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Original article

Periodontal microbiota of Sardinian children: comparing 200-year-old samples to present-day ones

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Abstract

Introduction: The microrganisms of the human oral cavity include more than 700 species or phenotypes of bacteria. Some "diseases of civilization" are strictly correlated to changes in the microbiome following the food revolution that occurred after WWII. For that reason, a precise recognition of the microbiome profile before and after this period should be useful to determine the health-compatible model of microbiome. The aim of this study was to compare the microbiome profiles (number of total cells, and pathogen types) of dental samples obtained from two distinct groups of children, a 200-year-old retrieved one and a present one.

Methods: Two different groups of samples have been studied. The first group was a set of 50 recent subgingival plaque samples obtained from children of age 2-8 years, 14 males and 36 females. They were enrolled by the Department of Dental Disease Prevention (University of Cagliari, in Sardinia, Italy) during standard dental care procedures. None reported periodontal disease and none had been under antibiotic therapy during the previous 6 months. The second group was an old retrieved group that included 24 teeth from 6 different 6- to 8-year-old crania fragments; they were obtained from a 200-year-old charnel-house located in Villaputzu, a city close to Cagliari. Representative periodontal bacteria have been identified by a previously published real-time PCR procedure (Sokransky et al., 1998) in which *P. gingivalis* and *T. forsythia* (red complex), *A.*

actinomycetemcomitans (green complex) and *F. nucleatum* (orange complex) were detected. In addition, the title of each pathogen was expressed as a percentage of the total bacteria (biofilm) in the sample.

Results and discussion: The profile of periodontal microbiomes, between recent/ancient samples showed a significant difference relative to Sokransky's red complex bacteria ($p < 0.05$). In all analyzed periodontal strains, the pathogenic bacteria *P. gingivalis* and *T. forsythia* showed the highest title in the recent group.

Conclusions: Our hypothesis is that the transfer of "commensal-pathogen" as an absolute number on the oral biofilm might be linked to the distinct alimentary habits of the two populations. Some diet rich in reducing agents, such as processed meat-based foods, might be able to increase the average number of pathogen anaerobic bacteria in the oral microbiota. The outcome would be an increase of the oral systemic diseases reported with these pathogens. Our data suggest that the ancient Sardinian population was able to control the pathogen oral anaerobic biofilm by some diet rich in antioxidant compounds. Further investigations are required to focus on the genetic profile and the health status of this ancient population but it appears that molecular microbiology might be considered as the "time machine" in oral biology.

Keywords

Subgingival plaque, microbiota, children, ancient population.

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Introduction

The microrganisms of the human oral cavity include more than 700 species or phenotypes of bacteria. Some "diseases of civilization" are strictly correlated to changes in the microbiome following the food revolution that occurred after WWII. For that reason, a precise recognition of the microbiome profile before and after this period should be useful to determine the health-compatible model of microbiome. Molecular biology can be applied to ancient human findings in order to detect relevant genetic data in different fields of medicine. The use of dental tissues as the most representative source of ancient DNA has recently been described [1-3]. The dental structure is characterized as a complex architecture; it is able to incorporate and preserve the nucleic acids from both the host and its ancient oral microbiota. Analysing these samples with molecular biology tools may provide a powerful method for studying ancient human habits, epidemiological diseases, human migrations and genetic drift [3]. The aim of this study was to investigate possible differences in the subgingival microbiota on the hard tissues of dental samples obtained from two distinct groups of children: a retrieved 200-year-old group and a present-day one.

Methods

The present-day group included a set of 50 recent subgingival plaque samples obtained from 14 boys and 36 girls aged between 2 and 8 years. They were enrolled by the Department of Dental Disease Prevention (University of Cagliari, in Sardinia, Italy) during standard dental care procedures. All parents signed an informed consent form before the children took part in the microbiological analysis. None reported periodontal disease and none of the children had undergone antibiotic therapy during the previous 6 months. Their health status was recorded and the subgingival plaque was immediately stored at -20°C following a previously described method [4, 5]. The retrieved ancient group included 24 teeth from 6 different 6- to 8-year-old crania fragments obtained from the 200-year-old charnel-house located in Villaputzu, a small town close to Cagliari (**Fig. 1**). The location of these bones appeared well maintained and any sign of humidity was recorded during the sampling.

These samples were gently cleaned with a cotton swab to remove the superficial soil. For each cranium, bones and teeth status was recorded; signs of periodontal diseases or caries were also recorded. The teeth were then extracted from the dental alveoli of the respective jaws

