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Salivary IgA antibody to malondialdehyde–acetaldehyde associates with mild periodontal pocket depth

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Abstract

Objective: Oxidized epitopes such as malondialdehyde–acetaldehyde (MAA) play a crucial role in the progression of atherosclerosis through activation of the humoral immune response. The exact mechanism of the association between atherosclerosis and periodontal diseases is not fully understood. The aim of the current study is to evaluate the association of oral humoral immune response to oxidized epitopes with parameters of periodontal disease.

Materials and methods: The Parogene cohort consist of patients who have undergone coronary angiography due to cardiac symptoms. In this study, 423 patients were randomly selected for an extensive oral examination. Salivary Immunoglobulin A to oxidized epitopes and bacterial antigens was determined by chemiluminescence immunoassay.

Results: In a binary logistic regression model adjusted with periodontal disease confounders, periodontal pocket depth (PPD) 4–5 mm associated with salivary IgA antibodies to MAA-LDL ($p = 0.034$), heat shock protein 60 of *Aggregatibacter actinomycetemcomitans* ($p = 0.045$), *Porphyromonas gingivalis* ($p = 0.045$), *A. actinomycetemcomitans* ($p = 0.005$), *P. intermedia* ($p = 0.020$), and total IgA ($p = 0.003$).

Conclusions: The current study shows the association of salivary IgA to MAA-LDL with PPD 4–5 mm in a cohort of patients with chronic coronary artery disease. Humoral immune cross-reactivation to oxidized epitopes such MAA-LDL could partly explain the link of periodontitis with systemic diseases.

KEYWORDS
IgA, MAA, malondialdehyde–acetaldehyde, periodontal pocket depth, periodontitis

1 | INTRODUCTION

Periodontitis is a chronic inflammatory condition where dysbiosis of oral microbiome has a central role in the disease progression by altering oral the immune system (Hajishengallis, 2015). The biofilm retained in the gingiva initiates local inflammation; clinically, gingivitis is observed as increased bleeding on probing (BOP) (Kinane et al., 2017). Periodontitis occurs when untreated gingivitis causes periodontal attachment loss, which is clinically is diagnosed by deepened periodontal pockets (Kinane et al., 2017).

Neutrophils are predominant cells in the host immune response to bacterial dysbiosis leading to periodontitis (Hajishengallis, 2015). It has been shown that in patients with periodontitis neutrophils are hyperactive and exhibit excess reactive oxygen species (ROS) formation (Matthews et al., 2007). The increased levels of ROS have a major role in host microbial defense (Nathan & Cunningham-Bussel, 2013). The ROS reactions are unspecific; during oxidative reactions, biological structures such as cell membrane lipids are modified, and malondialdehyde (MDA) is the main product of lipid peroxidation (Binder et al., 2016). MDA is presented on the surface of apoptotic cells and damaged structures such as oxidized low-density lipoproteins (OxLDLs) (Binder et al., 2016). It has been shown that patients with chronic periodontitis or aggressive periodontitis express high levels of MDA compared to controls (Baltacioglu et al., 2014). MDA and acetaldehyde are highly reactive and form malondialdehyde–acetaldehyde (MAA). MAA epitopes are highly stable and immunogenic entities (Thiele et al., 2015). MAA epitopes are present in inflamed periodontal lesions (Bright et al., 2018). Previously, we have shown that healthy subjects possess salivary IgA antibodies to MAA epitopes (Akhi et al., 2017).

Local dysbiosis of microbiota has a crucial role in the initiation and development of periodontitis (Lamont et al., 2018). *Porphyromonas gingivalis* is an anaerobic gram-negative bacterium and plays a central role in the pathogenesis of periodontitis by subverting the host immune system and shifting the microbial community to a dysbiotic mode (Hajishengallis, 2015). *Porphyromonas gingivalis* possesses various virulence factors such as fimbriae, lipopolysaccharide, and gingipains (Bostanci & Belibasakis, 2012). Gingipains have proteolytic activity and are classified according to their substrate specificity as arginine-specific gingipains (Rgp) and lysine-specific gingipains (Kgp) (Bostanci & Belibasakis, 2012). Immunization of murine model of periodontitis with RgpA prevents *P. gingivalis*-induced oral bone loss (Gibson & Genco, 2001). Previously, we have shown that monoclonal IgM antibody to MDA-LDL recognizes Rgp44 of the gingipain hemagglutinin/ adhesion domain (Turunen et al., 2012), and immunization of $LDLR^{-/-}$ mice with Rgp44-induced IgM antibodies to MAA-LDL (Kyrklund et al., 2018). Healthy human subjects have salivary IgA antibodies to Rgp44 and the antibodies show cross-reactivity with MAA epitopes (Akhi et al., 2017). We have also shown that monoclonal IgM antibody to *Aggregatibacter actinomycetemcomitans* virulence factor heat shock protein 60 (*Aa*-HSP60) cross-reacts with MAA-LDL (Kyrklund et al., 2020; Wang et al., 2016).

