Porphyromonas gingivalis as a Possible Risk Factor in the Development/Severity of Acute Alcoholic Hepatitis

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Bacterial infection is frequently observed in patients with alcoholic liver disease (ALD). We examined a possible role of *Porphyromonas gingivalis* in the development/progression and severity of disease in patients with acute alcoholic hepatitis (AAH). Plasma specimens from 47 patients with AAH (16 moderate, Model for End-Stage Liver Disease [MELD] score <20; 31 severe, MELD score >20) and 22 healthy controls (HCs) were collected. Clinical, drinking history (lifetime drinking history [LTDH]), and demographic data were collected. Antibody tests for immunoglobulin (Ig) G, IgM, and IgA against two *P. gingivalis* strains were performed. Between-group comparisons and within-group association analyses were carried out. Patients with severe AAH showed significantly higher plasma levels of IgG, IgA, and IgM against two *P. gingivalis* strains (W83 and 33277) compared to HCs. Patients with moderate AAH also had significantly elevated anti-*P. gingivalis* IgA concentrations for both strains compared to HCs. Male patients with moderate AAH showed a significant inverse association in LTDH and anti-*P. gingivalis* IgM. The aspartate aminotransferase:alanine aminotransferase ratio was positively associated with IgM of both strains in male patients with moderate AAH. Female patients with severe AAH showed a significant association between MELD scores and W83 IgM. *Conclusion:* Antibody response to *P. gingivalis* in AAH is elevated. Significantly elevated plasma anti-*P. gingivalis* IgG, IgA, and IgM in severe AAH provide preliminary data that *P. gingivalis* could be a novel risk factor in the development/severity of AAH. (*Hepatology Communications* 2019;3:293-304).

Alcoholic liver disease (ALD) is a major cause of morbidity, mortality, and health care expenditures in the United States and worldwide.[1] Acute alcoholic hepatitis (AAH) is an especially severe form of ALD that can carry a high short-term mortality risk.[1] Multiple factors, such as drinking pattern, sex, viral hepatitis infection, iron overload, and malnutrition, are contributing factors associated with the development/progression of ALD.[1] However, the role of oral bacterial infections as potential risk factors in the development/progression of ALD has not been thoroughly investigated.

Periodontal diseases (PDs) are induced by dysbiotic oral bacterial communities and affect the supporting structures of the teeth, including the gingiva, alveolar bone, and periodontal ligament. *Porphyromonas gingivalis* is a major pathogen of severe PD. It functions as a keystone pathogen[2] that not only sets the stage for the entire cascade of PD by altering the local immune microenvironment but also enters the blood circulation, is disseminated throughout the body, and contributes to multiple systemic diseases,[3] such as diabetes,[4] atherosclerosis,[5] rheumatoid arthritis,[6] and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH).[7,8] However, its role in ALD, particularly in AAH, is not clear.

P. gingivalis is known to cause alterations in immunoglobulin (Ig) response.[9] Changes in serum Ig levels occur frequently in liver disease.[10] Patients with ALD frequently show an increase in IgA serum levels, and IgA can be deposited in a continuous pattern along the hepatic sinusoids.[11] With the commencement of organ injury, IgM (primary response) rises significantly, whereas the IgG (secondary) response is slower. After several weeks, the IgM levels decrease and IgG rises.[12] Detection of serum Ig levels has been used to assist in the diagnosis of liver diseases.[13]

Because bacterial infections are common in patients with advanced ALD,[14] we determined the plasma anti-*P. gingivalis*-specific antibody profiles (IgG, IgM, and IgA) in order to determine whether *P. gingivalis* is associated with the development/severity of AAH as an independent risk factor. We also evaluated the roles of sex and lifetime drinking history (LTDH) in association with *P. gingivalis* antibody responses and AAH.[15]

Participants and Methods

STUDY PARADIGM

This investigation was a single time point evaluation of patients and healthy volunteers. We assessed blood samples, clinical data, relevant medical history, clinical markers of progression, and severity of AAH and drinking history. All authors had access to the study data and had reviewed and approved the final manuscript. We analyzed laboratory markers from plasma samples and compared the factors between patients with severe and moderate AAH and healthy volunteers. We detected anti-*P. gingivalis* antibody responses from the plasma samples and examined the association of anti-*P. gingivalis* antibody responses with clinical measures of disease severity. This study was approved by the institutional review board (protocol No.12.0427) of the University of Louisville.

STUDY PARTICIPANTS

Patients with AAH (31 patients with severe AH with Model for End-Stage Liver Disease [MELD] score ≥20 and 16 patients with moderate AAH with MELD score <20) and 22 healthy volunteers were included in this clinical study. This investigation is part of a large national multisite clinical trial (clinicaltrials.gov: NCT01809132) supported by the National Institute on Alcohol Abuse and Alcoholism (NIAAA). All patient participants were diagnosed with AAH. Patients were 21 to 66

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years of age, completed the consenting process for participation in the study, and did not have active drug abuse. Healthy controls (HCs) were of similar age and did not have liver disease or any comorbid conditions (heart, kidney, lung, neurologic or psychiatric illness, sepsis), and none had any acute or chronic inflammatory process. Pregnant and lactating women, prisoners, and other individuals with potential vulnerability were excluded from the study.

SPECIMEN COLLECTION

Whole blood (approximately 8 mL) was collected, and plasma was apportioned into 1-mL aliquots and stored at –80°C until use. Freeze–thaw cycles were avoided to maintain the integrity of the plasma samples.

