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Immunologic burden links periodontitis to acute coronary syndrome

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ABSTRACT

Background and aims: Periodontitis, a common polymicrobial inflammatory disease in the tooth supporting tissues, is a risk factor for coronary artery disease. One of the proposed underlying mechanisms is the systemic immune response to periodontal infection. We studied how serum antibodies against seven periodontal pathogens and their subgingival levels associate with each other, periodontitis, and coronary artery disease.

Methods: The Parogene cohort included 505 Finnish patients (mean age 63 y) who underwent coronary angiography, and clinical and radiographic oral examinations. Coronary diagnosis was defined as no significant coronary artery disease (<50% stenosis, n = 152), stable coronary artery disease (≥50% stenosis, n = 184) and acute coronary syndrome (n = 169). Levels of subgingival *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, Tannerella forsythia, Campylobacter rectus,* and *Fusobacterium nucleatum* were determined by checkerboard DNA-DNA hybridization. Serum antibody (IgA/IgG) levels were analyzed with enzyme-linked immunosorbent assay (ELISA). Aggregate IgA/IgG burdens were calculated by summing and standardizing the serum antibody levels.

Results: Patients with active periodontitis were characterized by higher levels of subgingival bacteria and corresponding IgA/IgG response. Quartiles 2–4 of serum IgA/IgG burden indicated higher risk for acute coronary syndrome (OR 1.84, 95%CI 1.01–3.35 for IgA; OR 1.87, 95%CI 1.01–3.46 for IgG) independently of established cardiovascular risk factors, body mass index, number of teeth, subgingival bacterial levels and periodontal diagnosis.

Conclusions: Our findings support the hypothesis that the association between periodontitis and cardiovascular diseases is partly mediated by the immunologic response for periodontal pathogens.

1. Introduction

Periodontitis is a chronic inflammatory disease in the tooth supporting tissues. It is one of the most common diseases worldwide; the severe form has a global age-standardized prevalence of 11.2% [1]. Periodontitis is preceded by gingivitis, and progresses as a complex interplay between putative periodontal pathogens and host response in susceptible individuals. If left untreated, the resulting resorption of alveolar bone leads to increased tooth mobility and eventually loss of teeth [2]. The deleterious effects of periodontitis are, however, not restricted to the oral cavity. Periodontitis may cause bacteremia, endotoxemia, and systemic low grade inflammation [3].

Even though periodontitis has been regarded as a polymicrobial infection for a century, the exact influence of periodontal pathogens is still under research. Some species, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella*

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intermedia, Tannerella forsythia, Campylobacter rectus and *Fusobacterium nucleatum* form complexes that associate with each other in a virulent manner, and are traditionally considered as periodontal pathogens [4]. The development of sequencing techniques has enabled identification of new putative periodontal pathogens. *Porphyromonas endodontalis* is one of the proposed candidates with a moderate grade of evidence [5]. The emerging concept of periodontal pathogenesis emphasizes the role of polymicrobial synergy caused by keystone pathogens and dysbiosis in the subgingival biofilm community [6].

The confounder independent association between periodontitis and atherosclerotic vascular disease is widely recognized and consistent through diverse populations [7,8]. One of the mechanistic explanations is the activation of adaptive immunity towards invasive periodontal pathogens, resulting in molecular mimicry by cross-reactive T, or B lymphocytes [9]. Several periodontal pathogens incorporate antigens, which share homology with human epitopes, and the resulting auto-reactive antibodies may induce endothelial dysfunction, systemic inflammation and promote atherogenesis [3,10]. These oral pathogens promote atherogenesis both directly, by virulence factors and endothelial invasion, which activates pro-atherogenic mechanisms, and via immunologic mechanisms, such as cross-activation between host- and pathogen antigens [11]. Therefore, high serum antibody levels to periodontal pathogens are likely to have both direct effects on the development of coronary artery disease (CAD) and simultaneously indicate periodontal infection [12]. From a clinical perspective, systemic antibody responses to periodontal pathogens have been proposed as surrogate markers for periodontitis [13–15].

It has been described repeatedly that cardiovascular risk associates with the inflammatory burden consisting of multiple microbial species [16–18]. Periodontitis is a polymicrobial disease, its pathogens share common surface antigens with the host, and some of the bacterial species have been associated with atherosclerosis. The relationships of the aggregate periodontal pathogen burden, their immune responses and CAD have not been reported before. We investigated these associations utilizing a well characterized population having angiographically verified coronary artery disease diagnosis as well as comprehensive clinical oral examinations with data on a select group of periodontal pathogens and serum antibody levels.

