The detection of subgingival microflora contents of type 2 diabetes patients with periodontitis and its correlation with gingival crevicular fluid cytokine levels

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ABSTRACT

Objective: To study subgingival microflora contents of type 2 diabetes patients with periodontitis and its correlation with gingival crevicular fluid cytokine levels. Methods: Type 2 diabetes patients with periodontitis and simple periodontitis patients treated in our hospital from March 2012 to August 2014 were selected for study and enrolled in diabetes with periodontitis group and simple periodontitis group respectively. Then subgingival microflora contents and levels of cytokines in gingival crevicular fluid were detected. Results: (1) Subgingival microflora: compared with simple periodontitis group, contents of porphyromonas gingivalis aeromonas, actinomycetes with haemophilus, Forsyth Tanner bacteria, middle Prairie Waugh bacteria and Treponema denticola of diabetes with periodontitis group were higher; (2) Antioxidants and oxidation products: compared with simple periodontitis group, MPO, CAT, SOD, GSH and VitC contents of diabetic with periodontitis group were lower; MDA and 8-OHdG contents were higher; (3) Signaling molecules: compared with simple periodontitis group, SFRP1, Fas, Fasl, Wnt5a, NF-kb and p38 contents of diabetic with periodontitis group were higher. Conclusion: Contents of pathogens in subgingival microflora of type 2 diabetes patients with periodontitis significantly increase; antioxidants are extensively consumed and oxidation products are largely generated; there is dysfunction of multiple signaling pathways.

1. Introduction

Type 2 diabetes with periodontitis is a common complication in patients with diabetes. Diabetes can directly increase the risk of periodontitis and gingivitis. Unfavorable blood glucose control is a risk factor that causes the development of periodontal inflammation and the aggravation of tissue destruction[1]. Gingival crevicular fluid is a good medium for many bacteria. Massive reproduction of bacteria and its attachment to the teeth can form dental plaque, which will lead to the occurrence of periodontitis. Porphyromonas gingivalis aeromonas, actinomycetes with haemophilus, Forsyth Tanner bacteria, middle Prairie Waugh bacteria and Treponema denticola are five known periodontal pathogens. Exploring pathogen contents of type 2 diabetes patients with periodontitis and simple periodontitis patients is helpful to clarify the pathogenesis of the disease. Meantime, the development of periodontitis is accompanied by changes of a variety of components in gingival crevicular fluid and involves oxidative stress, inflammatory response, bone absorption, apoptosis, and so on. In the following research, detection of subgingival microflora contents of type 2 diabetes patients with periodontitis and its correlation with levels of cytokines in gingival crevicular fluid were analyzed.

2. Object and methods

2.1. Research objects

Prospective studies were designed. Type 2 diabetes patients with periodontitis and simple periodontitis patients treated in our hospital from March 2012 to August 2014 were selected for study. 30
cases of type 2 diabetes patients with periodontitis and 30 cases of simple periodontitis patients were randomly screened and enrolled in diabetes with periodontitis group and simple periodontitis group respectively. Diabetes with periodontitis group included males/females: 18/12 cases with age range of (57.34±7.71); simple periodontitis group included males/females: 19/11 cases with age range of (57.86±6.94). There were no differences between two groups’ general data (P>0.05).

2.2. Sample collecting methods

2.2.1. Subgingival microflora collecting methods

On the day of enrolling, subgingival microflora of both groups was collected. Details were as follows: subgingival plaque of all patients was collected from the same location. At first, wet insulation treatment was conducted. Supragingival plaque was removed and dental surface was dried off with air gun; then sterile filter paper point was inserted into the bottom of the periodontal pocket and kept still for 20 s to fully wash off the subgingival plaque. Then it was taken out and put in 1.5 mL EP tube. After adding 1 mL PBS solution, it was preserved at -80 °C.

2.2.2. Gingival crevicular fluid collecting methods

After subgingival microflora collection was finished, subgingival plaque was completely cleared. Then dental surface and gum were dried off. Sterile filter paper was taken and inserted into gingival sulcus, kept still for 60 s and taken out. Weights of the filter paper before and after sampling were weighed and subtracted to get the weight of gingival crevicular fluid. PBS at a volume of 10 g/L was added to wash filter paper. Washing liquid was collected and preserved at -80 °C.

2.3. Index detecting methods

Genomic DNA extraction kit from TIANGEN Company was used to extract genomic DNA in the plaque. Primer of 16s-rDNA was designed and PCR amplification was conducted, products for agarose gel electrophoresis. After getting the DNA bands corresponding to different microflora, microflora contents were calculated. RIP was used to detect contents of MPO, CAT, SOD, GSH, VitC, MDA and 8-OhdG in GCF. RNA in GCF was extracted through Trizol, RT for PCR amplification; mRNA contents of SFRP1, Fas, FasL, Wnt5a, NF-kb and p38 were detected.

