A cross-sectional study assessing the relationship between non-alcoholic fatty liver disease and periodontal disease

Satsuki Sato1,10, Yohei Kamata1,10, Takaomi Kessoku2,10, Tomoko Shimizu1,10, Takashi Kobayashi2, Takeo Kurihashi3, Shogo Takashiba4, Kazu Hatanaka4, Nobuhiro Hamada5, Toshiro Kodama6, Takuma Higurashi2, Masataki Taguri7, Masato Yoneda2, Haruki Usuda8, Koichiro Wada8, Atsushi Nakajima2, Toshiya Morozumi9 & Masato Minabe9

The risk factors for non-alcoholic fatty liver disease (NAFLD) progression are not completely known. Porphyromonas gingivalis infection is a risk factor for systemic diseases. We investigated the association of P. gingivalis infection with the risk of non-alcoholic steatohepatitis progression. Here, hematological tests, periodontal examination, and saliva collection were performed for 164 patients with NAFLD. P. gingivalis was identified in saliva using polymerase chain reaction. Hepatic steatosis and stiffness were evaluated using vibration-controlled transient elastography (VCTE) and magnetic resonance imaging. In patients with NAFLD, P. gingivalis positivity (P. gingivalis ratio ≥ 0.01%) in saliva correlated with liver stiffness determined using magnetic resonance elastography (MRE; p < 0.0001). A P. gingivalis ratio of 0.01% corresponds to 100,000 cells/mL and indicates the proportion of P. gingivalis in the total number of bacteria in the oral cavity. Patients with NAFLD and advanced fibrosis on MRE showed significantly elevated endotoxin activity; those who had >10 periodontal pockets with depths ≥ 4 mm had significantly increased hepatic stiffness on both VCTE and MRE.

Non-alcoholic fatty liver disease (NAFLD) is now recognized as one of the most common chronic liver diseases1. It is common knowledge that people with high alcohol consumption rates develop fatty livers; however, recent studies have found that fatty liver also occurs in individuals who consume little or no alcohol2. NAFLD is an umbrella term for simple fatty liver (in which fat is deposited in the liver with little or no inflammation) and non-alcoholic steatohepatitis (NASH), which is a risk factor for fibrosis and liver cancer. It is believed that insulin resistance, oxidative stress, inflammatory cytokines, and other factors contribute to the progression of simple fatty liver to NASH, which finally leads to cirrhosis and hepatocellular carcinoma. Thus, NAFLD is an important disease, affecting approximately 30% of the population in Japan3,4. It has been reported that NAFLD...
is closely associated with the constituents of metabolic syndrome, such as obesity, type 2 diabetes mellitus, hypertension, and hyperlipidemia. In addition, this disease has been noted to co-occur with other diseases such as sleep apnea syndrome and hypothyroidism. It is clear that diet modification is effective in patients with NAFLD; however, no gold standard has been established for drug therapy.

Periodontal disease refers to chronic inflammation of the supporting tissues of teeth. It is associated with many metabolic diseases such as diabetes mellitus, cardiovascular disease, and NAFLD. In animal studies, periodontal disease has been found to induce increased blood concentrations of proinflammatory molecules and increase the oxidative stress that occurs because of these mediators. These studies support the hypothesis that periodontal disease may be a risk factor for NAFLD. Animal studies have demonstrated a relationship between Porphyromonas gingivalis and fatty liver disease, and human studies have shown the involvement of P. gingivalis in NAFLD progression; however, the relationship between NAFLD and periodontal disease in humans remains unclear.

We hypothesized that deep periodontal pockets and P. gingivalis bacterial content would be associated with hepatic fibrogenesis. Therefore, this study aimed to investigate the relationship between the pathogenesis of NAFLD and intraoral findings.

**Results**

According to the sample size calculation, 164 patients who met the eligibility criteria were identified and included between August 2015 and April 2019.

Of the 164 participants, P. gingivalis represented ≥ 0.01% and < 0.01% of all bacteria in the saliva of 45 and 119 patients, respectively.

A P. gingivalis ratio of 0.01% corresponds to approximately 100,000 cells/mL and indicates the ratio of P. gingivalis to the total number of bacteria in the oral cavity quantified by performing polymerase chain reaction (PCR) of the DNA obtained from saliva samples. The baseline demographics and disease characteristics of the patient groups with a P. gingivalis ratio ≥ 0.01% and a P. gingivalis ratio < 0.01% in saliva were almost similar (Table 1). Liver stiffness measured by magnetic resonance elastography (MRE) was significantly higher in the group with a P. gingivalis ratio ≥ 0.01% than in the group with a P. gingivalis ratio < 0.01% in saliva (3.0 ± 1.3 vs. 3.7 ± 1.0, p = 0.0064, Table 1). In addition, there was a significant difference between these groups in terms of the level of the inflammatory marker, ferritin (215 ± 198 vs. 282 ± 142, p = 0.04, Table 1).

The results of logistic regression analysis using MRE ≥ 3.4 kPa as the dependent variable for all participants showed that MRE ≥ 3.4 kPa (OR = 4.05; p < 0.01), endotoxin activity assay (0.2 ± 0.12 vs. 0.1 ± 0.05, respectively; p < 0.0001), and antibody titers for P. gingivalis SU63 (2.0 ± 2.6; P. gingivalis in saliva < 0.01% group: 0.5 (1.5) indicated that this study population had a stable periodontal disease condition (Table 1, Fig. 1).

The high elasticity group (MRE ≥ 3.4 kPa) included 64 participants, and the low elasticity group (MRE < 3.4 kPa) included 100 participants. We found significant differences between these two groups in terms of the presence of oral P. gingivalis (6.0 ± 1.3 vs. 0.2 ± 0.5 × 10^6 cells/mL, respectively; p < 0.01), results of endotoxin activity assay (0.2 ± 0.12 vs. 0.1 ± 0.05, respectively; p < 0.001), and antibody titers for P. gingivalis FDC381 (1.0 ± 2.4 vs. 0.3 ± 1.1, respectively; p = 0.0477) and SU63 (1.6 ± 2.6 vs. 0.7 ± 1.7, respectively; p < 0.019). These data suggest that an increased liver stiffness (determined by MRE) is associated with a high number of P. gingivalis bacteria in the oral cavity, increased endotoxin levels, and high antibody titers for P. gingivalis FDC381 and SU63 (Table 2, Fig. 2a–e).