Observational studies support an association between periodontitis and CAD, but the mechanism behind the association is not fully understood (Lockhart et al., 2012). The immune response to oxidized epitopes has a prominent role in atherosclerosis development and MAA-LDL is an antigen model of oxidized epitopes (Sage et al., 2019). Recently, in the present population, we have shown the association of salivary IgA to MAA-LDL and Rgp44 with coronary artery disease (CAD) and acute coronary syndrome (ACS) (Akhi et al., 2019). The current study evaluated the link between oral mucosal humoral immune response to oxidized epitopes and clinical parameters of periodontal disease in a coronary artery disease cohort.

2 | MATERIALS AND METHODS

2.1 | Population

The current study subjects are a sub-population of the Corogene (n = 5297) study. The Corogene study consisted of patients who underwent coronary angiography for any reason in Helsinki University Hospital (Vaara et al., 2012). Ten percent of the patients were randomly invited to an extensive oral examination in the Parogene cohort (Buhlin et al., 2011). All subjects signed an informed consent. The study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the ethics committee of the Helsinki University Central Hospital. The current study is comprised of 423 patients (83.3% of the original cohort of 508) with available saliva samples and periodontal status. Patients' health records were obtained from the hospital registry and their diseases were classified according to medication (Vaara et al., 2012). The patients' coronary artery disease status was classified as follows: no coronary artery disease (no-CAD) (no significant coronary artery disease; stenosis <50%), chronic CAD (stenosis >50% in at least one coronary artery), and acute coronary syndrome (ACS) (Stenosis >50%, chest pain and electrocardiographic changes). Prior to oral examination, patients were asked to fill a questionnaire to review their smoking status. Patients were considered as current smokers if they had quit smoking less than 6 months ago or were currently smoking (Buhlin et al., 2011).

2.2 | Oral examination

The oral examination was performed by two calibrated periodontists as previously described (Buhlin et al., 2011). The oral examination included palpation of masticatory muscles, temporomandibular joint, and lymph nodes. Bleeding on probing (BOP) was registered. The periodontal pocket depth (PPD) was measured from six sites of teeth. The subgingival bacterial samples were obtained from the deepest periodontal pocket of each dentate quadrant. The microbiological analysis was performed by DNA–DNA hybridization assay as previously described (Mäntylä et al., 2013). Oral panorama radiographs have been evaluated by an experienced oral radiologist. The patients' alveolar bone loss (ABL) was evaluated from radiographs and categorized as: no bone loss (none-ABL), ABL in cervical third of root (mild-ABL), ABL in mid-third of root (moderate-ABL), ABL in apical-third of root (severe-ABL), and total bone support loss (total-ABL).

2.3 | Antigens and immunoassay

MAA-LDL and CuOx-LDL were generated as previously described (Hörkkö et al., 1999; Veneskoski et al., 2011). Bacterial antigens were prepared using the following bacterial strains: *Prevotella intermedia* (ATCC 25611), *Porphyromonas endodontalis* (ATCC 35406), *Tannerella forsythia* (ATCC 43037), *Aggregatibacter actinomycetemcomitans* serotypes a, b, c, d, e, f and nonserotypeable strain x (ATCC 29523,

ATCC 43718, ATCC 33384, IDH 781, IDH 1705, CU 1000, C59A) and *Porphyromonas gingivalis* serotypes a, b, c (ATCC 33277, W50, OMGS 434) (Hyvärinen et al., 2009; Pussinen et al., 2002). *Porphyromonas gingivalis* and *A. actinomycetemcomitans* antigens were a mix of the listed strains. Bacteria were cultured anaerobically supplemented with 5%–10% blood. Bacteria were heat-killed by incubation in PBS (60°C) for 1 h. The *A. actinomycetemcomitans* heat shock protein (*Aa*-HSP60) and recombinant *P. gingivalis* virulence factor, gingipain Rgp44, were prepared as previously described (Turunen et al., 2012; Wang et al., 2016). Salivary IgA to oxidized LDL and bacterial antigens was determined with chemiluminescence immunoassay as previously described (Akhi et al., 2017). Triplicate measurement of saliva samples was performed. The saliva was diluted as follows: 1:250 for total IgA, 1:50 for IgA to oxidized antigens, 1:20 for *Aa*-HSP60, and 1:10 for bacterial antigens. Chemiluminescence immunoassay results were shown in relative light units (RLU) per 100 milliseconds (ms).