CLINICAL, DEMOGRAPHIC, AND DRINKING DATA

Collected data consisted of age, sex, body mass index, drinking history (using LTDH), medical assessments at admission (specific for the study to rule out any significant comorbid conditions, e.g., heart, lung, psychiatric illnesses, sepsis), and medical history. Confirmatory tests for AAH (laboratory and imaging) and markers of liver disease severity (Child-Turcotte-Pugh [CTP], MELD, and Maddrey discriminant function [DF]) were also performed. The laboratory panel included a comprehensive metabolic panel (including liver panel) and coagulation assessment. Other liver diseases were excluded using blood tests recommended by the NIAAA Alcoholic Hepatitis Consortia.[16]

BACTERIAL STRAINS AND GROWTH CONDITIONS

P. gingivalis strains W83 and ATCC 33277 were purchased from ATCC and cultured anaerobically at 37°C. Bacteria culture medium was trypticase soy broth supplemented with yeast extract (1 mg/mL), hemin (5 µg/mL), and menadione (1 µg/mL).[17] Bacteria were harvested in the exponential phase (optical density $[OD]_{600}$ = 1) and washed 3 times with phosphate-buffered saline (PBS) before use.

P. GINGIVALIS WHOLE-CELL ENZYME-LINKED IMMUNOSORBENT ASSAY FOR PLASMA ANTIBODY DETECTION

Overnight-cultured *P. gingivalis* bacteria were washed 3 times with PBS, and then 2×10^7 *P. gingivalis* were deposited on a polystyrene enzyme-linked immunosorbent assay (ELISA) plate in 0.1-M sodium carbonate buffer (pH 9.5) at room temperature for 2 hours. After three washes, the plate was blocked with PBS (pH 7.0) containing 5% fetal bovine serum for 1 hour. After washing 3 times with 0.05% PBS containing 0.05% Tween 20, the plate was incubated with 100 µL of a patient's plasma diluted 1:200 for 2 hours. After washing 5 times, the plate was incubated with 100 µL of 1:3,000 horseradish peroxidase (HRP)-coupled goat anti-human IgG (catalog No. 62-8420; Invitrogen) or 1:5,000 HRP-coupled goat anti-human IgM (catalog No. A6907; Sigma), or 1:10,000 HRP-coupled goat anti-human IgA (catalog No. A0296; Sigma) antibodies, specifically, for 1 hour. After seven washes, color was developed with tetramethylbenzidine for 30 minutes and stopped with 2 M H_2SO_4. Absorbance was read at OD_{450}.[18]

STATISTICAL ANALYSIS

Factorial 1-way analysis of variance was used to determine significance between group differences for clinical, demographic, drinking data, and *P. gingivalis* antibody levels. Linear regression was used for association analysis. Outcomes for association analyses were presented using significance level and model fit (adjusted R^2). All statistical analyses were performed using IBM SPSS (version 25.0; Chicago, IL). Normally distributed data were expressed as mean ± SD. Statistical significance was set at $P \leq 0.05$.

Results

PATIENT/HEALTHY VOLUNTEER CHARACTERIZATION

Demographic information and laboratory data of 47 subjects with AAH and 22 healthy volunteers are summarized in Table 1. Subjects with AAH

TABLE 1. CLINICAL PRESENTATION OF AAH AND HEALTHY CONTROLS

Variables	Healthy Controls			Moderate AAH			Severe AAH			Significant Difference
Sex	Male	Female	Total	Male	Female	Total	Male	Female	Total	
Numbers (n)	12	10	22	10	6	16	18	13	31	
Ages (years)	43.7 ± 16.4	51.1 ± 15.2	47.2 ± 15.9	47.4 ± 13.2	51.3 ± 9	48.9 ± 11.6	50.1 ± 10.2	44.8 ± 7.2	47.2 ± 9.6	
Hispanic or Latino (%)		NA		20% (2/10)	16.7% (1/6)	18.8% (3/16)	33.3% (6/18)	14.3% (2/14)	25% (8/32)	
AST (U/L)	27.77 ± 4.83	23.75 ± 7.74	25.84 ± 6.58[†‡]	184.9 ± 97.4	116 ± 32.0	155.4 ± 82.2[†]	138.4 ± 73.8	129.7 ± 80	134.4 ± 75.6[‡]	[†]$P = 0.0001$ (Mo vs. HC); [‡]$P = 0.0001$ (S vs. HC)
ALT (U/L)	27.54 ± 7.24	22 ± 4.67	24.88 ± 6.65[†‡]	95.6 ± 57.5	46.5 ± 18.9	74.6 ± 50.5[†§]	51.2 ± 28.3	37.7 ± 18.7	45 ± 25[‡§]	[†]$P = 0.0001$ (Mo vs. HC); [‡]$P = 0.026$ (S vs. HC); [§]$P = 0.005$ (Mo vs. S)
Alkaline phosphatase (IU/L)		NA		165.4 ± 105	192.5 ± 99.7	177 ± 99.7	192 ± 98.8*	172.3 ± 46.5*	183.1 ± 78.9	*$P = 0.036$ (M vs. F)[‖]; [§]$P = 0.0001$ (Mo vs. S)
Serum total bilirubin (µmol/L)		NA		8.45 ± 6.29	7.4 ± 4.1	8.1 ± 5.4[§]	20.4 ± 8.2	14.9 ± 7.6	17.9 ± 8.3[§]	
Creatinine (mg/dL)		NA		0.75 ± 0.29	0.52 ± 0.08	0.66 ± 0.26	0.98 ± 0.33	0.85 ± 0.74	0.92 ± 0.55	
Albumin (g/dL)		NA		2.62 ± 0.49	2.45 ± 0.58	2.55 ± 0.51	2.49 ± 0.32	2.35 ± 0.57	2.43 ± 0.45	
Globulin (g/dL)		NA		3.65 ± 0.76	3.82 ± 1.14	3.72 ± 0.9	3.44 ± 0.73	3.63 ± 0.85	3.53 ± 0.8	
A/G ratio		NA		0.73 ± 0.14	0.68 ± 0.27	0.71 ± 0.20	0.77 ± 0.19	0.72 ± 0.35	0.74 ± 0.28	
WBC (x10^9/L)		NA		9.31 ± 6.54	9.88 ± 6.36	9.58 ± 6.19	14.7 ± 6.85	12.75 ± 7.88	13.8 ± 7.28	
INR		NA		1.4 ± 0.5	1.35 ± 0.12	1.38 ± 0.39[§]	1.86 ± 0.39	1.9 ± 0.36	1.88 ± 0.37[§]	
AST/ALT	1.05 ± 0.24	1.08 ± 0.24	1.06 ± 0.24[†‡]	2.29 ± 1.35	3.13 ± 2.17	2.65 ± 1.72[†]	2.93 ± 1.14*	3.79 ± 1.96*	3.32 ± 1.60[‡]	*$P = 0.010$ (M vs. F)[‖]; [†]$P = 0.002$ (Mo vs. HC); [‡]$P = 0.0001$ (S vs. HC)
MELD score		NA		17.3 ± 1.9	16.8 ± 2.3	17.1 ± 2.03[§]	24.83 ± 5.23	24.13 ± 4.6	24.52 ± 4.9[§]	[§]$P = 0.0001$ (Mo vs. S)
DF		NA		19.8 ± 17.9	19.2 ± 6.1	19.6 ± 14.3[§]	55.3 ± 21.8	53.9 ± 17.4	54.6 ± 19.6[§]	[§]$P = 0.0001$ (Mo vs. S)
CTP		NA		9 ± 1.41	9.17 ± 0.75	9.08 ± 1.12[§]	11.11 ± 1.49	11 ± 1.41	11.06 ± 1.44[§]	[§]$P = 0.0001$ (Mo vs. S)