Among the 164 study participants, 52 and 112 had less than 10 (3 ± 3 sites) and more than 10 (26 ± 17 sites) periodontal pockets with depths ≥ 4 mm, respectively. The liver stiffness values were significantly higher in the group with > 10 PPD sites than in the corresponding group with < 10 PPD sites (3.6 ± 1.5 vs. 3.0 ± 1.0 kPa, respectively; p = 0.0095; Table 3, Fig. 3a–d). The mean of PPD > 4 mm was 4.0 mm (± 0.8). Moreover, there were significant differences between these groups regarding the periodontal conditions. These data indicate that liver stiffness increases in case of a greater number of deep periodontal pockets.

The results of logistic regression analysis using MRE ≥ 3.4 kPa as the dependent variable for all participants are shown in Table 4. The prevalence of NAFLD in advanced fibrosis was associated with weight [odds ratio (OR) = 0.948; p = 0.011], bleeding on probing (BOP) (OR = 1.045; p = 0.001), P. gingivalis ≥ 0.01% OR = 4.05; p = 0.04), and intima-media thickness (IMT) mean (OR = 1.21; p = 0.03), and PPD (OR = 2.045; p = 0.026).

We also constructed receiver operating characteristic curves for patients using at least 0.01% P. gingivalis in saliva and the number of periodontal pockets with depths ≥ 4 mm as independent variables. When periodontal pockets with depths ≥ 4 mm were observed in at least five sites, the area under the receiver operating characteristic curve, sensitivity, and specificity were 0.73, 0.82, and 0.65, respectively. Similarly, area under the curve of 0.73, sensitivity of 0.41, and specificity of 0.81 were obtained in case the number of periodontal pockets with depths ≥ 4 mm were greater than or equal to 10 (Fig. 4a,b).

Patients with NAFLD who had > 10 periodontal pockets with depths ≥ 4 mm on periodontal examination had significantly increased liver stiffness on both vibration-controlled transient elastography (VCTE) and MRE. Furthermore, in patients with liver stiffness ≥ 3.4 kPa, there were significant differences in salivary and serum P. gingivalis antibody levels, which may have been related to P. gingivalis’ endotoxin.

**Discussion**

This is a study to demonstrate that the association between NAFLD pathology (especially liver stiffness), P. gingivalis bacteria contents, and oral findings (PPD). This observational study examining the relationship between periodontal disease and NAFLD pathogenesis produced three major findings. First, it demonstrated that the group with ≥ 0.01% P. gingivalis in saliva had a higher MRE-determined liver stiffness than the group with < 0.01%.
| Variables | All (n = 164) | P. gingivalis < 0.01% (n = 119) | P. gingivalis ≥ 0.01% (n = 45) | p-value |
|-----------|--------------|-------------------------------|-------------------------------|---------|
| **Demographics** | | | | |
| Age (years) | 57 (15) | 55 (15) | 60 (15) | 0.043 |
| Male (%) | 92 (56) | 70 (59) | 22 (49) | 0.255 |
| **Comorbidities** | | | | |
| Type 2 diabetes (%) | 56 (34) | 41 (34) | 15 (33) | 0.893 |
| Hyperlipidemia (%) | 59 (36) | 46 (39) | 13 (29) | 0.248 |
| Hypertension (%) | 57 (35) | 43 (36) | 14 (31) | 0.55 |
| Hyperuricemia (%) | 15 (9) | 9 (8) | 6 (13) | 0.255 |
| Cardiovascular disease (%) | 6 (4) | 6 (5) | 0 (0) | 0.126 |
| Thyroid disease (hypothyroidism) (%) | 3 (2) | 2 (2) | 1 (2) | 0.819 |
| **Concomitant drug use** | | | | |
| Anti-diabetic (%) | 57 (35) | 41 (34) | 16 (35) | 0.896 |
| DPP4-inhibitor (%) | 39 (24) | 26 (22) | 13 (29) | 0.348 |
| Metformin (%) | 31 (19) | 21 (18) | 10 (22) | 0.507 |
| Sulfonylurea (%) | 17 (10) | 11 (9) | 6 (13) | 0.446 |
| Anti-lipidemic (%) | 56 (34) | 43 (36) | 13 (29) | 0.386 |
| Anti-hypertensive (%) | 56 (34) | 42 (35) | 14 (31) | 0.617 |
| Anti-platelet (%) | 0 (0) | 0 (0) | 0 (0) | |
| Laxatives, regular use (%) | 0 (0) | 0 (0) | 0 (0) | |
| **Liver imaging** | | | | |
| CAP on VCTE (dB/m) | 305 (60) | 310 (56) | 295 (69) | 0.168 |
| LSM on VCTE (kPa) | 7.7 (4.3) | 7.3 (4.4) | 8.6 (3.8) | 0.114 |
| Liver fat content on MRI-PDFF (%) | 12.4 (6.6) | 13.0 (6.6) | 10.8 (6.4) | 0.091 |
| Liver stiffness on MRE (kPa) | 3.2 (1.2) | 3.0 (1.3) | 3.7 (1.0) | 0.0064 |
| **Endotoxin** | | | | |
| EAA (× 10²) | 0.1 (0.09) | 0.1 (0.08) | 0.2 (0.08) | 0.001 |
| **Metabolic factors** | | | | |
| Weight (kg) | 72.6 (15.6) | 73 (16) | 71 (15) | 0.473 |
| BMI (kg/m²) | 27 (5.3) | 27.3 (5.0) | 26.1 (6.0) | 0.177 |
| Glucose (mg/dL) | 109 (35) | 112 (38) | 101 (26) | 0.093 |
| Insulin (μU/mL) | 20 (28) | 21 (31) | 15 (18) | 0.183 |
| HOMA-R | 6 (10) | 7 (11) | 4 (8) | 0.2 |
| **Liver function** | | | | |
| Platelet count | 22 (8) | 22 (9) | 20 (4) | 0.182 |
| AST (U/L) | 37 (21) | 35 (21) | 42 (22) | 0.056 |
| ALT (U/L) | 50 (38) | 48 (40) | 54 (38) | 0.434 |
| GGT (U/L) | 77 (97) | 73 (69) | 86 (147) | 0.447 |
| ALP (U/L) | 241 (91) | 238 (88) | 250 (99) | 0.476 |
| T.Bil (mg/dL) | 1.2 (4.8) | 1.3 (5.7) | 0.7 (0.3) | 0.428 |
| **Lipids** | | | | |
| Tcho (mg/dL) | 198 (40) | 199 (38) | 196 (44) | 0.591 |
| LDL-C (mg/dL) | 119 (85) | 121 (98) | 112 (35) | 0.56 |
| HDL-C (mg/dL) | 43 (82) | 40 (95) | 50 (15) | 0.459 |
| TG (mg/dL) | 182 (130) | 189 (143) | 162 (86) | 0.244 |
| **Inflammatory markers** | | | | |
| hsCRP (mg/L) | 0.2 (0.3) | 0.2 (0.4) | 0.1 (0.2) | 0.512 |
| Ferritin (ng/mL) | 233 (187) | 215 (198) | 282 (142) | 0.042 |
| **Fibrosis marker** | | | | |
| Type IV collagen 7x (ng/mL) | 4.2 (1.0) | 4.2 (1.0) | 4.3 (1.0) | 0.483 |
| **Periodontal assessment** | | | | |
| PPD (mm) | 2.5 (0.5) | 2.4 (0.5) | 2.7 (0.5) | 0.0091 |
| CAL (mm) | 2.5 (0.5) | 2.4 (0.5) | 2.7 (0.5) | 0.0091 |
| BOP (site) | 23 (30) | 19 (28) | 32 (31) | 0.0118 |
| Stability of teeth | 0 | 0 | 0 | |
| PPD ≥ 4 mm (site) | 14 (23) | 12 (22) | 22 (23) | 0.01 |
| Oral bacteria, P. gingivalis (× 10⁶ cells/mL) | 0.4 (1.0) | 0.2 (0.5) | 1.2 (1.6) | <0.0001 |
| **Continued** | | | | |
P. gingivalis migrates relatively easily from the oral cavity into the blood circulation\textsuperscript{39,40}; therefore, its endotoxin (present in the oral cavity) can reach the liver\textsuperscript{41}. Second, it found that the group with MRE-determined liver stiffness values of ≥ 3.4 kPa had higher serum P. gingivalis FDC381 and SU63 antibody levels. P. gingivalis is an obligate anaerobic gram-negative bacillus with strong adhesion; therefore, it forms a biofilm with other gram-negative bacteria associated with periodontal disease. In addition to producing a proteolytic enzyme called gingipains, P. gingivalis expresses lipopolysaccharides (which are endotoxins of the outer bacterial membrane) and is an anaerobic bacterium associated with periodontal disease progression. Inflammation caused by the cytokines and proteins produced during P. gingivalis infection is associated with liver stiffness\textsuperscript{42,43}. Furthermore, the histological lesions in NASH are unevenly distributed throughout the liver parenchyma; therefore, liver biopsy errors can result in substantial stratification and staging inaccuracies\textsuperscript{44}. We consider it important to perform non-invasive, safe, low-cost, and short-term clinical trials as proof-of-concept studies. Many studies have reported that the steatosis grade could be evaluated using the controlled attenuation parameter\textsuperscript{45–50} (which is based on the properties of ultrasonographic signals acquired with VCTE) or magnetic resonance imaging (MRI) proton density fat fraction\textsuperscript{51,52} (which is an MRI-based method for quantitatively assessing hepatic steatosis and is available for several types of MRI scanners). Moreover, VCTE and MRE have excellent diagnostic abilities for steatosis and fibrosis in patients with NAFLD\textsuperscript{53,54}. We consider that non-invasive evaluation of the pathogenesis of NAFLD using VCTE and MRI may replace invasive methods, such as liver biopsy. For this reason, we included non-invasive methods for assessing NASH/NAFLD progression as secondary endpoints and compared the results with those derived from liver biopsies.

