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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.06.011

Caries-related factors and bacterial composition of supragingival plaques in caries free and caries active Algerian adults

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ARTICLE INFO

Article history:
Received 15 Jan 2016
Received in revised form 15 Feb, 2nd revised form 8 Mar 2016
Accepted 22 Apr 2016
Available online 11 Jun 2016

Keywords:
Supragingival plaque
Caries-free
Caries-active
Bacterial composition
Algerian adults
Cultural level
Food intake

ABSTRACT

Objective: To compare oral hygiene practices, education and social background, food intake and oral malodor of Algerian adults suffering from dental caries with normal controls, and to determine and compare the bacterial composition of the supragingival plaques from the above-mentioned groups.

Methods: Participants completed a questionnaire and were clinically examined for dental caries using decayed, missing and filled teeth index according to the criteria laid down by the World Health Organization. Supragingival plaque samples were collected from 50 caries-free adults (CF) and 50 caries-active adults (CA). Standard procedures of culture and identification of aerobic and anaerobic bacteria were used. Data were analyzed using *Chi*-square test.

Results: A total of 117 bacterial strains were isolated from supragingival plaques in CF group subjects, 76 (64.96%) of them belonged to 9 aerobic genera, and 41 (35.04%) to 9 anaerobic genera ($P < 0.05$). While in the second group, 199 strains were isolated, 119 (59.80%) of the strains belonged to 10 aerobic genera and 80 (40.20%) to 10 anaerobic bacteria ($P < 0.05$). *Streptococcus mutans*, *Enterococcus faecium*, *Aerococcus viridans*, *Actinomyces meyeri*, *Lactobacillus acidophilus* and *Eubacterium limosum* showed a significantly higher prevalence in the CA group ($P < 0.05$). The findings revealed that CA group had a high sugar intake (80%). A significantly higher frequency of tooth brushing ($P < 0.000$) and a significantly less self-reported oral malodor ($P < 0.000$) and tooth pain ($P < 0.000$) were found in CF group, while there was no association of socioeconomic levels and intake of meal snacks with dental caries.

Conclusions: This study confirms the association of some aciduric bacteria with caries formation, and a direct association of sugar intake and cultural level with dental caries. Furthermore, oral hygiene practices minimize the prevalence of tooth decay.

1. Introduction

Dental plaque happens to be a diverse community of the microorganisms found on the tooth surface [1]. Greater than 700 microbial species inhabit the oral cavity [2]. The formation of dental plaque in a healthy individual involves an ordered pattern of colonization by a range of bacteria, and once a dynamic balance is established, the composition of the resident microbiota of each site remains relatively stable over time [3]. Dental caries is one of the most common chronic and multifactorial diseases affecting the human population [4]. It is considered by the World

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The study protocol was performed according to the Helsinki declaration and approved by the Tlemcen University Hospital Committee for research on human subjects. All participants were informed about the goals of this study and were asked to give their written consent.

Foundation Project: Supported by the National Committee of Research Program and Assessment (Cnepru-code I02020130110).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Health Organization as one of the most important global oral health burdens that affects people of various age groups all over the world.

Cariogenic plaques result when acidogenic and aciduric bacterial species increase following high frequency of exposure to carbohydrate. The microbial metabolism of such carbohydrates will result in the acidification of the biofilm, which in turn may lead to acid-induced demineralization of the dental hard tissues [5]. Moreover, the prevalence and incidence of dental caries in a population is influenced by a number of risk factor such as sex, age, socioeconomic status, dietary patterns and oral hygiene habits [6].

There are three microbial hypotheses regarding the etiology of dental caries [7], namely the specific plaque hypothesis, the non-specific plaque hypothesis, and the ecological plaque hypothesis. The specific plaque hypothesis has proposed that only a few species of the total microflora are actively involved in disease [8]. Of which the most relevant were mutans streptococci [main species: *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus*] and lactobacilli [9]. Contrary to the specific plaque hypothesis, the non-specific plaque hypothesis suggests that caries is the consequence of the overall interaction of all the groups of bacteria within plaque [10]. The ecological plaque hypothesis suggests that caries is the result of an imbalance in the microflora due to ecological stress, resulting in an enrichment of certain disease-related micro-organisms [8].

