Comparison of periodontal microbiological patterns in Italian spouses

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Key words  Periodontitis, Transmission oral pathogens, Real time PCR, Periodontal bacteria, Periodontal disease

Summary

Purpose: The aim of this study is to evaluate whether periodontopathogens are transmitted from husband to wife or vice versa.

Materials and methods: We tested the microbiological profile of 9 couples married for at least 10 years suffering from periodontitis. The microbiological analysis provides the quantification by Real-Time PCR of six main periodontopathogens, including P. gingivalis, T. denticola, T. forsythia, F. nucleatum subsp. polymorphum, P. intermedia, A. actinomycetemcomitans and genotype of P. gingivalis FimA in 90 subgingival plaque samples.

Results: The microbiological profiles highlighted a quite similar composition of oral microbial flora among husband and wife. Statistical results revealed a very high Pearson correlation values for the microbiological profiles in all 9 spouses. Additionally, five couples out of nine showed statistically similar values for the microbiological profile as determined by the Wilcoxon rank Sign test. We provided also a strong validation for the horizontal transmission of oral pathogens in the detection of the same genotype of P. gingivalis FimA in the spouses.

Conclusions: The presence of periodontitis in one member of the couple is a strong indicator of risk for the colonization of the spouse by periodontopathogenic bacteria. This study confirms that periodontal disease can be transmitted suggesting the importance of an early detection of oral pathogens in familial pattern of periodontitis to clarify the source of infection in order to assess correct prevention protocols based on potential infectivity within spouses.

Parole chiave  Parodontite, Trasmissione patogeni orali, Real time PCR, Batteri parodontali, Malattia parodontale

Riassunto

Scopo: La finalità di questo studio era verificare la trasmissibilità per via orale dei batteri responsabili dell’insorgenza e della progressione della malattia parodontale.
Introduction

Periodontitis is a multifactorial disease in which bacteria play an essential role in disease development and progression. The periodontal pathogens are recently reclassified by Socransky in complexes according to pathogenicity and colonization strategies (Socransky et al. 1998). The presence in periodontal pockets of anaerobic bacteria species that constitute the “red complex”, such as Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia, is mainly associated with advanced periodontitis, while the presence of bacteria of the “orange complex”, such as strains of the species Fusobacterium nucleatum ssp. polymorphum and Prevotella intermedia, is associated with either initial and moderate forms of periodontitis or with the recovery phase (Socransky et al. 1998). These bacteria thrive in periodontal pockets but they can also frequently recovered from saliva and they can be transmitted from person to person. The levels of periodontal pathogens found in saliva were demonstrated directly related to the presence of periodontitis. It’s also reported that the periodontal treatment eradicates or markedly decreases the levels of periodontal bacteria in saliva (von Troil-Linden et al. 1995a).
Most studies have been focalized to provide evidences of the transmission of two strong markers of periodontal disease in adult subjects (P. gingivalis and Aggregatibacter (ex Actinobacillus) actinomycetemcomitans), between spouses (van Steenbergen et al. 1993, Petit et al. 1993a, Ozmeric et al. 1999, Van Winkelhoff et al. 2005). Furthermore, the simultaneous isolation of P. gingivalis occurs more often in couples of which one spouse suffers from periodontitis (von Troil-Linden et al. 1995b). The studies of Petit et al. demonstrated also the possible transmission of P. gingivalis from parents to children (Petit et al. 1993b). The heterogeneous virulence of P. gingivalis is dependent on its clonal diversity. P. gingivalis fimbriae (FimA) are filamentous components on the cell surface that play an important role in the colonization and invasion of periodontal tissues. On the basis of the nucleotide sequence, fimA gene can be classified into six types (I to V and Ib) (Amano et al. 2004) and the presence of type II FimA organisms is associated with the more severe form of periodontitis because of a significantly greater adhesive and invasive capabilities to epithelial cells than other FimA type clones.

The theories on the transmission of P. gingivalis between individuals have been based on molecular biology techniques such as restriction enzyme analysis (REA) of whole chromosomal DNA, amplified fragments length polymorphisms (AFLP) (Rijnsburger et al. 2007). With the routine use of molecular biology techniques, commercial tests based on 16S rRNA gene Real-Time polymerase chain reaction (Real-Time PCR, or qPCR) offer high sensitivity and the possibility of species-specific detection. Consequently, tests providing quantitative and qualitative analysis for the major etiologic agents of periodontitis (Kuboniwa et al. 2004, Sanz et al. 2004) are useful to obtain a more accurate confirmation of the earlier observations on the transmission of periodontopathogens among spouses.

