72） | | 9.00（4.00，11.00） | |
| Systemically healthy controls with periodontitis group | 2.35（2.06，2.71） | 0.044 | 3.25（2.86，3.63） | 0.567 | 18.00（14.00，34.00） | 0.409 |
| T2DM with periodontitis group | 2.65（2.38，3.01） | | 3.23（2.76，3.93） | | 14.00（6.50，25.00） | |

PD: probing depth; CAL: attachment level; BOP: probing bleeding; IQR: inter-quartile range

2.2　*各组龈沟液中抗菌肽水平比较*

比较各组龈沟液中抗菌肽HBD-2和LL-37的水平，如表2所示，全身健康牙周炎组HBD-2和LL-37水平均高于健康对照组（$P < 0.05$）；T2DM牙周健康组HBD-2水平低于健康对照组（$P < 0.05$）；T2DM牙周健康组HBD-2和LL-37水平低于全身健康牙周炎组（$P < 0.05$）；T2DM合并牙周炎组HBD-2水平低于全身健康牙周炎组（$P < 0.05$）；其余组间差异均无统计学意义（$P > 0.05$）。

2.3　*牙周炎龈沟液中抗菌肽水平与牙周临床指标的相关性*

对全身健康牙周炎组和T2DM合并牙周炎组的龈沟液中抗菌肽水平与牙周临床指标进行Spearman相关性分析。如表3所示，全身健康牙周炎组中HBD-2与PD呈正相关（$P < 0.05$），HBD-2与CAL呈正相关（$P < 0.05$），HBD-2与BOP%的相关性无统计学意义（$P > 0.05$）；LL-37与PD、CAL呈正相关（$P < 0.05$），LL-37与BOP%的相关性无统计学

**表2**　各组龈沟液中抗菌肽水平

Table 2　Antimicrobial peptide levels in gingival crevicular fluid of each group　　Median(IQR)

| Groups | HBD-2 (pg/mL) | LL-37 (pg/mL) |
|---|---|---|
| Healthy control group | 42.72(28.29,70.73) | 11.06(5.16,30.41) |
| Systemically healthy controls with periodontitis group | 83.07(66.65,148.82)[1)] | 19.93(6.49,60.07)[1)] |
| T2DM with periodontal health group | 34.80(23.43,54.31)[1)2)] | 9.06(3.17,32.88)[2)] |
| T2DM with periodontitis group | 42.02(23.32,106.40)[2)] | 13.78(4.84,58.94) |
| *H* | 10.451 | 7.727 |
| *P* | 0.015 | 0.041 |

1): compared with the healthy control group group, $P < 0.05$; 2): compared with the systemically healthy controls with periodontitis group, $P < 0.05$

意义($P > 0.05$)。如表4所示,T2DM合并牙周炎组龈沟液中抗菌肽HBD-2、LL-37与牙周临床指标相关性无统计学意义($P > 0.05$)。

**表3**　全身健康牙周炎组龈沟液中抗菌肽水平与牙周临床指标的相关性

Table 3　Correlation between the antimicrobial peptide levels in the gingival crevicular fluid and the periodontal clinical indicators in systemically healthy controls in the periodontitis group

| | PD | | CAL | | BOP% | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| HBD-2 | 0.466 | <0.001 | 0.338 | 0.012 | 0.239 | 0.081 |
| LL-37 | 0.234 | 0.043 | 0.279 | 0.039 | -0.140 | 0.534 |

PD: probing depth; CAL: attachment level; BOP: probing bleeding; HBD-2: human beta defensins-2

**表4**　中老年T2DM合并牙周炎组龈沟液中抗菌肽水平与牙周临床指标的相关性

Table 4　Correlation between the antimicrobial peptide levels in gingival crevicular fluid and the periodontal clinical indicators in elderly patients with T2DM periodontitis

| | PD | | CAL | | BOP% | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| HBD-2 | -0.251 | 0.079 | -0.174 | 0.226 | -0.119 | 0.410 |
| LL-37 | -0.116 | 0.448 | -0.193 | 0.205 | -0.019 | 0.899 |

PD: probing depth; CAL: attachment level; BOP: probing bleeding; HBD-2: human beta defensins-2