Data are means ± SD, analyzed by SPSS 1-way analysis of variance. *Indicates difference between male and female AAH. [†,‡,§] indicate differences between HCs, moderate AAH, and severe AAH. [‖]M vs. F in severe AAH group.

Abbreviations: F, female; INR, international normalized ratio; M, male; Mo, moderate AAH; S, severe AAH; WBC, white blood cell.

were divided into the following two groups based on MELD scores: moderate subjects (MELD score <20) and severe subjects (MELD score ≥20). There were no differences in age, sex, or ethnicity among the three groups.

As expected, compared to HCs, patients with severe AAH exhibited significantly higher alanine aminotransferase (ALT) (P = 0.0001), higher plasma total bilirubin (P = 0.0001), higher international normalized ratio (P = 0.0001), higher percentage of ascites (P = 0.0001), higher DF (P = 0.0001), and higher CTP score (P = 0.0001).

Plasma Anti-*P. gingivalis* IgG, IgA, IgM Antibody Responses Assessed by AAH Severity

Compared to HCs, plasma IgG levels were significantly higher in the total group of patients with severe AAH and in male patients with severe AAH for both *P. gingivalis* strains (Fig. 1A-D) but not in female patients with AAH (Fig. 1E,F). Plasma IgG levels in severe AAH were significantly higher for both *P. gingivalis* strains (Fig. 1A,B) compared to moderate AAH. We did not find any statistical differences in plasma IgG levels between moderate AAH groups and HCs.

Compared to HCs, all patients with severe AAH as well as male and female patients with severe AAH separately had significantly increased IgA responses for both *P. gingivalis* strains (Fig. 2A-F). The levels were significantly higher in all patients with moderate AAH compared to HCs for both *P. gingivalis* strains (Fig. 2A,B) and separately in the male group (Fig. 2C) and the female group (Fig. 2E) for the W83 strain but not for the 33277 strain (Fig. 2D,F).

Plasma IgM levels were significantly higher in all patients with severe AAH compared to HCs for both *P. gingivalis* strains (Fig. 3A,B) but not in male patients (Fig. 3C,D) and female patients (Fig. 3E,F) separately.

Association of Plasma Anti-*P. gingivalis* IgM Responses and LTDH

All patients with AAH showed a significant inverse association in LTDH and IgM antibodies to the two *P. gingivalis* strains (33277, Fig. 4A; W83, Supporting Fig. S1A). All male patients with AAH

but not female patients with AAH (data not shown) showed a significant inverse association (33277, Fig. 4B; W83, Supporting Fig. S1B). We further examined this association within each AAH group. We found that the association between LTDH and IgM antibody levels was only significant in male patients with moderate AAH (33277, Fig. 4C; W83, Supporting Fig. S1C), whereas male patients with severe AAH did not show any significant association between LTDH and IgM antibodies (data not plotted). There was also no association in female patients with AAH.

When we separated patients into two groups by LTDH (group 1, <20 years of drinking; group 2, ≥20 years of drinking) and examined IgM, all patients with AAH showed a significantly higher IgM value in group 1 (33277, Fig. 4D; W83, Supporting Fig. S1D). In patients with severe AAH, IgM was also significantly higher among those with fewer years of drinking (33277, Fig. 4E; W83, Supporting Fig. S1E), whereas in patients with moderate AAH, this association was observed for strain 33277 (Fig. 4F) but not for W83 (Supporting Fig. S1F).