Third, the group with ≥ 10 periodontal pockets with depths ≥ 4 mm had increased liver stiffness compared to the group with < 10 periodontal pockets with depths ≥ 4 mm. The presence of more than 10 periodontal pockets with depths > 4 mm suggests high morbidity in patients with periodontal disease\textsuperscript{55}. The factor involved in PPD and liver stiffness in this study may have been age, and not sex. With increasing age, the prevalence of periodontal

| Variables                                      | All (n = 164) | P. gingivalis < 0.01% (n = 119) | P. gingivalis ≥ 0.01% (n = 45) | p-value |
|------------------------------------------------|---------------|---------------------------------|--------------------------------|---------|
| Antibody titer for P. gingivalis FDC381        | 0.6 (1.8)     | 0.5 (1.9)                       | 0.8 (1.4)                      | 0.28    |
| Antibody titer for P. gingivalis SU63          | 0.9 (2.0)     | 0.5 (1.5)                       | 2.0 (2.6)                      | 0.0001  |
| IMT mean (R)                                   | 0.9 (0.3)     | 0.9 (0.3)                       | 1.0 (0.3)                      | 0.0402  |
| IMT mean (L)                                   | 0.9 (0.3)     | 0.9 (0.2)                       | 1.0 (0.3)                      | 0.008   |
| IMT max (R)                                    | 1.1 (0.5)     | 1.1 (0.5)                       | 1.2 (0.4)                      | 0.239   |
| IMT max (L)                                    | 1.1 (0.5)     | 1.1 (0.6)                       | 1.2 (0.5)                      | 0.346   |

Table 1. Baseline characteristics of the trial population. ALP alkaline phosphatase, ALT alanine aminotransferase, BMI body mass index, BOP bleeding on probing, CAL clinical attachment level, CAP controlled attenuation parameter, DPP4 dipeptidyl peptidase-4, EAA endotoxin activity assay, GGT gamma-glutamyl transferase, HDL-C high-density lipoprotein cholesterol, HOMA-R homeostasis model assessment-estimated insulin resistance, hsCRP high-sensitivity C-reactive protein, IMT (R: right, L: left) intima-media thickness (R, L), LDL-C low-density lipoprotein cholesterol, LSM liver stiffness measurement, MRE magnetic resonance elastography, MRI magnetic resonance imaging, PDFF proton density fat fraction, PPD periodontal pocket depth, T.Bil total bilirubin, Tcho total cholesterol, TG triglycerides, VCTE vibration-controlled transient elastography. *Data are reported as means (SDs) or numbers (percentages). The p-values were determined using the t-test.