2.4. Statistical methods

Detected data was input by SPSS18.0 software, differences between two groups for t test. Differences were considered to be statistically significant at a level of P<0.05.

3. Results

3.1. Pathogen contents

That pathogens massively reproduce and become dominant microflora is the key link that causes periodontitis. In the research, PCR was used to amplify 16s-rDNA in subgingival microflora. Then image information was obtained through gel electrophoresis. Microflora contents corresponded to different bands were calculated. T test analysis of the results showed that compared with simple periodontitis group, contents of porphyromonas gingivalis aeromonas, actinomycetes with haemophilus, Forsyth Tanner bacteria, middle Prairie Waugh bacteria and Treponema denticola of diabetes with periodontitis group were higher.

3.2. Antioxidants and oxidation products

Oxidative stress response hyperthyroidism is an important link that causes type 2 diabetes with periodontitis. The process mainly involves extensive consumption of antioxidants and large generation of oxidation products. In the research, contents of antioxidants and oxidation products in GCF were detected. T test analysis of the results showed that compared with simple periodontitis group, contents of antioxidants MPO, CAT, SOD, GSH and VitC of diabetes with periodontitis group were lower; contents of oxidation products MDA and 8-OhdG were higher.

3.3. Signaling molecules

In the progressing process of periodontitis, there are abnormal bone metabolism and inflammatory response. Multiple signaling pathways participate in the regulation of bone metabolism and inflammatory response. In the research, mRNA contents of related
signaling molecules in GCF were detected. T test analysis of the results showed that compared with simple periodontitis group, contents of SFRP1, Fas, FasL, Wnt5a, NF-k B and p38 of diabetic with periodontitis group were higher.

4. Discussions

In recent years, studies on the correlation between diabetes and periodontitis have been constantly deepened. Some scholars believe that the microvascular theory that explains the complications of diabetes can also explain the occurrence of type 2 diabetes with periodontitis. Peripheral vessels of periodontal tissue are extremely abundant. Advanced glycation end products will reach periodontal tissue with bloodstream and gather, which can cause endothelial injury in microcirculation, activate oxidative stress response and inflammatory cascade reaction and promote expressions of a variety of protease molecules, eventually leading to structure damage in microcirculation, activate oxidative stress response and many other links caused by diabetes. Specific pathological process of oxidative stress is the abnormal generating and clearing process of free radicals and extensive consumption of antioxidants, thus causing local tissue injury[7]. Antioxidants in the body include non-enzymes and enzymes. The former ones mainly refer to glutathione (GSH), vitamin A and vitamin C; the latter ones mainly refer to myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD)[8]. When antioxidants were constantly consumed, tissues will be attacked and damaged by oxygen free radicals. Lipids, under the effect of oxygen free radicals, generate malonaldehyde (MDA); DNA, under the effect of oxygen free radicals, generates 8-hydroxydeoxyguanosine (8-OhdG)[9]. Type 2 diabetes itself will induce the micro-inflammatory and activated oxidative stress state of the body, ROS being constantly generated; excessively reproduced pathogens in local periodontium will act on human gingival fibroblast and through combination with CD14-TLR-4, activate downstream signaling molecules AP-1 and NK- B, thus continuing to generate ROS through macrophages, neutrophils, and others[10]. Excessive generation of ROS and constant consumption of antioxidants will eventually result in periodontal tissue destruction and periodontitis[11]. In the research, contents of oxidative stress response related molecules in GCF were detected. Results showed that compared with simple periodontitis group, contents of signaling molecules in GCF of both groups.

Table 3.
Comparison of contents of signaling molecules in GCF of both groups.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Diabetes with periodontitis group</th>
<th>Simple periodontitis group</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO (U/L)</td>
<td>25.62±4.52</td>
<td>41.94±6.49</td>
<td>7.982</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td>45.62±6.71</td>
<td>79.14±9.49</td>
<td>10.702</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td>51.23±6.96</td>
<td>93.45±10.21</td>
<td>8.392</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GSH (U/L)</td>
<td>37.84±4.96</td>
<td>56.71±7.02</td>
<td>6.918</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VitC (U/L)</td>
<td>15.62±1.95</td>
<td>29.82±4.20</td>
<td>9.203</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MDA (U/L)</td>
<td>75.52±9.42</td>
<td>31.29±5.20</td>
<td>12.398</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8-OhdG (U/L)</td>
<td>103.42±14.52</td>
<td>60.19±7.88</td>
<td>8.029</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

In the research, PCR was used to amplify 16s-rDNA. Then gel electrophoresis was conducted. Contents of different flora were analyzed through image information. Results showed that compared with simple periodontitis group, contents of Porphyromonas gingivalis aeromonas, actinomyces with haemophilus, Forsyth Tanner bacteria, middle Prairie Waugh bacteria and Treponema denticola of diabetes with periodontitis group were higher, which indicated that massive reproduction of pathogens would cause occurrence of type 2 diabetes with periodontitis.