Two closely related species of mutans streptococci, namely *S. mutans* and *Streptococcus sobrinus*, are associated with dental caries in humans [11]. Based on current research, it is believed that the bacteria of the genus *Lactobacillus* are important in further caries development, especially in the dentin [12]. However, it is now recognized that a large number of species naturally present in oral plaque biofilm produce acid from dietary carbohydrate and that the consequent caries-associated microbiota is complex [13].

Since oral bacteria are considered as one of the etiologic factors involved in caries development, various microbial studies have been conducted to better understand this dental problem.

Because of a lack of data available for detecting the bacterial diversity of dental caries in the Algerian adults population, the present study was performed to determine all cultivable bacterial species associated with health and dental caries of permanent teeth in adults, to determine the associations of specific bacterial species or bacterial communities with healthy and carious teeth, and to reveal the relation of food intake, oral hygiene practices, cultural level, socioeconomic status and oral malodor with dental caries among Algerian adults.

2. Materials and methods

2.1. Subject population

The study protocol was performed according to the Helsinki declaration and approved by the Tlemcen University Hospital Committee for research on human subjects. All participants were informed about the goals of this study and were asked to give their written consent.

One hundred healthy patients, aged 20–35 years were recruited from the dental clinic at the Departments of Dentistry, University of Tlemcen. They completed a medical and dental history questionnaire and signed an informed consent document. The questionnaire consisted of two parts. The first part consisted of general information such as age, sex and educational qualification. The second part consisted of six questions related to oral health, attitude and practices.

Subjects underwent dental examination for caries prevalence by a dentist, who applied the World Health Organization's caries diagnostic criteria to determine the decayed, missing, filled teeth (DMFT) index [14]. The subjects were divided into two groups: caries-free (CF) group (DMFT = 0, n = 50) and caries-active (CA) group ($4 \leq$ DMFT ≤ 8, n = 50).

Exclusion criteria included antibiotic therapy in the previous 3 months, any systemic diseases and having less than 24 permanent teeth.

2.2. Plaque sampling

Samples were collected in the morning, 12 h after tooth brushing and 2 h after the last food and/or drink intake.

The supragingival plaque samples of CF and CA groups were collected by isolating the teeth with sterile cotton rolls and rubbing the vestibular face of the smooth and proximal surfaces of the central and lateral incisor, canines, premolars, and the first and second molars with a sterile swab. Each sample was pooled in 1 mL of pre-reduced brain heart infusion broth, (pH 7.2, Oxoid, Basingstoke, UK) and transported immediately to microbiology laboratory located next to the dental clinic.

2.3. Microbiological processing

The specimens were processed in the laboratory for the cultivation of aerobic and anaerobic bacteria.

First, the samples were vortexed for 30 s and the suspension was serially diluted (10^{-1}–10^{-4}) with sterile brain heart infusion broth (pH 7.2, Oxoid, Basingstoke, UK), aliquots (100 μL) of each dilution and the undiluted suspension were inoculated onto non-selective and selective media. Enriched blood agar [Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% laked blood] was used for the isolation of facultative and anaerobic bacteria. Plates were incubated anaerobically for 5–7 days at 37 °C in an environment consisting of 80% N_2, 10% CO_2, and 10% H_2, and aerobically for 24–48 h at 37 °C. Selective media such as MacConkey (Oxoid, Basingstoke, UK) used for the isolation of enterobacteria and Chapman (Oxoid, Basingstoke, UK) for staphylococci were also inoculated and incubated under an aerobic condition for 24–48 h at 37 °C.

Bacterial identification was based on the colony morphology and pigmentation, Gram staining and the biochemical reactions (API 20 A, API 20 Strep, API 20 E, API 20 Staph) (Biomérieux, Marcy l'Etoile, France) [15,16]. Antibiotics sensitivity test was also used to confirm biochemical test results.

2.4. Statistical analysis

Statistical analysis was performed on SPSS v.20.0 statistics software (SPSS Inc., Chicago, IL, USA). Simple frequency tables and descriptive statistics were processed and analyzed by *Chi*-square test (χ^2). Statistical significance was set at $P < 0.05$.

3. Results

A total number of 100 individuals were examined for dental caries. One half (50%) was CA, whereas the other half was CF.

The questionnaire data revealed that there were 13 males (26%) and 37 females (74%) in the CF group, in which 41 (82%) persons were aged between 20 and 25 years and 9 (18%) within 25–30 years age group. Whereas in the second group, there were 16 (32%) males and 34 (68%) females, in which 31 (62%) of