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Salivary features of periodontitis and gingivitis in type 2 diabetes mellitus

Jeffrey L. Ebersole[1]✉, Sreenatha S. Kirakodu[2], Xiaohua D. Zhang[3], Dolph Dawson III[2,4] & Craig S. Miller[2,4]

Type 2 diabetes mellitus (T2DM) is associated with cellular abnormalities, tissue and organ dysfunctions, and periodontitis. This investigation examined the relationship between the oral microbiome and salivary biomarkers in T2DM patients with or without periodontitis. This cohort (35–80 years) included systemically healthy non-periodontitis (NP; n = 31), T2DM without periodontitis (DWoP; n = 32) and T2DM with periodontitis (DWP; n = 29). The oral microbiome [Operational Taxonomic Units (OTUs)] (16 s rRNA sequencing) and targeted host salivary biomarkers (immunoassays) were assessed. We identified 47 OTUs that were significantly different in abundance between NP samples and any disease subset or between disease subgroups. The most unique microbiome patterns were observed in the DWP group. Differences in genera/species abundance were also observed when T2DM patients were stratified by extent of periodontal inflammation and disease (i.e., generalized versus localized gingivitis/periodontitis). Salivary biomarkers showed significant elevations in MMP-8, MMP-9, resistin, IL-1β, IL-6, IFNα, and BAFF (THFSR13b) comparing generalized to localized periodontitis. Salivary analytes showed significant positive correlations with specific microbiome members, predominantly in DWP patients. Odds ratio analyses reinforced that a panel of biologic markers (IL-6, MMP-8) and bacteria (e.g., *Bacteroidetes*, *Fusobacteria*, *Spirochaetes*) discriminated the severity and extent of periodontal disease in this diabetic population.

Keywords Diabetes, Microbiome, Inflammation, Periodontitis, Saliva

Type 2 diabetes mellitus (T2DM) is a chronic inflammatory disease with elevated systemic biomolecules of inflammation that are central to the pathogenesis of the disease[1–5]. Beyond the identification of periodontitis as a sequela of T2DM, localized inflammatory proteins are routinely increased in the gingival crevicular fluid and saliva of periodontitis affected T2DM patients[6–10]. Within this oral environment the host responses are driven by, and reflect, an altered microbiome with dysbiotic changes of opportunistic pathogens and pathobionts revealing a lesional process[11–15].

As variations in the characteristics of the oral microbiome and associated host responses have been related to extrinsic and intrinsic (e.g., T2DM, age) effectors of the host ecosystem[16–19], there remain gaps in our knowledge concerning the biologic mechanisms that are controlling these features in the oral cavity. Additionally, mechanical therapeutics in systemically normal individuals have been shown to substantially impact the microbiome and help transition its characteristics to one more consistent with periodontal health[20–22]. Nevertheless, existing data indicate that the prevalence and severity of periodontitis is increased in T2DM[23–27].

This investigation cross-tabulated and specifically compared the microbiome and biomarkers in saliva of T2DM patients with or without periodontitis. These findings provide insight into the unique biological features of localized and generalized gingivitis and periodontitis in the presence of T2DM.

Results

Oral clinical features in diabetes

Figure 1 provides the periodontal characteristics and BMI relative to the various groups/subgroups of subjects. More specifically the five subgroups denote a systemically healthy, non-periodontitis groups (NP). Within the

[1]Department of Biomedical Sciences, School of Dental Medicine, University of Nevada Las Vegas, Las Vegas, NV 89131, USA. [2]Center for Oral Health Research, College of Dentistry, University of Kentucky, Lexington, KY, USA. [3]Department of Biostatistics, University of Kentucky, Lexington, KY, USA. [4]Department of Oral Health Practice, Center for Oral Health Research, College of Dentistry, University of Kentucky, Lexington, KY, USA. ✉email: jeffrey.ebersole@unlv.edu