2.4 | Statistics

For the statistical analysis, data with skewed distributions were logarithmically transformed and geometric means were calculated. The differences between periodontal parameters groups were analyzed with one-way analysis of variance (ANOVA). The association of periodontal parameters with salivary IgA antibodies to antigens was analyzed by binary logistic regression model adjusted with clinical confounders: gender, age, smoking, coronary artery disease, diabetes (yes/no), and total DNA probe. For the binary logistic regression model, patients' data were categorized into binary form as follows: PPD 4–5 mm, 0: 0–6 periodontal pockets, 1: more than 6 periodontal pockets; PPD 6 mm, 0: 0–3 periodontal pockets, 1: more than 3 periodontal pockets; ABL, 0: none-mild, 1: moderate to severe alveolar bone loss. For statistical analyses, SPSS statistics software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) was used. *p*-value below 0.05 was considered as significant. The numbers of variables missing data are shown in supplemental Table 1.

TABLE 1 Baseline characteristics of the population

Characteristics	Mean (SD)
Age, y	62.96 (9.2)
BMI, kg/m^2	27.85 (5.1)
BOP %	37.27 (18.9)
Number of teeth	21.41 (7.5)
Total DNA probe[a]	86.44 (76.6)
Saliva total IgA, µg/mL	361.59 (161.1)
	N (%)
Gender (men)	282 (66.7)
Smoking	
Never	202 (47.8)
Former	168 (39.7)
Current	53 (12.5)
Diabetes	92 (21.7)
Dyslipidemia	338 (79.7)
Hypertension	265 (62.6)
CAD diagnosis	
No CAD	127 (30.0)
CAD	155 (36.6)
ACS	139 (32.9)

Abbreviations: ACS, acute coronary syndrome; BMI, body mass index; BOP, bleeding on probing; CAD, coronary artery disease.
[a]Unit, count ×10^5.

3 | RESULTS

The basic characteristics and risk factors of the study subjects are shown in Table 1. The population was elderly, with a mean age of 63.0 years, and 66.7% of the study subjects were male. Over half of (52.2%) were former or current smokers. Dyslipidemia medication was used by 79.7% of the cohort, 62.6% had been prescribed

TABLE 2 Salivary total IgA antibody according to clinical parameters of periodontal disease

	N	Mean of salivary IgA µg/ml (SD)
Tertiles of BOP%		
0–25	135	348.1 (172.1)
26–44	151	358.3 (156.7)
44–100	137	378.5 (154.2)
		p = 0.185
Sites with PPD 4–5 mm		
0	43	301.7 (128.9)
1–6	122	336.3 (138.4)
7–16	139	377.7 (169.3)
17–	119	390.4 (174.9)
		***p* = 0.001**
Sites with PPD ≥6 mm		
0	234	339.0 (149.6)
1–3	94	381.6 (162.4)
4–6	38	360.9 (173.2)
7–	57	421.6 (179.9)
		***p* = 0.005**
Alveolar bone loss		
None	103	338.0 (136.5)
Mild	192	360.0 (166.4)
Moderate	105	389.1 (170.2)
Severe - total	22	341.8 (157.1)
		p = 0.098

Note: The difference of salivary total IgA levels according to periodontal parameters was analyzed by one-way analysis of variance (ANOVA). Statistically significant *p*-values are bolded.

hypertension medication, and 21.7% of the participants had diabetes.

3.1 | Salivary total IgA and periodontal disease

The association of salivary total IgA with periodontal parameters was examined (Table 2). The total salivary IgA levels increased with the number of sites with periodontal pocket depth (PPD) 4–5 mm ($p < 0.001$) and PPD ≥6 mm ($p = 0.005$).

3.2 | Salivary cross-reactive IgA antibodies to MAA-LDL and periodontal disease

Salivary IgA antibodies to MAA-LDL increased with the number of sites of PPD 4–5 mm ($p = 0.015$) (Figure 1a). Salivary IgA to periodontal pathogen virulence factors Rgp44 ($p < 0.001$) and *Aa*-HSP60 ($p = 0.034$) increased with number of sites of PPD 4–5 mm (Figure 1a), but such association was not observed with the number of sites with PPD ≥ 6 mm (Figure 1b). In addition, salivary IgA to Rgp44 increased with the levels of alveolar bone loss ($p = 0.030$) (Figure 1c). Salivary IgA to CuOx-LDL ($p = 0.020$) decreased with the levels of bleeding on probing (Figure 1d).