2. Materials and methods

2.1. Population

The Corogene cohort consists of 5297 Finnish patients who underwent coronary angiography between June 2006 and March 2008 at the Helsinki University Central Hospital as previously described [19]. A random gender-stratified subsample (N = 505) was enrolled for extensive clinical, and radiographic oral examinations. These individuals constitute the Parogene cohort used in this study. The study complies with the principles of the Declaration of Helsinki and adheres to the STROBE guidelines for observational studies. Informed consent was obtained from all study subjects. The study design was approved by the Helsinki University Central Hospital ethics committee (approval reference number 106/2007).

2.2. Examinations

The oral examinations were performed by two calibrated periodontal specialists (PM and KB) who were unaware of the coronary status. The data collection has been described in detail [20]. Among other parameters "bleeding on probing" (BOP) was registered as a proxy for active gingival inflammation [21]. Periodontal pocket probing depth (PPD) was measured from six sites on each tooth and number pockets with PPD 4–5 mm and PPD ≥6 mm were registered [20]. Periodontal inflammatory burden index was calculated by adding the number of periodontal pockets indicating mild to moderate periodontitis (PPD 4–5 mm) to the weighted number of periodontal pockets indicating more advanced periodontitis (number of periodontal pockets with PPD ≥6 mm multiplied by two) as previously reported [20,22]. This index takes into account the enlargement of inflamed subgingival surface area in deepened periodontal pockets. Alveolar bone loss (ABL), a hallmark of periodontitis, and the number of teeth present were determined from panoramic radiographs. Periodontal diagnosis was defined as "healthy" (no ABL and BOP<25%, n = 46), "gingivitis" (no ABL and BOP≥25%, n = 65), "history of periodontitis" (mild-severe ABL and BOP<25%, n = 92) and "active periodontitis" (mild-severe ABL, BOP≥25%, n = 269). These definitions for the periodontal diagnosis have been reported earlier [23]. Edentulous subjects (n = 33) lack periodontal diagnosis.

Subgingival bacterial samples were collected from the deepest periodontal pocket in each quadrant [24]. Bacterial levels of seven studied periodontal pathogens, *A. actinomycetemcomitans, P. gingivalis, P. endodontalis, P. intermedia, T. forsythia, C. rectus* and *F. nucleatum,* were analyzed with checkerboard DNA-DNA hybridization [25,26]. Bacterial prevalence was defined as level >0.

Information on weight, height and smoking was obtained from questionnaires. Subjects were considered having hypertension, dyslipidemia or diabetes mellitus if they had respective mediations. Coronary diagnosis was acquired from the coronary artery angiography, symptoms, and laboratory determinations as follows: no significant CAD/controls (<50% stenosis, n = 152), stable CAD (≥50% stenosis, n = 184), acute coronary syndrome (ACS; ≥50% stenosis, an episode of typical chest pain, and elevated levels of a cardiac biomarkers, n = 169) [19]. Blood samples were drawn and stored at −80 C°.

Serum levels of immunoglobulin A and G (IgA/IgG) against whole cell antigen of all studied bacteria were determined with enzyme-linked immunosorbent assay (ELISA) [27]. When the antigen composed of several strains, the bacterial suspensions with aligned densities were mixed in equal volumes before coating the plates. The serum samples were analyzed as duplicates and two dilutions. The mean absorbance values from four wells were calculated and normalized per reference serum samples on each plate and used as a continuous variable [28,29]. *A. actinomycetemcomitans* and *P. endodontalis* IgA/IgG determinations have been described earlier [30,31] and the remaining laboratory analyses were performed for this study. The used bacterial strains (DNA-DNA hybridization/ELISA), ELISA dilutions and inter-assay coefficients of variations are displayed on Supplemental Table 1. We summed the levels of subgingival *A. actinomycetemcomitans* (two strains) and *F. nucleatum* (four strains) after controlling that the prevalence of any single strain does not affect the corresponding serum antibody response (non-significant in Mann-Whitney U and Students T- tests, data not shown).

2.3. Statistical analyses

The statistical analyses were performed with the SPSS statistics software (version 24; IBM Corp, Armonk, NY, USA). Comparison between groups was performed with the Mann-Whitney *U* test (non-normal) for continuous variables and χ^2 test for categorical variables. The levels of subgingival bacteria and their corresponding serum antibodies were standardized with Z-score prior to analysis to increase normality and to facilitate comparison between