Pathogenesis of diabetes with periodontitis has not been fully clarified. Oxidative stress may play an important role in the occurrence and development of the disease. It can participate in microcirculation damage, periodontal tissue injury and destruction and many other links caused by diabetes. Specific pathological process of oxidative stress is the abnormal generating and clearing process of free radicals and extensive consumption of antioxidants, thus causing local tissue injury[7]. Antioxidants in the body include non-enzymes and enzymes. The former ones mainly refer to glutathione (GSH), vitamin A and vitamin C; the latter ones mainly refer to myeloperoxidase (MPO), catalase (CAT) and superoxide dismutase (SOD)[8]. When antioxidants were constantly consumed, tissues will be attacked and damaged by oxygen free radicals. Lipids, under the effect of oxygen free radicals, generate malonaldehyde (MDA); DNA, under the effect of oxygen free radicals, generates 8-hydroxydeoxyguanosine (8-OhdG)[9]. Type 2 diabetes itself will induce the micro-inflammatory and activated oxidative stress state of the body, ROS being constantly generated; excessively reproduced pathogens in local periodontium will act on human gingival fibroblast and through combination with CD14-TLR-4, activate downstream signaling molecules AP-1 and NK- B, thus continuing to generate ROS through macrophages, neutrophils, and others[10]. Excessive generation of ROS and constant consumption of antioxidants will eventually result in periodontal tissue destruction and periodontitis[11]. In the research, contents of oxidative stress response related molecules in GCF were detected. Results showed that compared with simple periodontitis group, contents
of antioxidants MPO, CAT, SOD, GSH and VitC of diabetic with periodontitis group were lower and contents of oxidation products MDA and 8-OHdG were higher, which indicated that there was oxidative stress response hyperthyroidism in type 2 diabetes patients with periodontitis; antioxidants in GCF were extensively consumed and oxidation products were largely generated.

Recent studies have shown that dysfunction of multiple signaling pathways is related to the progression of periodontitis. Progressing process of periodontitis is accompanied by abnormal bone metabolism. Inhibited osteoblasts and enhanced osteoclasts will lead to alveolar bone absorption. Wnt signaling pathway and Fas/FasL signaling pathway participate in the regulation of bone metabolism. Wnt signaling pathway can interact with downstream signaling molecule LRP-5, activate osteoblasts and promote osteogenesis; secreted frizzled related protein SFRP1 is a specific antagonist molecule of Wnt signaling pathway and can inhibit Wnt signaling pathway, cause function loss of osteoblasts and induce local bone destruction[12]. Fas/FasL is a pair of regulatory molecules in apoptosis process. They can recruit Caspase-8 through downstream DED domain and form death-inducing signaling complex to induce apoptosis. In periodontal tissue, they can participate in bone metabolism process through inducing osteoblast apoptosis; increased expressions of Fas/FasL will increase osteoblast apoptosis, thus leading to the absorption and destruction of alveolar bone[13].

Apart from abnormal apoptosis and alveolar bone metabolism, local inflammatory cell infiltration is also an important feature of patients with periodontitis. MAPK is the most important signaling pathway in the body that regulates inflammation; p38, a member of MAPK family, can promote the aggregation and activation of inflammatory cells and also induce adhesion between inflammatory cells and endothelial cells. It has specific pro-inflammatory effects[14]. In Wnt family, Wnt5a is a new inflammation related signaling molecule. It is activated under the effect of nuclear transcription factor NF-κB, thus participating in inflammatory response of periodontal tissue in autocrine and paracrine manners[15]. In the research, contents of related signaling molecules in GCF were detected. Results showed that compared with simple periodontitis group, SFRP1, Fas, FasL, Wnt5a, NF-κB and p38 contents of diabetes with periodontitis group were higher, which indicated that there was dysfunction of signaling pathways in type 2 diabetes patients with periodontitis.

Based on above discussions and it can be concluded that contents of pathogens in subgingival microflora of type 2 diabetes patients with periodontitis significantly increase; antioxidants are extensively consumed and oxidation products are largely generated; there is dysfunction of multiple signaling pathways.

References