3.3 | Salivary IgA antibodies to oral pathogen and periodontal disease

Salivary IgA antibodies to *P. gingivalis* ($p = 0.001$), *A. actinomycetemcomitans* ($p = 0.031$), and *P. intermedia* ($p < 0.001$) increased significantly according to the number of sites with periodontal pocket depth 4–5 mm (Figure 2a). Salivary IgA to *P. gingivalis* increased significantly according to periodontal pocket depth ≥6 mm ($p = 0.001$)

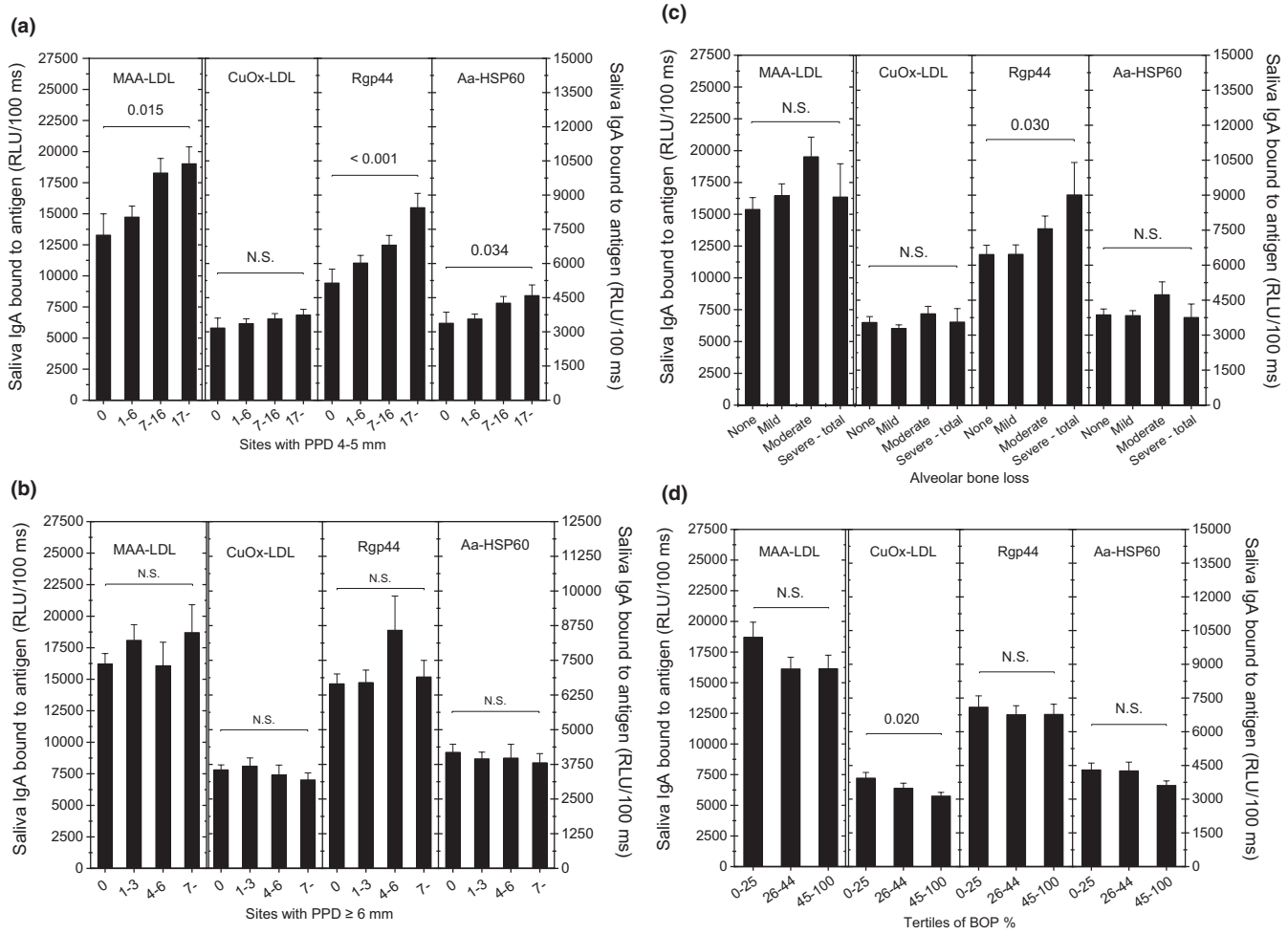


FIGURE 1 Level of salivary IgA antibody to cross-reactive epitopes according to periodontal status. (a) Sites with periodontal pocket depth (PPD) 4–5 mm, (b) Sites with periodontal pocket depth ≥6 mm (c) Levels of periodontal alveolar bone loss (ABL) (d) Percentage of periodontal sites with bleeding on probing (BOP%). The error bar represents standard error of the mean (SEM). Parameters due to skewed distributions were logarithmically transformed. The difference between groups was analyzed by one-way analysis of variance (ANOVA). *Aa*-HSP60, *Aggregatibacter actinomycetemcomitans* heat shock protein 60; CuOx-LDL, copper-oxidized low-density lipoprotein; MAA-LDL, malondialdehyde acetaldehyde–modified low-density lipoprotein; NS, not significant; Rgp44, *Porphyromonas gingivalis* A hemagglutinin domain; RLU, relative light unit

and alveolar bone loss (p = 0.001) (Figure 2b,c). Salivary IgA antibody to periodontal pathogens did not associate with levels of bleeding on probing levels (Figure 2d).