Figure 1. Liver stiffness in groups of patients with < 0.01% and ≥ 0.01% P. gingivalis in saliva. MRE, magnetic resonance elastography.
| Variables                        | All (n = 164) | MRE < 3.4 kPa (n = 100) | MRE ≥ 3.4 kPa (n = 64) | p-value |
|---------------------------------|---------------|-------------------------|------------------------|---------|
| Demographics                    |               |                         |                        |         |
| Age (years)                     | 57 (15)       | 56 (14)                 | 60 (14)                |         |
| Male (%)                        | 92 (56)       | 57 (57)                 | 25 (39)                |         |
| Comorbidities                   |               |                         |                        |         |
| Type 2 diabetes (%)             | 56 (34)       | 33 (33)                 | 23 (36)                |         |
| Hyperlipidemia (%)              | 59 (36)       | 38 (38)                 | 21 (31)                |         |
| Hypertension (%)                | 57 (35)       | 35 (35)                 | 22 (34)                |         |
| Hyperuricemia (%)               | 15 (9)        | 9 (9)                   | 6 (9)                  |         |
| Cardiovascular disease (%)      | 6 (4)         | 3 (3)                   | 3 (0)                  |         |
| Thyroid disease (hypothyroidism) (%) | 3 (2)        | 1 (1)                   | 2 (0)                  |         |
| Concomitant drug use            |               |                         |                        |         |
| Anti-diabetic (%)               | 57 (35)       | 32 (32)                 | 25 (35)                |         |
| DPP4-inhibitor (%)              | 39 (24)       | 22 (22)                 | 17 (27)                |         |
| Metformin (%)                   | 31 (19)       | 21 (21)                 | 10 (16)                |         |
| Sulfonylurea (%)                | 17 (10)       | 10 (10)                 | 7 (11)                 |         |
| Anti-lipidemic (%)              | 56 (34)       | 37 (37)                 | 19 (30)                |         |
| Anti-hypertensive (%)           | 56 (34)       | 34 (34)                 | 22 (34)                |         |
| Anti-platelet (%)               | 0 (0)         | 0 (0)                   | 0 (0)                  |         |
| Laxatives, regular use (%)      | 0 (0)         | 0 (0)                   | 0 (0)                  |         |
| Liver imaging                   |               |                         |                        |         |
| CAP on VCTE (dB/m)              | 305 (60)      | 309 (57)                | 297 (67)               |         |
| LSM on VCTE (kPa)               | 7.7 (4.3)     | 6.3 (3.2)               | 10.9 (4.8)             | < 0.0001|
| Liver fat content on MRI-PDFF (%)| 12.4 (6.6)   | 12.5 (7.2)              | 12.2 (5.5)             |         |
| Liver stiffness on MRE (kPa)    | 3.2 (1.2)     | 2.4 (0.5)               | 4.3 (1.3)              | < 0.0001|
| Endotoxin                       |               |                         |                        |         |
| EAA (× 10^2)                    | 0.1 (0.09)    | 0.1 (0.05)              | 0.2 (0.12)             | <0.0001 |
| Metabolic factors               |               |                         |                        |         |
| Weight (kg)                     | 72.6 (15.6)   | 75 (15)                 | 69 (15)                | 0.022   |
| BMI (kg/m²)                     | 27 (5.3)      | 27.9 (4.9)              | 26.6 (4.5)             |         |
| Glucose (mg/dL)                 | 109 (35)      | 113 (33)                | 111 (44)               |         |
| Insulin (µU/mL)                 | 20 (28)       | 20 (26)                 | 18 (18)                |         |
| HOMA-R                          | 6 (10)        | 6 (8)                   | 6 (11)                 |         |
| Liver function                  |               |                         |                        |         |
| Platelet count                  | 22 (8)        | 23 (9)                  | 19 (6)                 | 0.0239  |
| AST (U/L)                       | 37 (21)       | 36 (17)                 | 46 (24)                | 0.0065  |
| ALT (U/L)                       | 50 (38)       | 54 (44)                 | 55 (27)                |         |
| GGT (U/L)                       | 77 (97)       | 71 (70)                 | 100 (145)              |         |
| ALP (U/L)                       | 241 (91)      | 236 (58)                | 268 (135)              |         |
| T.Bil (mg/dL)                   | 1.2 (4.8)     | 1.5 (7.0)               | 0.8 (0.6)              |         |
| Lipids                          |               |                         |                        |         |
| Tcho (mg/dL)                    | 198 (40)      | 199 (39)                | 202 (40)               |         |
| LDL-C (mg/dL)                   | 119 (85)      | 127 (119)               | 115 (29)               |         |
| HDL-C (mg/dL)                   | 43 (82)       | 34 (117)                | 51 (20)                |         |
| TG (mg/dL)                      | 182 (130)     | 192 (140)               | 175 (99)               |         |
| Inflammatory markers            |               |                         |                        |         |
| hsCRP (mg/L)                    | 0.2 (0.3)     | 0.2 (0.4)               | 0.2 (0.3)              |         |
| Ferritin (ng/mL)                | 233 (187)     | 223 (207)               | 271 (147)              |         |
| Fibrosis marker                 |               |                         |                        |         |
| Type IV collagen 7x (ng/mL)     | 4.2 (1.0)     | 4.0 (0.7)               | 4.9 (1.2)              | <0.0001 |
| Periodontal assessment          |               |                         |                        |         |
| PPD (mm)                        | 2.5 (0.5)     | 2.4 (0.5)               | 2.6 (0.6)              | 0.0403  |
| CAL (mm)                        | 2.5 (0.5)     | 2.4 (0.5)               | 2.6 (0.6)              | 0.0403  |
| BOP (site)                      | 23 (30)       | 13 (18)                 | 38 (40)                | <0.0001 |
| Stability of teeth              | 0             | 0                      | 0                      |         |
| PPD ≥ 4 mm (site)               | 10 (14)       | 6 (12)                  | 16 (17)                | 0.0084  |
| Oral bacteria, P. gingivalis (× 10^6 cells/mL) | 0.4 (1.0) | 0.2 (0.5) | 6.0 (1.3) | 0.0101  |
| Continued                       |               |                         |                        |         |
Table 2. Baseline characteristics of the trial population. ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, BMI body mass index, BOP bleeding on probing, CAP controlled attenuation parameter, CAL clinical attachment level, DPP4 dipeptidyl peptidase-4, EAA endotoxin activity assay, GGT gamma-glutamyl transferase, HDL-C high-density lipoprotein cholesterol, HOMA-R homeostasis model assessment-estimated insulin resistance, hsCRP high-sensitivity C-reactive protein, IMT (R: right, L: left) intima-media thickness (R, L), LDL-C low-density lipoprotein cholesterol, LSM liver stiffness measurement, MRE magnetic resonance elastography, MRI magnetic resonance imaging, PDFF proton density fat fraction, PPD periodontal pocket depth, TBil total bilirubin, Tcho total cholesterol, TG triglycerides, VCTE vibration-controlled transient elastography. *Data are reported as means (SDs) or numbers (percentages). P-values were determined using the t-test.