Association of Plasma Anti-*P. gingivalis* IgM Responses and Aspartate Aminotransferase: ALT Ratio

The aspartate aminotransferase (AST):ALT ratio is considered to be a reliable marker for progression of liver injury.[19,20] The association of the AST:ALT ratio and 33277 IgM was not significant in the total group of patients with AAH (Fig. 5A); however, patients with moderate AAH did show a significant association (Fig. 5B). We also evaluated this correlation by sex. Although the AST:ALT ratio was significantly higher in female patients than in male patients in the severe AAH group (Table 1), we found a significant association in the AST:ALT ratio and 33277 IgM in male patients with AAH (Fig. 5C) and in male patients with moderate AAH (Fig. 5D). Female patients in both disease groups did not show any such association (data not shown).

Similarly, there was no significant association between the AST:ALT ratio and W83 IgM antibody in the total group of patients with AAH and patients with moderate AAH (Supporting Fig. S2A,B). Although there was no significant association in all male patients with AAH (Supporting
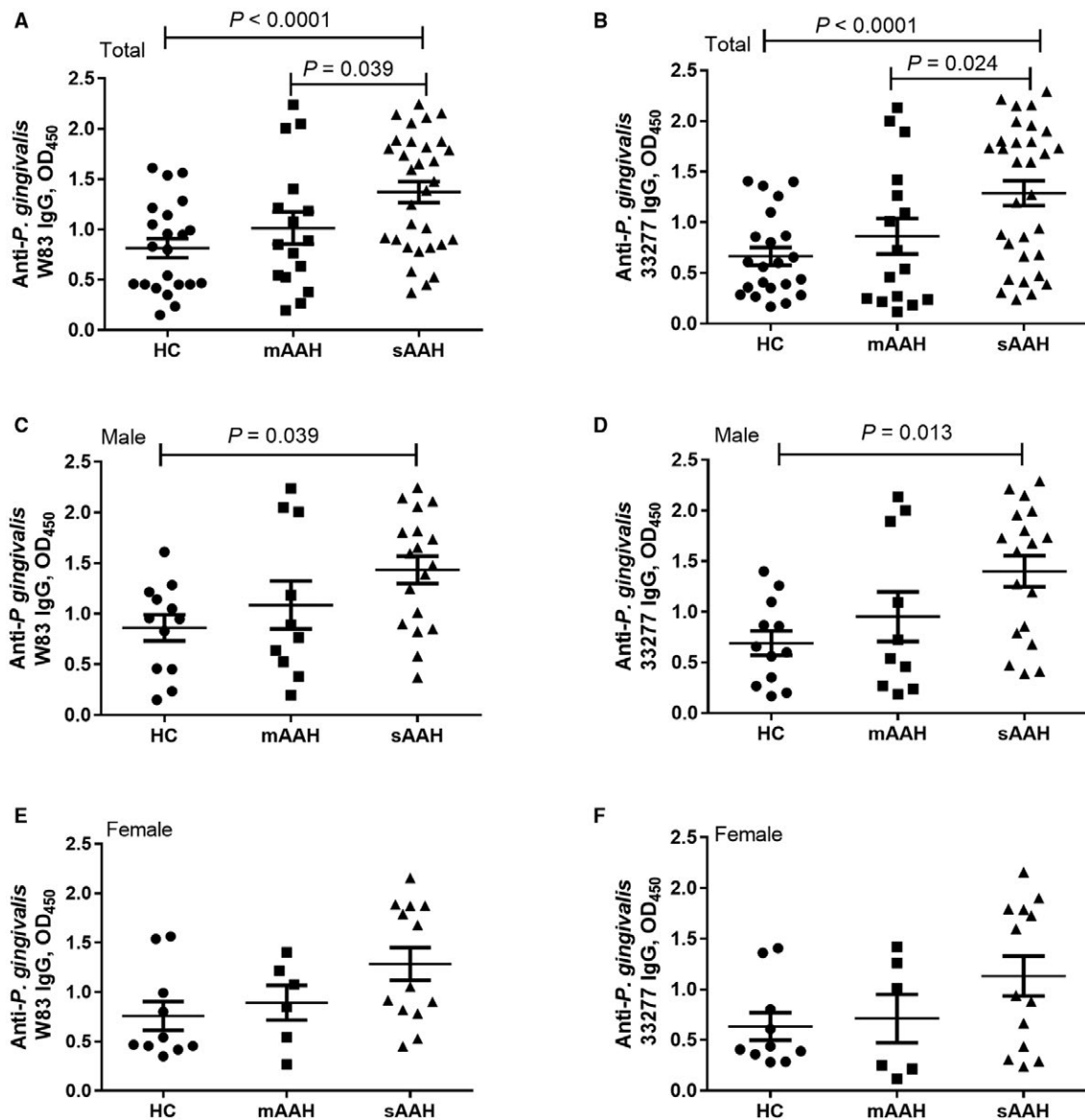
FIG. 1. Anti–*P. gingivalis* IgG and AAH severity. (A,B) Total AAH. (C,D) Male patients. (E,F,) Female patients. Anti–*P. gingivalis* W83 IgG levels in HCs, patients with moderate AAH, and patients with severe AAH in (A) total AAH, (C) male patients, and (E) female patients. Anti–*P. gingivalis* 33277 IgG levels in HCs, patients with moderate AAH, and patients with severe AAH in (B) total AAH, (D) male patients, and (F) female patients. Horizontal bars represent mean ± SD.