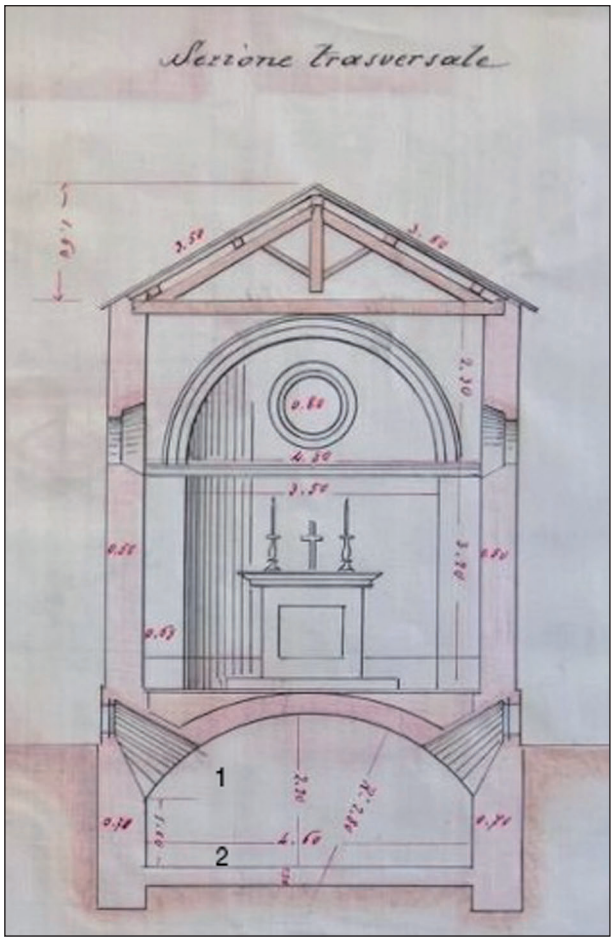

Figure 1. Schematic representation of the site of the ancient fragments: 1) charnel-house area, 2) pile of skeletal material in the recovery stratum.

(**Fig. 2**). Sample pretreatment and DNA extraction were performed following the procedures described by Bolnick et al. and Orrù et al. [4, 5]. Representative periodontal bacteria described by Socransky et al. [6] were identified by a previously published real-time PCR procedure [5] in which *P. gingivalis* and *T. forsythia* (red complex) [7], *A. actinomycetemcomitans* (green complex) [5] and *F. nucleatum* (orange complex) [8] were detected. In addition, the titer of each pathogen was expressed as a percentage of the co-respective total bacteria (biofilm) in the sample detected by a set of universal primers designed on *rrs* sequence of *E. coli* (**Fig. 3**); this approach prevented any possible errors due to DNA degradation of the ancient samples during their time in the ossuary, i.e. errors in the accuracy of the real-time PCR procedure [9].

The real-time PCR reaction was performed with a LightCycler® instrument (Roche Diagnostics, Mannheim, Germany) and a SYBR® Premix Ex Taq™ Kit (TaKaRa-Clontech®), according to the manufacturer's instructions [5].

Results and discussion

Observation showed that the 200-year-old group displayed different dental/bone diseases. These were extensive caries (21.4%), tooth wear (21%), dental calculus (7.1%), but no bone loss. These conditions are probably due to the lack of oral care and insufficient hygiene conditions. As described by Weyrich et al., the use of dental calculus (calcified tartar or plaque) was identified as a relevant recovery source of ancient DNA [10]. Indeed, subgingival and supragingival plaque is rich in calcium phosphates and silicates; it calcifies *in situ* during the host life while forming layered fossilized concretions known as dental calculus. These concretions lock other kinds of material located in the oral cavity and preserve it from taphonomic and environmental alterations. It thus remains intact for long periods of time, indeed over millennia. The same formation is present in recent samples (dental plaque) [1, 11, 12]. According to this approach, the profile of periodontal microbiomes showed a significant difference between recent/ancient samples relative to Sokransky's red complex bacteria ($p < 0.05$), while no statistical significance was observed for the other examined periodontal pathogens ($p > 0.05$). In all analyzed periodontal strains, the pathogenic bacteria *P. gingivalis* and *T. forsythia* showed the most important differences compared to other periodontal pathogens (**Fig. 3**). These strains are associated with severe infections in humans, such as periodontal disease, endocarditis, rheumatoid arthritis and pre-term birth [13, 14] and these human infections are often related to modern civilization [15]. In addition, a possible physiological activity for some periodontal bacteria, such as *F. nucleatum*, in the flavor and taste perception of vegetables, has been suggested [16]. Our hypothesis is that the transfer of "pathogen/commensal" as percentage amount in the oral biofilm might be linked to the distinct alimentary habits of the two populations. A diet rich in reducing agents, such as processed meat-based foods, might be able to increase the average number of anaerobic bacteria in the oral microbiota. The outcome would be an increase in the oral systemic diseases reported with these pathogens in the last decades. In this context our data suggest that the ancient Sardinian young population was able to control the pathogens in the oral anaerobic biofilm by a diet rich in antioxidant compounds.

Figure 2. Jaw from the ancient sample group, comprising 6- to 8-year-old jaws. 1) caries, 2) deciduous teeth, 3) permanent teeth, in eruption.