3.4 | Salivary IgA to MAA-LDL and oral pathogens associate with early stage of periodontitis

A statistical model adjusted with periodontal disease confounding factors gender, age, smoking, coronary artery disease, diabetes (yes/no), and total DNA probe was used to evaluate the association of salivary IgA to oxidized epitopes and periodontal pathogens with periodontal parameters. Early PPD 4–5 mm associated with salivary IgA to MAA-LDL (p = 0.034), *Aa*-HSP60 (p = 0.045), *P. gingivalis* (p = 0.045), *A. actinomycetemcomitans* (p = 0.005), *P. intermedia* (p = 0.020), and total IgA (p = 0.003) (Figure 3a). Periodontal pocket depth ≥6 mm associated with salivary total IgA (p = 0.046) (Figure 3b). The alveolar bone loss associated with saliva IgA antibody to *P. gingivalis* (p = 0.018) and *P. intermedia* (p = 0.037) (Figure 3c).

4 | DISCUSSION

In the current study, salivary IgA antibodies to MAA epitopes associated with early periodontal pocket depth (PPD) 4–5 mm, also salivary IgA levels to *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and virulence factor *Aa*-HSP60 also associated with early PPD 4–5. The salivary IgA antibody levels to periodontal pathogens *P. gingivalis* and *P. intermedia* associated with radiographically diagnosed alveolar bone loss.

Oxidized epitopes such as MDA are products of reactive oxidative species and present on apoptotic cell wall (Miller et al., 2011), and MAA epitopes are advanced malondialdehyde (MDA) lysine adducts (Weismann et al., 2011). In homeostasis, MDA are recognized and removed by the innate immune system (Binder et al.,