Fig. S2C), a highly significant association in the AST:ALT ratio and W83 IgM in male patients with moderate AAH was observed (Supporting Fig. S2D). This may be because the AST:ALT ratio is more predictive of severity in early liver disease. Female patients in either disease group did not show any such association. Thus, the interactions between IgM responses for both strains and the AST:ALT ratio were similar.

Association of Anti-*P. gingivalis* IgM Response and MELD Score

There was a positive association between W83 IgM response and MELD score in the total group of patients with severe AAH (Fig. 6A) and in female patients with severe AAH (Fig. 6B) but not in male patients with severe AAH (Fig. 6C). We did not find an association in male or female patients with moderate AAH (data
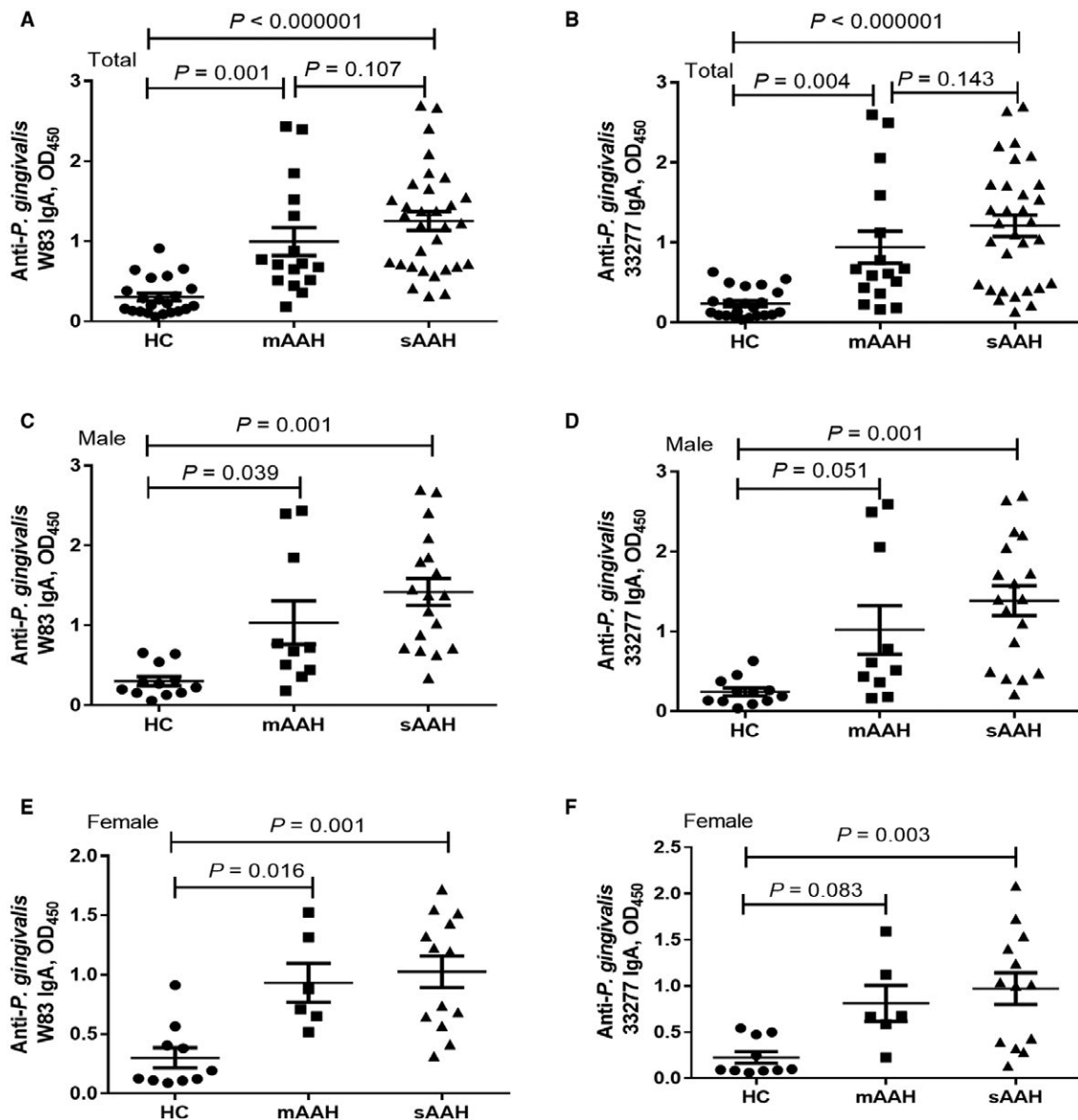
FIG. 2. Anti–*P. gingivalis* IgA and AAH severity. (A,B) Total AAH. (C,D) Male patients. (E,F) Female patients. Anti–*P. gingivalis* W83 IgA levels in HCs, patients with moderate AAH, and patients with severe AAH in (A) total AAH, (C) male patients, and (E) female patients. Anti–*P. gingivalis* 33277 IgA levels in HCs, patients with moderate AAH, and patients with severe AAH in (B) total AAH, (D) male patients, and (F) female patients. Horizontal bars represent mean ± SD.

not shown). There was also no association between IgG or IgA and MELD in either male patients or female patients in the severe or moderate groups.