## 3　讨　论

### 3.1　全身健康人群龈沟液中抗菌肽水平

牙周组织抵御微生物侵犯的第一道防线包括了牙龈上皮的物理屏障作用和其分泌的抗菌物质(如抗菌肽、溶酶体酶等)所形成的化学屏障[10]。龈沟液为牙龈上皮渗出液,内含牙龈上皮分泌的抗菌物质[11]。本研究发现,与健康对照组相比,全身健康牙周炎组龈沟液中的抗菌肽(包括HBD-2和LL-37)水平升高。Sidharthan和Öztürk等[12-13]发现牙周炎患者龈沟液中HBD-2水平明显高于牙龈炎患者和牙周健康人群,Soldati等[14]发现牙周炎龈沟液中LL-37的水平明显上升,吸烟会导致龈沟液中LL-37降低。但Yilmaz等[15]发现牙周炎时唾液中仅LL-37升高而HBD-2与牙周健康对照组无差异,这种不一致可能因为其采集的样本为唾液而非龈沟液,HBD-2由上皮细胞分泌较多,龈沟液局部环境表达浓度较高,但唾液的稀释作用导致了差异减小,或因为在受炎症影响的牙周组织中,蛋白水解酶活性显著升高,其对HBD-2的降解限制了组织中的HBD-2向唾液的分泌。当牙周致病菌入侵,脂多糖等毒素直接刺激上皮细胞分泌抗菌肽,同时活化单核巨噬细胞分泌白细胞介素-1β(interleukin-1β,IL-1β),IL-1β升高可以刺激活化中性粒细胞,中性粒细胞也可以分泌抗菌肽。HBD-2、LL-37均为诱导性表达的抗菌肽,LL-37主要由中性粒细胞表达,HBD-2更多由上皮细胞分泌[5]。带正电荷的抗菌肽除了静电吸引直接杀菌作用外,还是重要的免疫调剂。抗菌肽作为趋化因子同时诱导其他趋化因子的产生,可抑制脂多糖诱导的促炎细胞因子的产生,调节树突状细胞或T细胞,是先天免疫与获得性免疫的桥梁。Yang等[16]发现LL-37促进角质细胞自噬作用,可减少胞内牙周致病菌。还有研究表明,抗菌肽相关基因的改变也可能与牙周炎症有关,如抗菌肽HBD-2基因组低拷贝会增加严重慢性牙周炎的风险[4]。不同牙周状态下抗菌肽的变化也提示了抗菌肽作为牙周炎生物标志物的可能性。

### 3.2　中老年T2DM患者龈沟液中抗菌肽水平

本研究结果显示中老年T2DM合并牙周炎组的HBD-2显著低于全身健康的牙周炎组，LL-37虽呈降低趋势但无明显统计学差异。有体外研究显示，高葡萄糖浓度下角质细胞功能受损，HBD-2的表达明显下调[17]，免疫细胞分泌LL-37显著减少[18]。牙周炎时T2DM患者唾液中HBD-1，2，3明显低于全身健康人群[15]。目前关于T2DM患者龈沟液内LL-37水平的信息有限。人源阳离子抗菌肽-18（human cationic antimicrobial peptide-18，hCAP-18）是LL-37的前体，可通过外界刺激脱去保守片段成为LL-37活性片段。研究发现糖尿病患者牙龈组织中hCAP18的表达水平上调[19]，另有研究表明糖尿病会引起中性粒细胞功能障碍[8]，如趋化功能受损，这可能是导致T2DM患者牙周组织中hCAP18上调但龈沟液中LL-37活性片段分泌并没有增加的原因。抗菌肽在牙周炎患者龈沟液中会发生降解，特别是在存在牙龈卟啉单胞菌（*Porphyromonas gingivalis*，*P.g*）的位点，而T2DM人群的口腔*P.g*检出率明显高于无系统性疾病的人群[20]。*P.g*对抗菌肽的降解作用也可能是T2DM患者龈沟液中抗菌肽下调的原因之一。

### 3.3　龈沟液中抗菌肽水平与牙周临床指标相关性

牙周临床指标PD值和CAL值能直接反映牙周炎的破坏程度，本研究显示全身健康牙周炎组HBD-2、LL-37与CAL显著正相关，这与Ramamoorthy等[21]研究结果相似，提示炎症刺激了抗菌肽上调进而发挥其抗菌、免疫调节等作用。体外试验显示β防御素还可以促进人类间充质干细胞、成骨细胞和角质形成细胞的增殖[22]。Peng等[23]发现β防御素可促进骨再生和防止感染。有研究表明抗菌肽LL-37可增加牙龈成纤维细胞产生基质金属蛋白酶-1的组织抑制剂，可调节成纤维细胞在牙周组织重建的反应[24]。Yu等[25]发现LL-37可通过P2X7受体和MAPK信号通路减弱脂多糖（lipopolysaccharide，LPS）对成骨的抑制作用。以上体外试验均从机制层面解释了HBD-2和LL-37的牙周保护作用。此外，人工合成抗菌肽可用于治疗糖尿病足、牙周炎感染[26]。有研究发现激光照射诱导LL-37和HBD-2的表达，可促进组织愈合和清除*P.g*[27]，这也提示了HBD-2和LL-37在牙周组织中，尤其是细菌入侵、炎症反应时产生的保护作用。与全身健康牙周炎组不同，T2DM合并牙周炎组HBD-2和LL-37与牙周临床指标的相关性没有统计学意义，这可能是因为糖尿病对抗菌肽的抑制作用使得牙周炎症反应时抗菌肽无法升高至正常浓度发挥其牙周保护功能，导致了T2DM患者牙周破坏的进一步加重。

综上，全身健康人群患牙周炎时，抗菌肽参与到免疫调节中，对牙周病原微生物有明显的抑制和消灭作用，牙周破坏加重时，抗菌肽会进一步升高起到牙周保护作用。中老年T2DM患者龈沟液中抗菌肽HBD-2的减少可能是牙周感染加重的原因之一。抗菌肽LL-37与中老年T2DM患者牙周健康的关系还需更大样本量的临床试验进一步验证。

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