| Variables                          | All (n = 164) | MRE < 3.4 kPa (n = 100) | MRE ≥ 3.4 kPa (n = 64) | p-value |
|------------------------------------|--------------|------------------------|-----------------------|---------|
| Antibody titer for *P. gingivalis* FDC381 | 0.6 (1.8)    | 0.3 (1.1)              | 1.0 (2.4)             | 0.0477  |
| Antibody titer for *P. gingivalis* SU63 | 0.9 (2.0)    | 0.7 (1.7)              | 1.6 (2.6)             | 0.0192  |
| IMT mean (R)                       | 0.9 (0.3)    | 0.8 (0.2)              | 1.1 (0.3)             | < 0.0001|
| IMT mean (L)                       | 0.9 (0.3)    | 0.8 (0.2)              | 1.1 (0.3)             | < 0.0001|
| IMT max (R)                        | 1.1 (0.5)    | 1.0 (0.5)              | 1.4 (0.5)             | < 0.0001|
| IMT max (L)                        | 1.1 (0.5)    | 1.0 (0.5)              | 1.5 (0.6)             | < 0.0001|

Figure 2. Endotoxin activity (a), PPD (b), periodontal disease-causing bacteria, *P. gingivalis* (c), antibody titer for *P. gingivalis* FDC381 (d), and antibody titer for *P. gingivalis* SU63 (e) in the groups with MRE < 3.4 kPa and MRE ≥ 3.4 kPa. MRE magnetic resonance elastography, PPD periodontal pocket depth.
| Variables                                      | All (n = 164) | PPD ≥ 4 mm at < 10 sites (n = 112) | PPD ≥ 4 mm at ≥ 10 sites (n = 52) | p-value |
|------------------------------------------------|---------------|-----------------------------------|-----------------------------------|---------|
| **Demographics**                                |               |                                   |                                   |         |
| Age (years)                                    | 57 (15)       | 57 (15)                           | 56 (13)                           |         |
| Male (%)                                       | 92 (56)       | 66 (59)                           | 26 (50)                           |         |
| **Comorbidities**                              |               |                                   |                                   |         |
| Type 2 diabetes (%)                            | 56 (34)       | 36 (32)                           | 20 (38)                           |         |
| Hyperlipidemia (%)                             | 59 (36)       | 42 (38)                           | 17 (33)                           |         |
| Hypertension (%)                               | 57 (35)       | 40 (36)                           | 17 (33)                           |         |
| Hyperuricemia (%)                              | 15 (9)        | 10 (9)                            | 5 (10)                            |         |
| Cardiovascular disease (%)                     | 6 (4)         | 5 (4)                             | 1 (2)                             |         |
| Thyroid disease (hypothyroidism) (%)           | 3 (2)         | 1 (1)                             | 2 (4)                             |         |
| **Concomitant drug use**                       |               |                                   |                                   |         |
| Anti-diabetic (%)                              | 57 (35)       | 37 (33)                           | 20 (38)                           |         |
| DPP4-inhibitor (%)                             | 39 (24)       | 24 (22)                           | 15 (29)                           |         |
| Metformin (%)                                  | 31 (19)       | 20 (18)                           | 11 (21)                           |         |
| Sulphonylurea (%)                              | 17 (10)       | 10 (9)                            | 7 (13)                            |         |
| Anti-lipidemic (%)                             | 56 (34)       | 40 (36)                           | 16 (31)                           |         |
| Anti-hypertensive (%)                          | 56 (34)       | 40 (36)                           | 16 (31)                           |         |
| Anti-platelet (%)                              | 0 (0)         | 0 (0)                             | 0 (0)                             |         |
| Laxatives, regular use (%)                    | 0 (0)         | 0 (0)                             | 0 (0)                             |         |
| **Liver imaging**                              |               |                                   |                                   |         |
| CAP on VCTE (dB/m)                             | 305 (60)      | 305 (56)                          | 304 (68)                          |         |
| LSM on VCTE (kPa)                              | 7.7 (4.3)     | 7.0 (3.9)                         | 9.2 (4.8)                         | 0.0049  |
| Liver fat content on MRI-PDFF (%)              | 12.4 (6.6)    | 12.4 (6.8)                        | 12.1 (6.3)                        |         |
| Liver stiffness on MRE (kPa)                   | 3.2 (1.2)     | 3.0 (1.0)                         | 3.6 (1.5)                         | 0.0095  |
| **Endotoxin**                                  |               |                                   |                                   |         |
| EAA (× 10²)                                    | 0.1 (0.09)    | 0.1 (0.05)                        | 0.2 (0.13)                        | < 0.0001|
| **Metabolic factors**                          |               |                                   |                                   |         |
| Weight (kg)                                    | 72.6 (15.6)   | 72 (16)                           | 72 (16)                           |         |
| BMI (kg/m²)                                    | 27 (5.3)      | 27.0 (4.9)                        | 26.7 (6.1)                        |         |
| Glucose (mg/dL)                                | 109 (35)      | 109 (32)                          | 106 (40)                          |         |
| Insulin (μU/mL)                                | 20 (28)       | 21 (32)                           | 17 (19)                           |         |
| HOMA-R                                         | 6 (10)        | 6 (11)                            | 6 (11)                            |         |
| **Liver function**                             |               |                                   |                                   |         |
| Platelet count                                 | 22 (8)        | 22 (9)                            | 21 (6)                            |         |
| AST (U/L)                                      | 37 (21)       | 38 (22)                           | 35 (19)                           |         |
| ALT (U/L)                                      | 30 (38)       | 51 (40)                           | 45 (28)                           |         |
| GGT (U/L)                                      | 77 (97)       | 80 (109)                          | 65 (54)                           |         |
| ALP (U/L)                                      | 241 (91)      | 256 (102)                         | 231 (32)                          |         |
| T.Bil (mg/dL)                                  | 1.2 (4.8)     | 1.3 (5.8)                         | 0.8 (6.6)                         |         |
| **Lipids**                                     |               |                                   |                                   |         |
| Tcho (mg/dL)                                   | 198 (40)      | 201 (39)                          | 193 (43)                          |         |
| LDL-C (mg/dL)                                  | 119 (85)      | 124 (101)                         | 108 (31)                          |         |
| HDL-C (mg/dL)                                  | 43 (82)       | 40 (98)                           | 49 (19)                           |         |
| TG (mg/dL)                                     | 182 (130)     | 185 (135)                         | 178 (126)                         |         |
| **Inflammatory markers**                       |               |                                   |                                   |         |
| hsCRP (mg/L)                                   | 0.2 (0.3)     | 0.2 (0.4)                         | 0.2 (0.3)                         |         |
| Ferritin (ng/mL)                               | 233 (187)     | 228 (200)                         | 241 (151)                         |         |
| **Fibrosis marker**                            |               |                                   |                                   |         |
| Type IV collagen 7s (ng/mL)                    | 4.2 (1.0)     | 4.2 (0.9)                         | 4.4 (1.2)                         |         |
| **Periodontal assessment**                     |               |                                   |                                   |         |
| PPD (mm)                                       | 2.5 (0.5)     | 2.3 (0.3)                         | 3.0 (0.5)                         | < 0.0001|
| CAL (mm)                                       | 2.5 (0.5)     | 2.3 (0.3)                         | 3.0 (0.5)                         | < 0.0001|
| BOP (site)                                     | 23 (30)       | 12 (15)                           | 47 (40)                           | < 0.0001|
| Stability of teeth                             | 0             | 0                                 | 0                                 |         |
| PPD ≥ 4 mm (site)                              | 10 (14)       | 3 (3)                             | 26 (17)                           | < 0.0001|
| Oral bacteria, P. gingivalis (× 10⁶ cells/mL)  | 0.4 (1.0)     | 0.1 (0.3)                         | 0.9 (1.6)                         | < 0.0001|
| **Continued**                                  |               |                                   |                                   |         |
Inflammation caused by the cytokines and proteins produced in response to *P. gingivalis* infection is associated with liver stiffness. Therefore, the amount of bacteria in the oral cavity and serum antibody titers were examined in this cross-sectional study, and the relationship between periodontal disease and liver stiffness in patients with NAFLD was evaluated.