Discussion

It is generally well accepted that the gut microbiome plays an important role in the development and progression of several types of liver disease.[21] Intestinal dysbiosis has been observed by our group and others in experimental ALD/NAFLD in mice as well as humans.[21-23] There are initial reports suggesting that the oral microbiome may also play a role in liver disease.[7,8,24-26] In experimental animals, infection with *P. gingivalis* worsens steatohepatitis in mice fed a high-fat diet.[27] In humans, periodontitis is associated with increased hepatic fibrosis in subjects
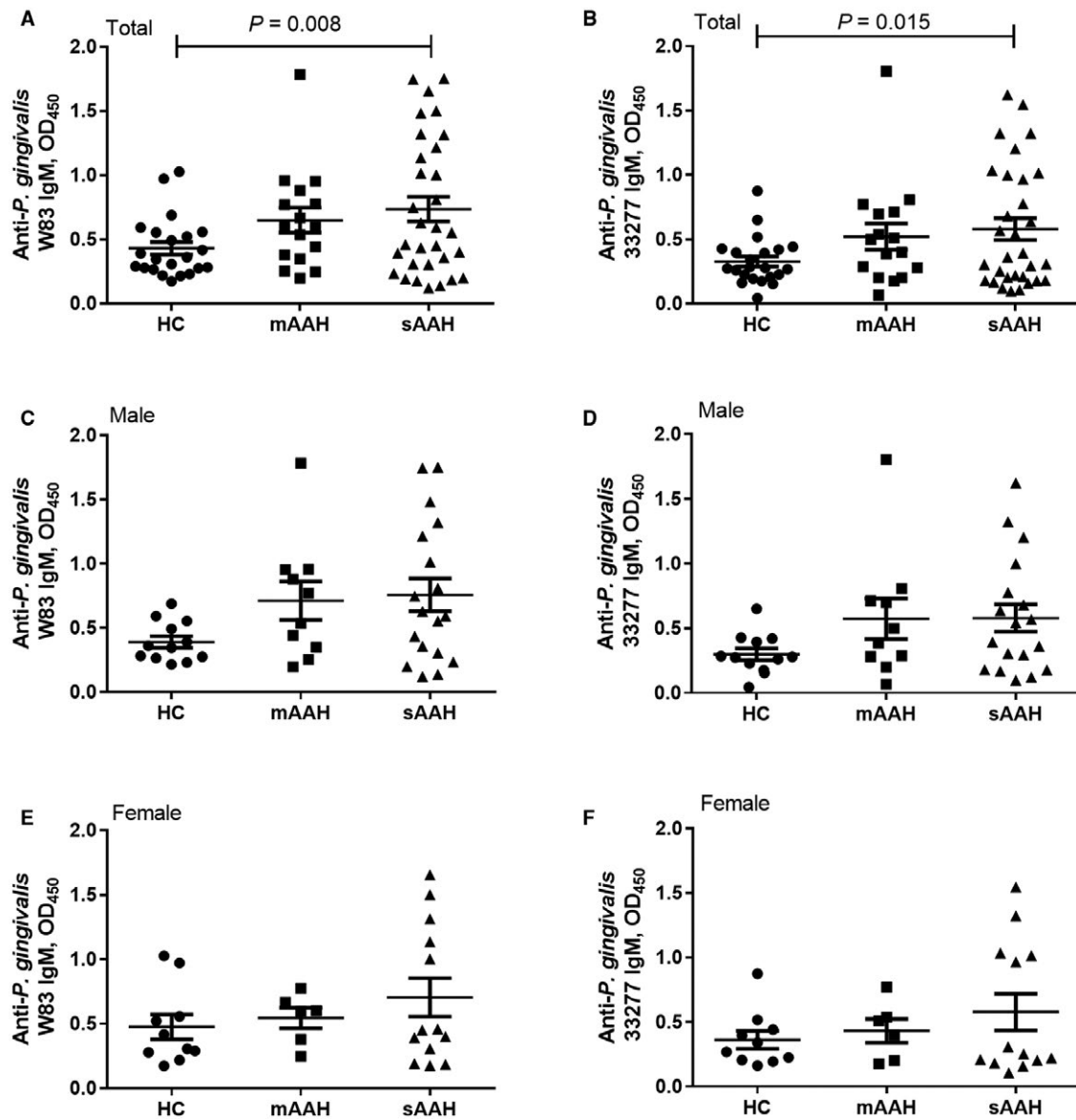
FIG. 3. Anti-*P. gingivalis* IgM and AAH severity. (A,B) Total AAH. (C,D) Male patients. (E,F) Female patients. Anti-*P. gingivalis* W83 IgM levels in HCs, patients with moderate AAH, and patients with severe AAH in (A) total AAH, (C) male patients, and (E) female patients. Anti-*P. gingivalis* 33277 IgM levels in HCs, patients with moderate AAH, and patients with severe AAH in (B) total AAH, (D) male patients, and (F) female patients. Horizontal bars represent mean ± SD.

with NAFLD.[28] Patients with cirrhosis have been reported to have increased mortality if they have periodontitis.[29] Importantly, patients with alcoholism have a pathogenic oral microbiome and worse PD than patients without alcoholism,[30] and patients with alcoholism with a smoking history have higher odds ratio of PD.[31] Thus, we hypothesized that patients with AAH would be a likely population to have their liver disease associated with (and likely negatively impacted by) PD.