This is the first study about microbial transmission between spouses that compares the quantization of six main periopathogens using Real Time PCR. The analysis of microbiological reports of spouses that highlights as the oral microbial flora of spouses is composed by the same periodontopathic bacteria in comparable proportions and the sub-typing of P. gingivalis, confirms that transmission of periodontal pathogens is possible and it is a risk factor for developing periodontal disease.
Materials and methods

Patients and sampling

In this study we tested 90 periodontal pockets sites from 9 couples married for at least 10 years suffering from periodontitis and that have never received periodontal treatments in their life. Sampling was carried out following the procedures reported in the BPA kit (Bacterial Periodontal Assessment, Biomolecular Diagnostic, Firenze Italy) after drying the area and removing supragingival plaque. Subgingival plaque samples were collected with sterile paper points inserted for one minute into the deepest pockets (choosing at least one pocket for each quadrant) and stored at 4°C in a sterile tube. Five samples per patient were taken from different sites having PPD ≥ 3mm and pooled together. Clinical examination was performed using Florida Probe (www.floridaprobe.com) a computerized periodontal probing that allows tests comparable over time and independent from the operator. Clinical characteristics of spouses are presented in Table 1.

Assessment of periodontopathic bacteria

DNA extraction was performed by using a QIAxtractor® (QIAGEN®, GMBH, Germany). The protocol recommended by the kit manufacturer for DNA extraction was followed precisely. After extraction, DNA was eluted in 50 µl of elution buffer and about 40 ng of DNA was used for bacterial detection by Real-Time PCR of the most important periodontopathogens. The amplification and detection of DNA with species-specific primers for the 16S rRNA gene by real-time PCR were performed by Biomolecular Diagnostic using SYBR-Green in a Rotor-Gene 3000 (Corbett) apparatus. The microbiological reports express bacterial titres as number of cells per plaque sample. The microbiological profile (Table 2) included: total bacterial number, percentage of pathogens, percentage of red complex bacteria, percentage of orange complex bacteria, titre of P. gingivalis, genotype of P. Gingivalis FimA, titres of T. denticola, T. forsythia, F. nucleatum ssp. polymorphum, P. intermedia. No A. actinomycetemcomitans were retrieved for all 18 patients.

Results

To evaluate the similarity in the microbiological profiles from the 9 spouses under study a Wilcoxon rank sign test and Pearson correlation were used. Wilcoxon rank
Table 1 - Clinical characteristics of the 9 couples under study*

<table>
<thead>
<tr>
<th>Couple nr</th>
<th>Sex</th>
<th>Age</th>
<th>PPD±SD</th>
<th>REC±SD</th>
<th>CAL±SD</th>
<th>BOP%</th>
<th>PUS%</th>
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<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>55</td>
<td>6,6±1,516</td>
<td>0,6±0,548</td>
<td>7,2±2,049</td>
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<td></td>
<td>f</td>
<td>54</td>
<td>6,6±0,894</td>
<td>0,6±0,548</td>
<td>7,6±1,816</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>63</td>
<td>6,2±1,304</td>
<td>1,1±0,224</td>
<td>7,3±1,483</td>
<td>100</td>
<td>0</td>
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<tr>
<td></td>
<td>f</td>
<td>61</td>
<td>5±1,225</td>
<td>2,4±0,962</td>
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<td>0</td>
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<tr>
<td>3</td>
<td>m</td>
<td>58</td>
<td>7,2±1,923</td>
<td>1,2±0,447</td>
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<td>80</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>58</td>
<td>3,8±0,447</td>
<td>0</td>
<td>3,8±0,447</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
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<td>1,4±0,548</td>
<td>8,6±1,140</td>
<td>100</td>
<td>40</td>
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<tr>
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<td>0,2±0,447</td>
<td>4,8±0,837</td>
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<td>m</td>
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<td>0,2±0,447</td>
<td>4,8±0,447</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>46</td>
<td>8,8±0,837</td>
<td>0,4±0,894</td>
<td>9,2±1,643</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>m</td>
<td>59</td>
<td>5,4±1,522</td>
<td>0,6±0,559</td>
<td>6±1,871</td>
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<tr>
<td>9</td>
<td>m</td>
<td>57</td>
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<td>1,1±0,224</td>
<td>6,9±1,516</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>57</td>
<td>6,2±0,837</td>
<td>0,9±0,548</td>
<td>7,1±1,342</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* SD: standard deviation; PPD: periodontal probing depth; REC: recession; CAL: clinical attachment loss; BOP: bleeding on probing; PUS: suppuration