NAFLD is common in patients with metabolic abnormalities, such as type 2 diabetes and obesity; its prevalence is rapidly increasing globally with the growing obese population. The main risk associated with this disease is that it progresses from liver cirrhosis to liver cancer over a long time without manifestation of accompanying subjective symptoms, and mortality due to cerebral or myocardial infarction is also high. It is thought that the pathology in patients with NASH progresses as periodontopathic bacteria enter the blood, leading to

---

**Table 3.** Baseline characteristics of the study population. ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase, BMI body mass index, BOP bleeding on probing, CAP controlled attenuation parameter, CAL clinical attachment level, DPP4 dipeptidyl peptidase-4, EAA endotoxin activity assay, GGT gamma-glutamyl transferase, HDL-C high-density lipoprotein cholesterol, HOMA-R homeostasis model assessment-estimated insulin resistance, hsCRP high-sensitivity C-reactive protein, IMT (R: right, L: left) intima-media thickness (R, L), LDL-C low-density lipoprotein cholesterol, LSM liver stiffness measurement, MRE magnetic resonance elastography, MRI magnetic resonance imaging, PDFF proton density fat fraction, PPD periodontal pocket depth, TBil total bilirubin, Tcho total cholesterol, TG triglycerides, VCTE vibration-controlled transient elastography. *Data are reported as means (SDs) or numbers (percentages). The p-values were determined using the t-test.*

| Variables                      | All (n = 164) | PPD ≥ 4 mm at < 10 sites (n = 112) | PPD ≥ 4 mm at ≥ 10 sites (n = 52) | p-value |
|-------------------------------|--------------|-----------------------------------|----------------------------------|---------|
| Antibody titer for *P. gingivalis* FDC381 | 0.6 (1.8) | 0.5 (1.5) | 0.8 (2.4) |          |
| Antibody titer for *P. gingivalis* SU63 | 0.9 (2.0) | 0.6 (1.3) | 1.5 (2.5) | 0.0029  |
| IMT mean (R)                  | 0.9 (0.3)   | 0.8 (0.2)   | 1.2 (0.2)  | <0.0001 |
| IMT mean (L)                  | 0.9 (0.3)   | 0.8 (0.2)   | 1.1 (0.3)  | <0.0001 |
| IMT max (R)                   | 1.1 (0.5)   | 1.0 (0.4)   | 1.5 (0.4)  | <0.0001 |
| IMT max (L)                   | 1.1 (0.5)   | 1.0 (0.5)   | 1.5 (0.5)  | <0.0001 |

**Figure 3.** LSM (a), MRE (b), antibody titer for *P. gingivalis* FDC381 (c), and antibody titer for *P. gingivalis* SU63 (d) in patients with periodontal pockets ≥ 4 mm at a depth of < 10 mm or ≥ 10 sites. LSM liver stiffness measurement, MRE magnetic resonance elastography, PPD periodontal pocket depth.
inflammation in the fatty liver. Therefore, it is necessary to actively interfere with the link between periodontal disease and NAFLD.

The strength of this study lies in the use of non-invasive MRI (in place of liver biopsy) to assess changes in liver fat content. Importantly, this non-invasive method revealed a seemingly mutual relationship between \textit{P. gingivalis} and liver stiffness.

The study has a certain limitation. It would be important to differentiate NASH from NAFLD in the patients enrolled in this study; however, currently, biopsy is not used to diagnose and differentiate liver tissue.

In summary, patients with NAFLD who had \textit{P. gingivalis} infection and deeper periodontal pockets had higher liver stiffness values than those who did not have periodontal disease.

Furthermore, the serum antibody titers for \textit{P. gingivalis} FDC381 and SU63 were high in the patient group with increased liver stiffness, and logistic regression analysis suggested that periodontal disease is associated with liver stiffness. These results indicate that periodontal disease may be associated with NAFLD. As this was a cross-sectional study, a causal relationship could not be ascertained. Additional randomized controlled trials are needed to investigate the relationship between periodontal disease and NAFLD, and further investigation of patients with NAFLD who do not have periodontal disease is needed. As this study showed that liver fibrosis and periodontal disease are likely to be related, future interventional studies with treatment for fibrosis as an endpoint will be necessary.