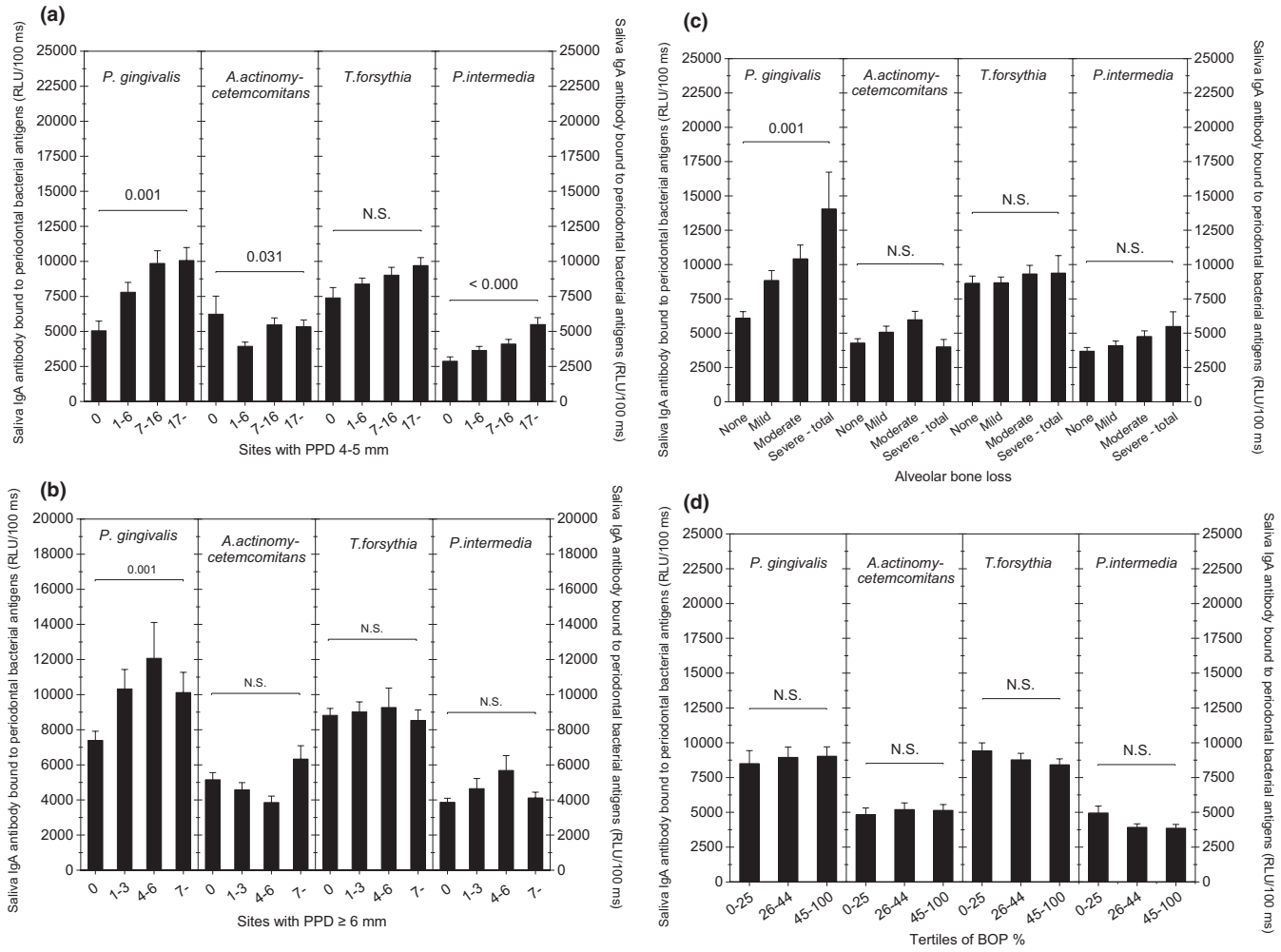


FIGURE 2 Level of salivary IgA antibody to periodontal pathogens according to periodontal status. (a) Sites with periodontal pocket depth (PPD) 4–5 mm, (b) Sites with periodontal pocket depth ≥6 mm (c) Levels of periodontal alveolar bone loss (ABL) (d) Percentage of periodontal sites with bleeding on probing (b). The error bar represents standard error of the mean (SEM). Parameters due to skewed distributions were logarithmically transformed. The difference between groups was analyzed by one-way analysis of variance (ANOVA). NS, not significant. RLU, relative light unit

2016). However, if the oxidized epitopes are not efficiently cleared, they can turn into damage-associated molecular patterns (DAMP) and initiate sterile inflammation (Binder et al., 2016). In a mouse model, MDA epitope neutralization reduced bone loss induced osteoporosis disease (Ambrogini et al., 2018). Our group has shown in cross-sectional studies that plasma IgA to MAA-LDL associates with obesity and markers of glucose metabolism (Vehkala et al., 2013) and plasma IgM antibody to MDA-LDL associates inversely with intima media thickness (Karvonen et al., 2003). A recent meta-analysis covering 14 studies and 991 participants showed that patients with periodontitis have high MDA levels in salivary and gingival crevicular fluids (Chen et al., 2019). Periodontal therapy decreases MDA levels in patients' gingival crevicular fluid (Wei et al., 2010). Here, we show that host response to oxidized epitopes may induce the salivary IgA antibody to MAA-LDL.

The exact mechanism behind the association of periodontal diseases with atherosclerosis remains unknown (Lockhart et al., 2012). The humoral immune response triggered by oxidized epitopes has a key role in atherogenesis (Sage et al., 2019). We have shown that atherosclerotic mouse model immunized with *P. gingivalis* and Rgp44 shows increased levels of plasma IgM antibody to MAA-LDL (Kyrklund et al., 2018). Recently, in the current population, we have shown the association of coronary artery disease (CAD) and acute coronary syndrome (ACS) with salivary IgA to MAA-LDL (Akhi et al., 2019). Also, we shown that salivary IgA antibody to MAA-LDL correlates and cross-reacts with periodontal pathogens, Rgp44, and *Aa*-HSP60. The Data provided by the current study contribute to the hypothesis that early stage periodontitis may enhance atherogenesis through the cross-reactivation of humoral immunity.

The hallmark of periodontitis is periodontal attachment loss due to inability of the host to resolve the biofilm-caused inflammation (Hajishengallis, 2015). During pro-inflammatory response, IgA antibodies against the biofilm are secreted (Kinane et al., 2017). Current understanding on the role of salivary IgA in periodontitis is limited. Individuals with IgA deficiency do not have increased risk for periodontal disease (Jorgensen et al., 2010). On the other hand, periodontal treatment consisting of scaling and root planing decreases the salivary total IgA in four weeks after the treatment, but does not influence long-term salivary IgA levels (Hagewald et al., 2003). Here, we show the association of total IgA with periodontal pocket depth of (PPD) 4–5 mm and PPD ≥ 6 mm. This finding supports the hypothesis that salivary total IgA may increase due to host humoral immune response in periodontitis.