We selected two strains of *P. gingivalis* to perform antibody ELISA detection. Strain 33277 represents the fimbriated/nonencapsulated lineage and possesses both the Mfa1 and FimA fimbriae, which are present in 20 of 21 *P. gingivalis* strains. Strain W83 represents the nonfimbriated/capsulated lineage.[32] Both strains are pathogenic in animal models of infection.[33] Detection of the antibodies showed a wide range of concentrations. Because we studied a group with a
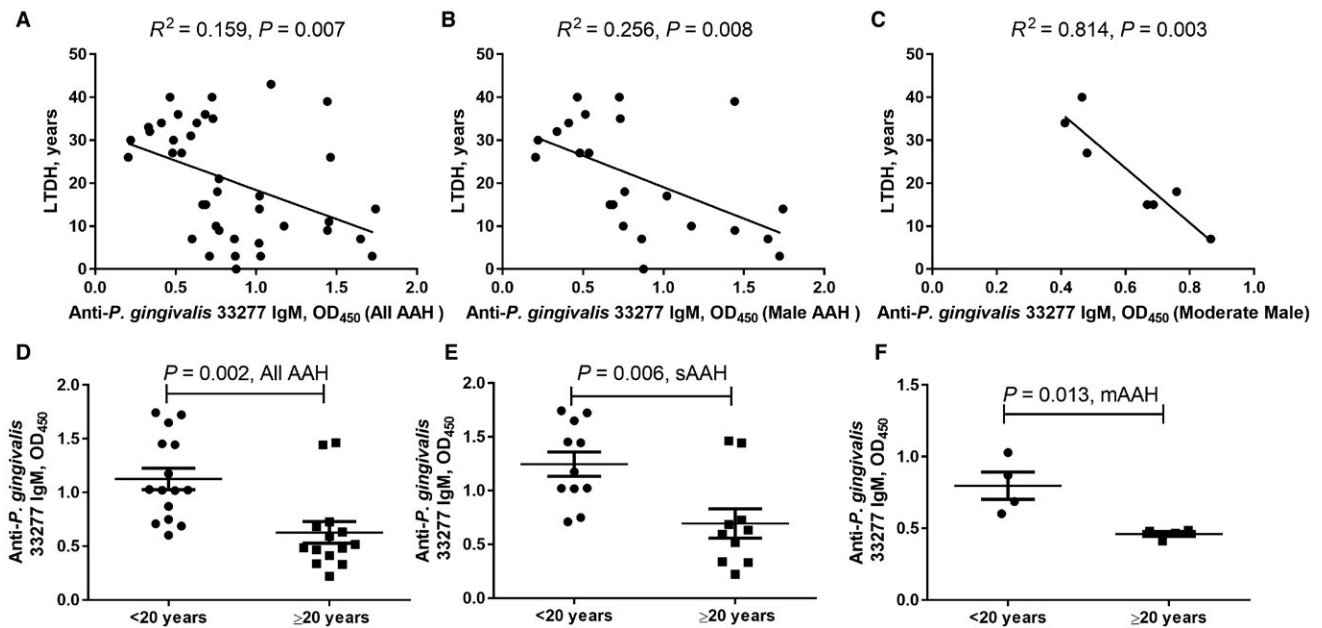
FIG. 4. Association of anti–*P. gingivalis* 33277 IgM response and LTDH in patients with AAH. IgM response in (A) all AAH, (B) all male AAH, and (C) male moderate AAH. Anti–*P. gingivalis* 33277 IgM comparison between LTDH <20 years of drinking and LTDH ≥20 years of drinking in (D) all AAH, (E) severe AAH, and (F) moderate AAH. (D-F) Horizontal bars represent mean ± SD.
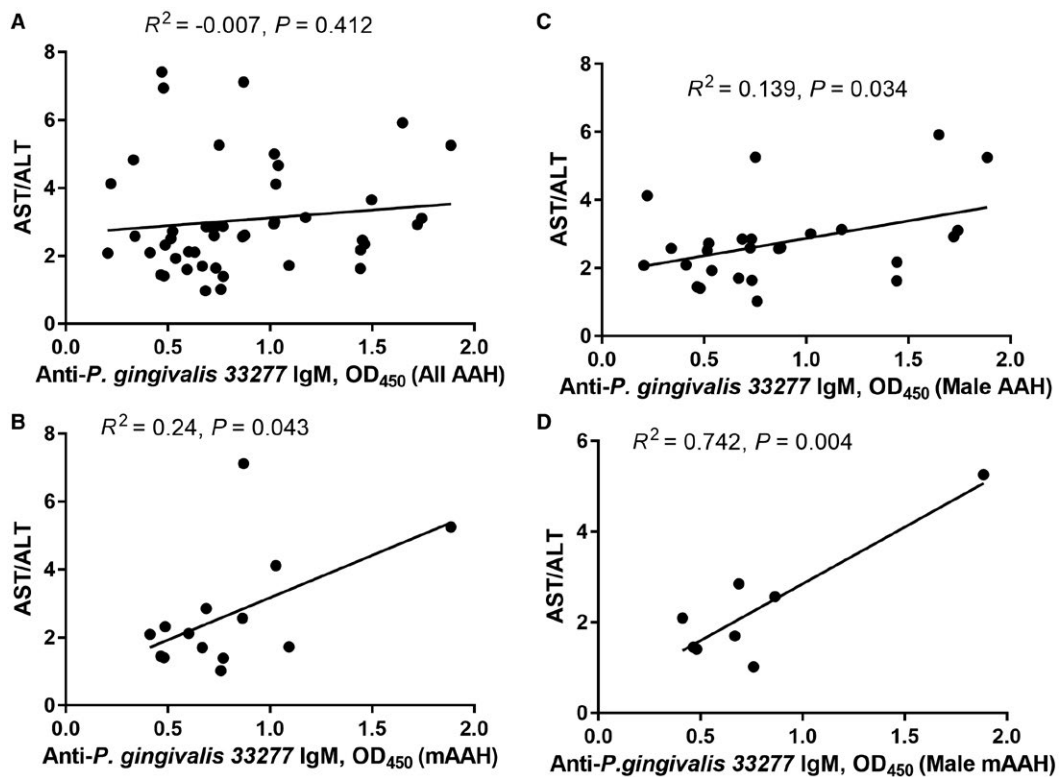