Table 4. Factors associated with NAFLD in advanced fibrosis. AST aspartate aminotransferase, BOP bleeding on probing, IMT intima-media thickness, NAFLD non-alcoholic fatty liver disease, PPD periodontal pocket depth.

| Covariate          | Odds ratio | 95% confidence interval | p-value |
|--------------------|------------|-------------------------|---------|
| Endotoxin          | 1.050      | 0.946–1.166             | 0.337   |
| Weight (kg)        | 0.948      | 0.907–0.991             | 0.011   |
| Age                | 0.998      | 0.951–1.048             | 0.954   |
| Platelet count (× 10^12/L) | 0.977 | 0.900–1.060             | 0.523   |
| AST (U/L)          | 1.013      | 0.984–1.044             | 0.341   |
| Type VII collagen 7s (ng/mL) | 1.705 | 0.889–3.271             | 0.098   |
| BOP                | 1.045      | 1.013–1.079             | 0.001   |
| \textit{P. gingivalis}  \geq 0.01% | 4.05   | 1.056–15.52             | 0.04    |
| IMT mean           | 1.21       | 1.056–1.387             | 0.003   |
| PPD (mm)           | 2.045      | 0.875–1.393             | 0.026   |

Figure 4. AUROC curves for patients with PPDs \(\geq 4\) mm at \(\geq 5\) (a) and \(\geq 10\) (b) sites. AUROC area under the receiver operating characteristic, PPD periodontal pocket depth.
Methods
The recruitment process for this study was carried out at Kanagawa Dental University Yokohama Clinic, Kanagawa Dental University, Iwasaki Internal Medicine Clinic, and Yokohama City University Hospital Cohort. Patient recruitment process was conducted over a period of 8 h per day, 5 days a week. Eligible patients were screened by the attending physicians and deputy researchers (gastroenterologists and periodontists). The design of our research required patient involvement; specifically, the development of the research question was based on experiences of the patients. The Kanagawa Dental University Committee for Research Screening approved this study (approval number: 323; approval date: August 21, 2015). All procedures were performed in accordance with the tenets of the Declaration of Helsinki.

Each participant was examined to assess the amount of periodontal disease-causing bacteria (e.g., P. gingivalis, T. foetus, and T. denticola) present in oral cavity, the degree of infection, and periodontal disease severity. All examinations were performed by dentists affiliated with Kanagawa Dental University Hospital and Kanagawa Dental University Yokohama Clinic. The amount of P. gingivalis in saliva was measured using quantitative PCR, and the infection level was evaluated using serum immunoglobin G (IgG) antibody titer tests for P. gingivalis FDC381 and SU63 based on enzyme-linked immunosorbent assays. The severity of periodontal disease was determined by assessing the PPD, clinical attachment level, level of gingival BOP at six sites per tooth using a calibrated periodontal probe (HU-FRIEDY PQ-OY Thin Williams, Hu-Friedy, Chicago, IL, USA), and the stability of the teeth. The patients with NAFLD recruited in this study met the inclusion and exclusion criteria listed in Supplementary Tables S1 and S2, respectively. Fatty liver, steatosis grade, and fibrosis stage were evaluated using non-invasive methods such as ultrasonography (VCTE) and MRI.

Registration. For patients judged to be eligible, the principal investigator or collaborator filled out a patient registration form with the necessary information and sent these data to the Patient Enrollment Center (Yokohama City University). The Patient Enrollment Center confirmed the patient's eligibility based on the registration form, registered the patient, and informed the main researcher or collaborator of the patient's identification and assignment numbers. All participants provided informed consent to participate in the study prior to the commencement of the study.

Sample size calculation. A retrospective analysis of patients with NAFLD and periodontitis (P. gingivalis ratio < 0.01% vs. P. gingivalis ratio ≥ 0.01%) in Yokohama City University Hospital showed a mean liver stiffness of 2.8 and 3.6 in the P. gingivalis < 0.01% and P. gingivalis ≥ 0.01% groups, respectively, on MRE. We decided to calculate the number of patients required for a proper analysis of the variance t-test based on these data. Considering the liver stiffness (determined with MRE) in the P. gingivalis < 0.01% and P. gingivalis ≥ 0.01% groups to be 2.8 and 3.6, respectively, with a common SD of 1.8, a total of 159 patients were needed to reach an 80% statistical power with a two-sided significance level of 5%. To compensate for dropouts, we proposed an increase in the number of patients to 164.

Statistical analyses. In this study, statistical analyses were performed mainly for the following items. As primary analysis, stratification analysis was performed using the P. gingivalis bacterial content, MRE, and periodontal pockets as indexes. At the secondary endpoint, the P. gingivalis IgG antibody titer in the blood was required for each participant, and the paired Wilcoxon signed-rank test was performed. Data are presented as means ± SDs or numbers (percentages). The significance level was set at p < 0.05. We used statistical software for all analyses (SAS version 9.4, SAS Institute, Cary, NC, USA).

Trial steering and data monitoring committees. The pilot committee consisted of three people appointed by independent clinical and basic researchers (general and palliative care specialists and statisticians at Yokohama City University School of Medicine). They provided overall supervision and ensured all registered examinations. These investigators were anonymous and randomly selected. The Independent Data Monitoring Committee comprised two members of staff from the Department of Biostatistics, Yokohama City University School of Medicine. The management team monitored the monthly progress of patients and data with phone, email, and/or web conferencing. If the oversight committee determined that field monitoring was necessary, a member of the Independent Data Monitoring Committee visited the site for face-to-face monitoring.

Ethics declarations. This study involves human participants and was approved by the Kanagawa Dental University Committee for Research Screening (Approval No: 323). All procedures were performed in accordance with the Declaration of Helsinki.

Consent to participate/Consent to publish. All participants provided informed consent to participate in the study prior to initiation of the study. Participants also signed a form indicating their agreement to the presentation of their data at conferences and in papers in a manner that does not disclose their personal information.

Data availability
All data generated or analyzed during this study are included in this published article and its Supplementary Information files.
References

1. Vernon, G., Baranova, A. & Younossi, Z. M. Systematic review: The epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment. Pharmacol. Ther. 34, 274–285 (2011).

2. Perumpail, B. J. et al. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J. Gastroenterol. 23, 8263–8276 (2017).

3. Jimba, S. et al. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. Diabet. Med. 22, 1141–1145 (2005).

4. Eguchi, Y. et al. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: A multicenter large retrospective study. J. Gastroenterol. 47, 586–595 (2012).