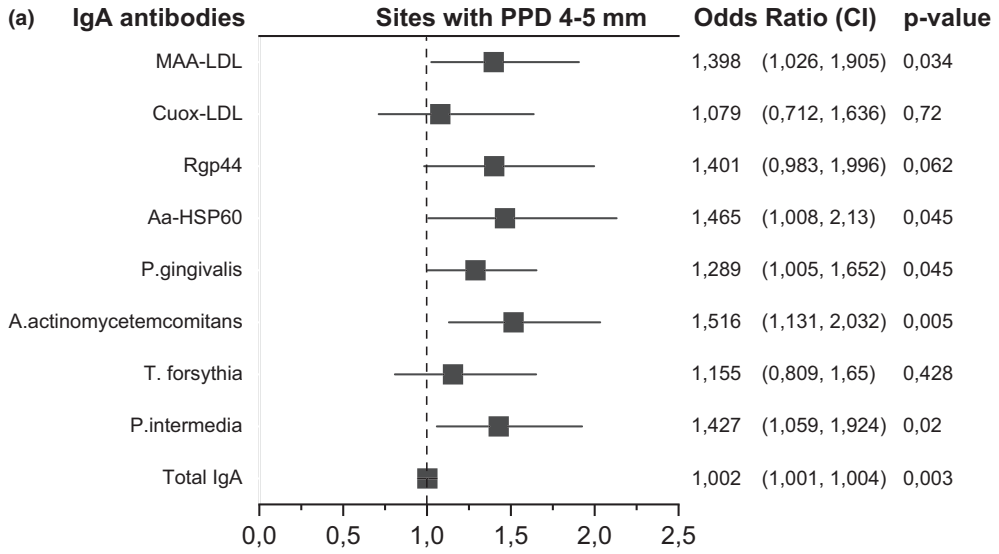
There are more than 700 bacteria species residing in the oral cavity, and IgA immunoglobulin has an essential role in microbiome homeostasis in the mucosa (Brandtzaeg, 2013; Dewhirst et al., 2010). Recent findings suggest the presence of two distinct humoral immunity responses in the gastrointestinal tract (Bunker & Bendelac, 2018). First, a homeostatic humoral response to commensal bacteria involving natural polyreactive IgA antibodies, and second, an induced IgA antibody to a specific pathogen with high affinity and specificity (Bunker et al., 2017). Here, we have shown increased levels of salivary IgA antibodies to *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia* in the early stages of periodontitis. Currently, it is unknown whether these antibodies are natural IgA antibodies or induced IgA antibodies due to specific pathogens, and this issue should be addressed in future studies.

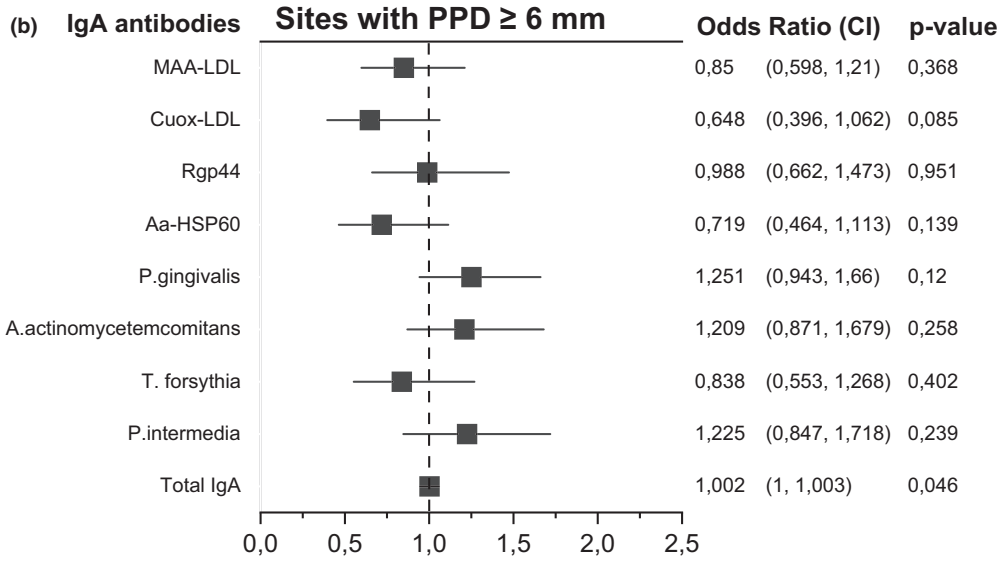
Earlier studies have shown the association of IgA antibodies to periodontal pathogens such as *P. gingivalis* and *A. actinomycetemcomitans* with periodontitis (Kinane et al., 1999). Here, salivary IgA to *A. actinomycetemcomitans* and *Aa*-HSP60 associated with early stage, and not with advanced phase of periodontitis lesions. It is worth mentioning that IgA to Rgp44 did not associate with periodontitis after being adjusted with risk factors. The data highlight the role of *A. actinomycetemcomitans* virulence factors in early-stage periodontitis lesions by activating the humoral immune response.