Sign test allow to evaluate if the overall microbiological data of male are similar to those of females in the same spouse, that is if one partner had similar, higher or lower values than the other partner. Then, Pearson correlation gave insight into correlation of profiles between males and females. Results of the analyses are reported in Table 3. Very high correlation values were obtained for the microbiological profiles in all 9 spouses. Additionally, five couples out of nine (1-2-5-6-9) showed statistically similar values for the microbiological profile as determined by the Wilcoxon rank Sign test. Subgingival dental plaque samples positive for *P. gingivalis* were also studied for fimA classification by a set of fimA type-specific primers as describe previously (Amano et al. 1999). *P. gingivalis* with type II fimA was detected in both spouses of 6 couple out of 9 (2-4-5-6-7-9).
Discussion

Table 3 - Wilcoxon rank sign test and Pearson correlation for the 9 spouses under study*

<table>
<thead>
<tr>
<th>Spouse</th>
<th>Wilcoxon rank Sign test</th>
<th>Pearson r correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.612 (n.s.)</td>
<td>0.999 ***</td>
</tr>
<tr>
<td>2</td>
<td>0.859 (n.s.)</td>
<td>0.996 **</td>
</tr>
<tr>
<td>3</td>
<td>0.037 (s.)</td>
<td>0.965 **</td>
</tr>
<tr>
<td>4</td>
<td>0.008 (s.)</td>
<td>0.955 **</td>
</tr>
<tr>
<td>5</td>
<td>0.086 (n.s.)</td>
<td>0.999 **</td>
</tr>
<tr>
<td>6</td>
<td>0.110 (n.s.)</td>
<td>0.955 **</td>
</tr>
<tr>
<td>7</td>
<td>0.05 (s.)</td>
<td>0.944 **</td>
</tr>
<tr>
<td>8</td>
<td>0.047 (s.)</td>
<td>1.000 **</td>
</tr>
<tr>
<td>9</td>
<td>0.208 (n.s.)</td>
<td>0.999 **</td>
</tr>
</tbody>
</table>

*(n.s.): not significant; (s.): significant

benefits. This study showed for the first time a large and quantitative microbiological profile of the members of couples providing also a strong validation for the horizontal transmission of oral pathogens in the detection of the same genotype of fim A P. gingivalis in the spouses. As shown in Fig.1 the microbiological profiles of each couple match both in species and in proportions as if the mouths of the couple had became a unique oral ecosystem. Our data about intrafamiliar transmission of oral pathogens through saliva are in accordance with literature regarding P. gingivalis, A. actinomycetemcomitans and P. intermedia (Matto et al. 1996; van Steenbergen et al. 1993, Petit et al. 1993a-b, Ozmeric et al. 1999, Van Winkelhoff et al. 2005), while there are no studies that take into account the transmission of the others pathogens object of our study (F. nucleatum, T. forsythia, T. denticola) nor even about a simultaneously detection of the six main periodontal bacteria. As shown in table 2 it is possible appreciate some differences in the percentage of pathogens between the members of couples 2-3-4-6-7, while the percentage relative of the red and orange complexes are the same. The severity of periodontitis like a multifactorial disease is related also to other factors such as genetical immunological profile and environmental risk factors exposure (Zhang et al. 2011; Nibali 2009). So we can speculate that the patient with an higher total bacterial load has a lower individual susceptibility to develop periodontal disease and he needs a higher percentage of periodontal pathogens to develop periodontitis with a grade of severity comparable to that of spouse. Conversely, the couples 5-8-9 show a comparable percentage of
Figure 1 - Comparison of microbiological profiles of the 9 couples. Y-axis provides the quantification obtained by Real-Time PCR (number of cells per plaque sample) for each bacteria in male (M) and in female (F) of each couple. In x-axis the name of bacteria (A.a., Aggregatibacter actinomycetemcomitans; Pg, Porphyromonas gingivalis; Tf, Tannerella forsythia; Td, Treponema denticola, Pi, P. intermedia; Fn ssp, F. nucleatum ssp. Polymorphum)
pathogens but the microbiological profile of a spouse shows a prevalence of red complex while the other shows a prevalence of orange complex suggesting a different evolution of periodontal disease (Socransky et al. 1998).

In conclusion, this is a first study that evaluating, by Real-Time PCR, a large microbiological profile based on detection of six periodontal pathogens, confirming that periodontitis can be transmitted in a familiar pattern. Thus, the presence of periodontitis in one member of the couple is a strong indicator of risk for the colonization of the spouse by periodontophatogens. This suggests the importance of an early detection of oral pathogens in familial pattern of periodontitis to clarify the source of infection in order to assess correct prevention protocols based on potential infectivity within spouses. Further studies on a larger number of subjects are necessary to confirm these results.

Acknowledgments

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Bibliography


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