5. Younossi, Z. M. et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–86 (2016).

6. Stepanova, M., Rafiq, N. & Younossi, Z. M. Components of metabolic syndrome are independent predictors of mortality in patients with chronic liver disease: A population-based study. Gut 59, 1410–1415 (2010).

7. Lonardo, A., Nascimbeni, F., Mantovani, A. & Targher, G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? J. Hepatol. 68, 335–352 (2018).

8. Sonmez, A. et al. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J. Clin. Lipidol. 9, 576–582 (2015).

9. Afar, M. M., Niyazi, F., Lopez, R., Dasarathy, S. & McCullough, A. J. The association of nonalcoholic steatohepatitis and obstructive sleep apnea. Eur. J. Gastroenterol. Hepatol. 29, 1380–1384 (2017).

10. Lugari, S., Mantovani, A., Nascimbeni, F. & Lonardo, A. Hypothyroidism and nonalcoholic fatty liver disease—A chance association? Horm. Mol. Biol. Clin. Invest. 41, 20180047 (2018).

11. Yoneda, M. et al. Involvement of a periodontal pathogen, Porphyromonas gingivalis on the pathogenesis of non-alcoholic fatty liver disease. BMC Gastroenterol. 12, 16 (2012).

12. Huang, M. A. et al. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: A pilot study. Am. J. Gastroenterol. 100, 1072–1081 (2005).

13. Toshimitsu, K. et al. Changes of anthropometric and biological parameters in patients with nonalcoholic steatohepatitis for dietary modification. J. Metab. Clin. Nutr. 12, 337–346 (2009).

14. Musso, G., Cassader, M., Rosina, F. & Gambino, R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of randomised trials. Diabetologia 55, 885–904 (2012).

15. Wang, R. T., Koretz, R. L. & Yee, H. F. Is weight reduction an effective therapy for nonalcoholic fatty liver?. Am. J. Med. 115, 554–559 (2003).

16. Sanyal, A. J. et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N. Engl. J. Med. 362, 1675–1685 (2010).

17. Mahady, S. E., Webster, A. C., Walker, S., Sanyal, A. & George, J. The role of theta-zidilinediones in non-alcoholic steatohepatitis—A systematic review and meta analysis. J. Hepatol. 55, 1383–1390 (2011).

18. Boeticher, E., Csako, G., Pucino, F., Wesley, R. & Loomba, R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. Aliment. Pharmacol. Ther. 35, 66–75 (2012).

19. Hasegawa, T., Yoneda, M., Nakamura, K., Makino, I. & Terano, A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: A pilot study. Aliment. Pharmacol. Ther. 15, 1667–1672 (2001).

20. Musso, G., Gambino, R., Cassader, M. & Pagano, G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology 52, 79–104 (2010).

21. Zein, C. O. et al. Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. Hepatology 54, 1610–1619 (2011).

22. Sanz, M. et al. Scientific evidence on the links between periodontal diseases and diabetes: consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International Diabetes Federation and the European Federation of Periodontology. J. Clin. Periodontol. 45, 138–149 (2018).

23. Holmlund, A., Lampa, E. & Lind, L. Poor response to periodontal treatment may predict future cardiovascular disease. J. Dent. Res. 96, 768–773 (2017).

24. Yamazaki, K. et al. Oral pathobiont-induced changes in gut microbiota aggravate the pathology of nonalcoholic fatty liver disease in mice. Front. Immunol. 12, 766170 (2021).

25. Ao, M. et al. Dental infection of Porphyromonas gingivalis induces preterm birth in mice. PLoS ONE 10, e0137249 (2015).

26. Hayashi, C., Gudino, C. V., Gibson, F. C. & Genco, C. A. Review: Pathogen-induced inflammation at sites distant from oral infection: Bacterial persistence and induction of cell-specific innate inflammatory pathways. Mol. Oral Microbiol. 25, 305–316 (2010).

27. Riewe, S. D. et al. Human trophoblast responses to Porphyromonas gingivalis infection. Mol. Oral Microbiol. 25, 252–259 (2010).

28. Roth, G. A. et al. Porphyromonas gingivalis infection and cell death in human aortic endothelial cells. FEMS Microbiol. Lett. 272, 106–113 (2007).

29. Hasegawa-Nakamura, K. et al. The possible mechanism of preterm birth associated with periodontopathic Porphyromonas gingivalis, J. Periodontol. Res. 46, 497–504 (2011).

30. Tatetsu, F. et al. Detection of Fusobacterium nucleatum in chorionic tissues of high-risk pregnant women. J. Clin. Periodontol. 39, 417–424 (2012).

31. Noack, B. et al. Periodontal infections contribute to elevated systemic C-reactive protein level. J. Periodontol. 72, 1221–1227 (2001).

32. Ibsenole, J. L. et al. Periodontitis in humans and non-human primates: Oral-systemic linkage inducing acute phase proteins. Ann. Periodontol. 7, 102–111 (2002).

33. Tamaki, N. et al. Short-term effects of non-surgical periodontal treatment on plasma level of reactive oxygen metabolites in patients with chronic periodontitis. J. Periodontol. 80, 901–906 (2009).

34. Kato, T. et al. Oral administration of Porphyromonas gingivalis alters the gut microbiome and serum metabolome. mSphere 3, e00460-18 (2018).

35. Tomofuji, T. et al. Chronic administration of lipopolysaccharide and proteases induces periodontal inflammation and hepatic steatosis in rats. J. Periodontol. 78, 1999–2006 (2007).

36. Kuraji, R. et al. Porphyromonas gingivalis induced periodontitis exacerbates progression of non-alcoholic steatohepatitis in rats. Clin. Exp. Dent. Res. 2, 216–225 (2016).

37. Socrazensky, S. S., Haf AJ, A. D., Cugini, M. A., Smith, C. & Kent, R. L. Microbial complexes in subgingival plaque. J. Clin. Periodontol. 22, 134–144 (1998).

38. Araujo, M. W. B. et al. Reproducibility of probing depth measurements using a constant-force electronic probe: Analysis of inter- and intraexaminer variability. J. Periodontol. 74, 1736–1740 (2003).
40. Ebersole, J. L.

39. Taylor, G. W.

institutional affiliations.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and
requests for materials should be addressed to Y. K.

Correspondence and requests for materials should be addressed to Y. K.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-17917-2.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022