FIG. 5. Association of anti–*P. gingivalis* 33277 IgM response with progression of liver severity determined by AST:ALT ratio. (A) All patients with AAH. (B) All patients with moderate AAH. (C) All male patients with AAH. (D) All male patients with moderate AAH.
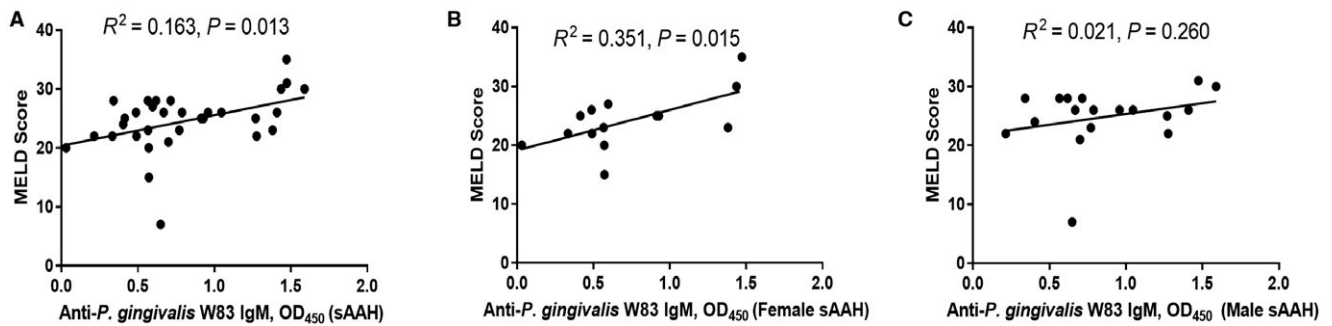
FIG. 6. Association of anti–*P. gingivalis* W83 IgM response with liver severity determined by MELD score in female patients with severe AAH. (A) All patients with severe AAH. (B) All female patients with severe AAH. (C) All male patients with severe AAH.

wide spectrum of alcohol hepatitis disease severity, it was not surprising to find a wide spectrum of antibody levels to *P. gingivalis.*

Elevated levels of Ig antibodies against *P. gingivalis* have a detectable protective effect against periodontal infections.[34,35] Elevated serum IgG against *P. gingivalis* has been reported in patients with chronic adult periodontitis, and levels decrease with appropriate therapy.[36] In addition, cardiovascular disease (CVD) and periodontitis are associated with levels of IgG to *P. gingivalis.*[37] *P. gingivalis* IgA levels also predict myocardial infarction and stroke independently of established CVD risk factors.[38] A significant correlation between fibrosis progression and *P. gingivalis* IgG titers has been reported in a study evaluating the effect of *P. gingivalis* infection as a risk factor in the progression of NASH.[8] Our data are consistent with this finding, with significantly elevated IgG, IgM, and IgA levels in patients with severe AAH.

P. gingivalis can activate pattern recognition receptors found on cells, such as macrophages/Kupffer cells, and initiate intracellular signaling and an inflammatory response. Receptor types of major importance for *P. gingivalis* are the toll-like receptors (TLR2 and TLR4) and nucleotide-binding oligomerization domains (NODs).[39] Activation of either TLR-2 or NOD1/NOD2 can activate nuclear factor kappa B with subsequent increased expression of many proinflammatory cytokines and possible liver injury. Interestingly, oral administration of *P. gingivalis* in mice caused intestinal dysbiosis, decreased intestinal tight junction proteins, and endotoxemia, which are all features of ALD.[40]

The IgM response represents active infection.[41] Anti-*P. gingivalis* IgM and length of drinking had a strong inverse association, suggesting an important role of heavy prolonged drinking in altering Ig immune responses, with longer drinking showing a lower immune response.[15] In the moderate AAH group, the IgM response was strongly correlated with the AST:ALT ratio, suggesting active infection of *P. gingivalis* was associated with progressive liver damage in these patients (most specifically in male patients).[42] There was no correlation between the AST:ALT ratio and IgM in severe AAH, indicating that this association is more meaningful in moderate AAH and is lost in the severe form of AAH.

We found that IgM was highly associated with the MELD score, especially in female patients. Female patients might be especially vulnerable to active infection of *P. gingivalis* and its negative impact on the liver.

There were some limitations in this study. Importantly, this was an association study and could not assess causality. It would be optimal to verify the specificity of the antibody response to *P. gingivalis.* In addition, detection of *P. gingivalis* in liver biopsy specimens from patients with AAH by polymerase chain reaction or immunohistochemistry should be a gold standard in the future, but liver biopsies are not routinely performed for the diagnosis of ALD in the United States.[43] Because little is known about the role of *P. gingivalis* in ALD development/progression, we analyzed immune responses to *P. gingivalis* as independent factors to estimate their individual effects. In addition, determining the presence or absence of PD in controls and subjects with

alcoholic hepatitis would be important in future studies. Studies with larger numbers of participants could further clarify the roles of other confounding factors and their interactions with *P. gingivalis,* sex, and drinking measures. Moreover, a longitudinal or intervention study could help to better delineate the role of *P. gingivalis* in the development/progression of AAH.

In summary, *P. gingivalis* may be associated with ALD and may function as a confounding factor in AAH.[25,26,44] Our data support the concept that infection with *P. gingivalis* is associated with both progression and severity of AAH, and this association was modestly impacted by sex. Further studies are indicated to determine whether treatment of PD may help prevent or attenuate ALD.

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