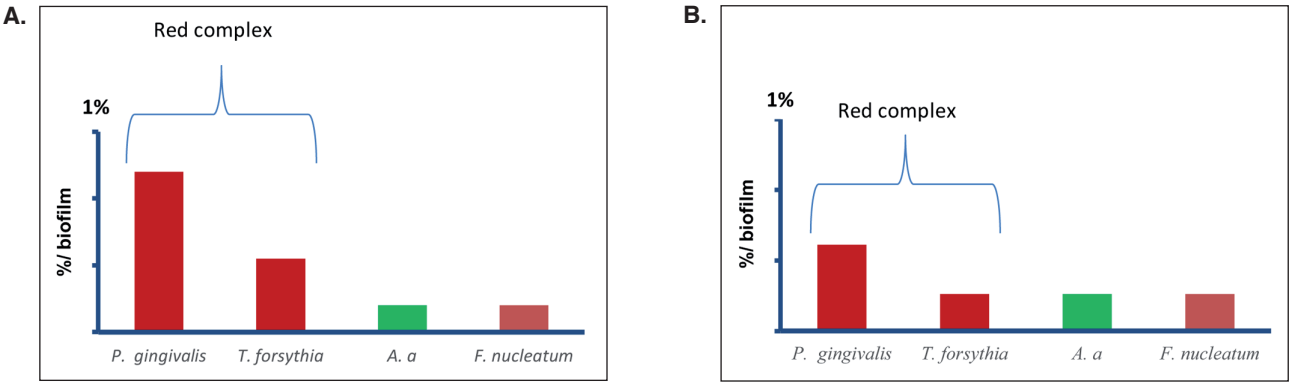

Figure 3. Percentage in the biofilm of each periodontal bacterium detected in the recent group (**A**), and in the ancient one (**B**).

A. a.: *A. actinomycetemcomitans.*

Conclusions

This preliminary study showed a difference in red complex bacteria titer in children that lived 200 years ago when compared to a present-day group. Further investigations are required to focus on the genetic profile and the health status of this ancient population but it appears that molecular microbiology might be considered as the "time machine" in oral biology.

Acknowledgements

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Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Warinner C, Speller C, Collins MJ. A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. Philos Trans R Soc Lond B Biol Sci. 2015;370(1660):20130376.
2. Warinner C, Speller C, Collins MJ, Lewis CM Jr. Ancient human microbiomes. J Hum Evol. 2015;79:125-36.
3. Higgins D, Rohrlach AB, Kaidonis J, Townsend G, Austin JJ. Differential nuclear and mitochondrial DNA preservation in post-mortem teeth with implications for forensic and ancient DNA studies. PLoS One. 2015;10(5):e0126935.
4. Bolnick DA, Bonine HM, Mata-Míguez J, Kemp BM, Snow MH, LeBlanc SA. Nondestructive sampling of human skeletal remains yields ancient nuclear and mitochondrial DNA. Am J Phys Anthropol. 2012;147(2):293-300.
5. Orrù G, Marini MF, Ciusa ML, Isola D, Cotti M, Baldoni M, Piras V, Pisano E, Montaldo C. Usefulness of real time PCR for the differentiation and quantification of 652 and JP2 Actinobacillus actinomycetemcomitans genotypes in dental plaque and saliva. BMC Infect Dis. 2006;6:98.
6. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
7. Narayanan D, Hamlet S, Cullinan M, Davies R, Ellwood R, Bird P, Seymour GJ. The distribution of Tannerella forsythia in an adolescent and adult population. J Periodontal Res. 2005;40(6):482-8.
8. Signat B, Roques C, Poulet P, Duffaut D. Fusobacterium nucleatum in periodontal health and disease. Curr Issues Mol Biol. 2011;13(2):25-36.
9. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill FR 3rd, Smith TF. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev. 2006;19(1):165-256.
10. Weyrich LS, Dobney K, Cooper A. Ancient DNA analysis of dental calculus. J Hum Evol. 2015;79:119-24.
11. Ozga AT, Nieves-Colón MA, Honap TP, Sankaranarayanan K, Hofman CA, Milner GR, Lewis CM Jr, Stone AC, Warinner C. Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. Am J Phys Anthropol. 2016;160(2):220-8.
12. Adler CJ, Dobney K, Weyrich LS, Kaidonis J, Walker AW, Haak W, Bradshaw CJ, Townsend G, Sołtysiak A, Alt KW, Parkhill J, Cooper A. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nat Genet. 2013;45(4):450-5, 455e1.
13. Kumar PS. From focal sepsis to periodontal medicine: a century of exploring the role of the oral microbiome in systemic disease. J Physiol. 2017;595(2):465-76.
14. Xin X, Junzhi H, Xuedong Z. [Oral microbiota: a promising predictor of human oral and systemic diseases]. [Article in Chinese]. Hua Xi Kou Qiang Yi Xue Za Zhi. 2015;33(6):555-60.
15. Choi BC, Hunter DJ, Tsou W, Sainsbury P. Diseases of comfort: primary cause of death in the 22nd century. J Epidemiol Community Health. 2005;59(12):1030-4.
16. Starkenmann C, Le Calvé B, Niclass Y, Cayeux I, Beccucci S, Troccaz M. Olfactory perception of cysteine-S-conjugates from fruits and vegetables. J Agric Food Chem. 2008;56(20):9575-80.