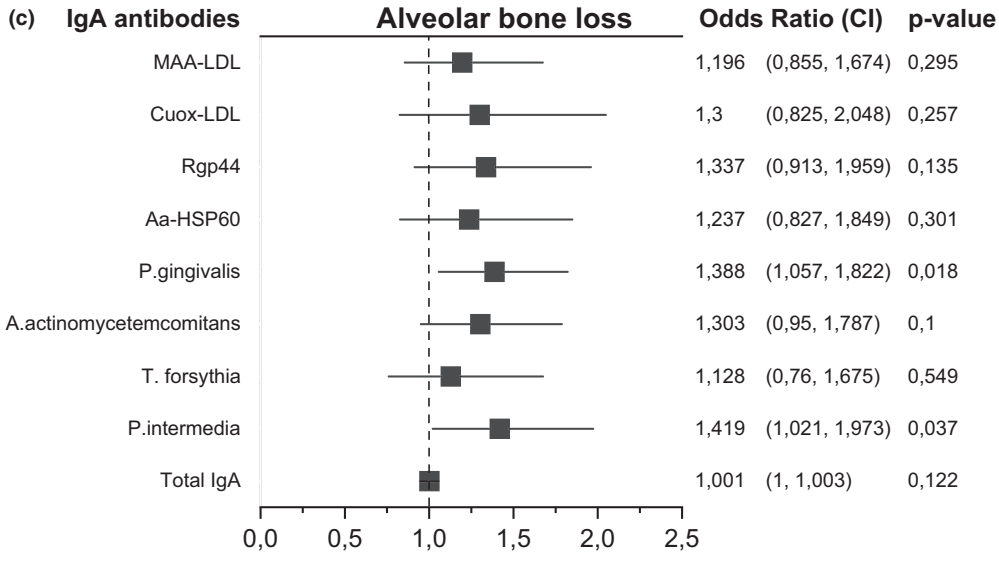
The strengths of the current study are the relatively large cohort and well verified health background of the cohort study subjects. The main limitation is the cross-sectional cohort setup and patient enrolment from an existing pool of patients. Due to skew distribution, the data were logarithmically transformed, and odd ratio are in logarithmic scale. Age may also be a limitation since there is generally a high level of oxidative stress in aging population (Liguori et al., 2018).

In conclusion, here we have shown the association of salivary IgA to MAA-LDL with periodontal parameters. Periodontitis has been linked with systemic diseases such as atherosclerosis and diabetes type 2 (Graves et al., 2020; Lockhart et al., 2012). The result of the current study shows that oxidative stress products formed during periodontitis may activate humoral immune response. Increased humoral immune response to oxidized epitopes such MAA-LDL could partly explain the link of periodontitis with systemic diseases.

FIGURE 3 Levels of salivary IgA antibodies to antigens in (a) Sites with periodontal pocket depth (PPD) 4–5 mm; (b) Sites with periodontal pocket ≥6 mm; (c) Periodontal alveolar bone loss were analyzed by multinomial logistic regression. The odd ratio variation is at logarithmic level. Model was adjusted with gender, age, smoking, coronary artery disease, diabetes, and total DNA probe. Data values were logarithm transformed due to skewed distributions except "Total IgA" which had normal distribution; odds ratio represents the difference in logarithmic scale. For the binary logistic regression model, patients' data were categorized into binary form as follows: PPD 4–5 mm, 0: 0–6 periodontal pocket, 1: more than 6 periodontal pockets; PPD 6 mm, 0: 0–3 periodontal pockets, 1: more than 3 periodontal pockets; ABL, 0: none-mild, 1: moderate to severe alveolar bone loss. *Aa*-HSP60, *Aggregatibacter actinomycetemcomitans* heat shock protein 60; CuOx-LDL, copper-oxidized low-density lipoprotein; MAA-LDL, malondialdehyde acetaldehyde-modified low-density lipoprotein; Rgp44, *Porphyromonas gingivalis* A hemagglutinin domain







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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS

Ramin Akhi: Conceptualization; Formal analysis; Methodology; Project administration; Visualization; Writing-original draft; Writing-review & editing. **Antti E Nissinen:** Conceptualization; Methodology; Writing-review & editing. **Chunguang Wang:** Conceptualization; Methodology; Writing-review & editing. **Mikael Kyrklund:** Conceptualization; Methodology; Writing-review & editing. **Susanna Paju:** Conceptualization; Methodology; Writing-review & editing. **Päivi Mäntylä:** Conceptualization; Methodology; Writing-review & editing. **Kare Buhlin:** Conceptualization; Methodology; Writing-review & editing. **Juha Sinisalo:** Conceptualization; Methodology; Writing-review & editing. **Pirkko Pussinen:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Writing-review & editing. **Sohvi Hörkkö:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Writing-review & editing.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Research data not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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