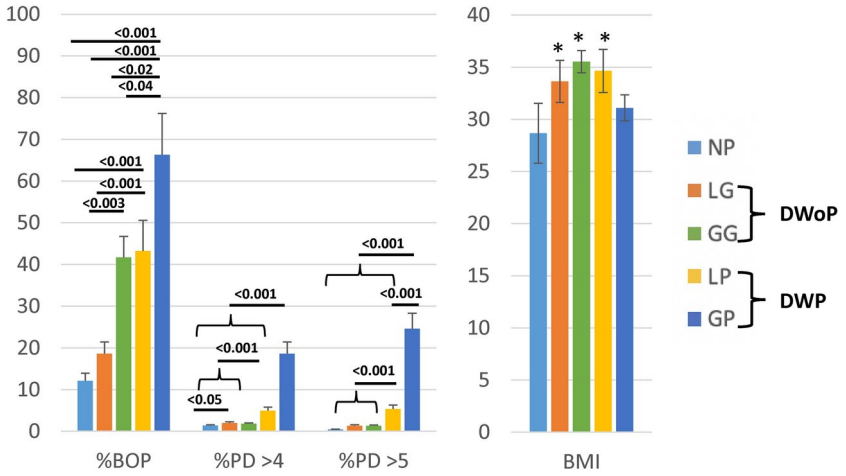

Fig. 1. Clinical features of patient groups (NP—non-periodontitis; DWoP—T2DM without periodontitis; DWP—T2DM with periodontitis). The DWoP group had localized (LG) or generalized (GG) gingivitis. The DWP group had localized (LP) or generalized (GP) periodontitis. The bars are the group means and the vertical brackets enclose 1 SEM. The connecting horizontal bars identify significant differences between the groups and the asterisk (*) for BMI values denotes significantly different than the NP group at $p < 0.05$ as determined by an ANOVA.

type 2 diabetes patients, a subgroup was included without periodontitis by exhibiting some level of gingivitis that was either determined to be localized (DWoP LG) or generalized (DWoP GG). Finally, in the T2DM cohort the subgroups with periodontitis were stratified into localized (DWP LP) or generalized (DWP GP) disease. In Fig. 1, the NP group showed a significantly lower BMI than most T2DM groups (e.g., DWoP, DWP), with no differences in HbA1c levels related to the clinical measures. In contrast and as expected, the clinical features of the destructive disease in the generalized periodontitis patients were significantly different from both the gingivitis and localized periodontitis groups. Additionally, BOP showed significantly greater levels with increasing severity of disease. Of note, most periodontally healthy individuals were in the NP group, and most gingivitis cases were in the DWoP group. Based upon the disease features in affected patients, an additional perspective evaluated the characteristics of the microbiome and host response patterns related to the magnitude of disease.

Microbiome characteristics in generalized and localized disease in diabetes

Supplemental Fig. 1 provides a summary of the individual sample OTU reads for the NP, GG, LG, GP, and LP salivary microbiomes. Generally, similar numbers of overall reads were identified across the subgroups. In another report we identified the top 104 OTUs based upon levels across the three study groups (NP, DWoP, DWP), and showed that this portfolio of microorganisms provide 96–98% coverage of the microbiome reads in the samples[28] (Supplemental Table 1).

Figure 2A presents the results for the individual NP subjects and shows *Firmicutes (Bacillota)* as the dominant phyla and the *Bacteroidetes* phyla was next most prevalent. *Proteobacteria* was elevated in some individuals, while a subset of NP subjects demonstrated elevated levels of *Spirochetes* and/or *Absconditabacteria* in their salivary microbiome. We next stratified the diabetic periodontitis (DWP) and gingivitis (DWoP) patients based on the clinical presentation (i.e., generalized or localized form) of periodontal inflammation and lesions to examine the phyla distribution. *Firmicutes* was the dominant phyla across the majority of DWoP patients (Fig. 2B). Figure 2C presents that *Firmicutes* was also the dominant phyla in DWP group although multiple patients showed elevated *Bacteroidetes*. A summary of these comparisons at the patient group level is presented in Supplemental Table 2. Striking was that the generalized periodontitis adults showed higher levels of *Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes,* and *Synergistetes* compared to the other groups, including the localized disease group, although these differences did not reach statistical significance. The data was also analyzed for alpha and beta diversity within the 5 subject groups. The results supported a higher microbial diversity via the inverse Simpson and Shannon indices particularly in the GP group. However, the Chao1 values indicated similar numbers of species in the NP, LG and GG samples, while the GP samples showed an increased number of species and LP fewer species present.

The evaluation of the microbiome differences was extended to focus on the individual microbial components in the various patient groups. Figure 3 illustrates the relative abundance of the targeted set of 104 OTUs in samples from the study population based on the level of oral disease (i.e., NP [healthy], LG, GG, LP, and GP). We identified 47 OTUs that were significantly different in abundance between NP and any disease subset or between disease subgroup categories (Table 1). There also were numerous OTU abundance differences (N = 18) between LG and LP that spanned altered commensal species, as well as taxa associated with periodontitis. Seven bacterial species differences in abundance were observed between GG and GP that were skewed towards more pathogenic species. Four bacterial taxa abundance differences were observed between LG and GG. Interestingly, only one species (*P. gingivalis*) differed in relative abundance between LP and GP, supporting the substantial role

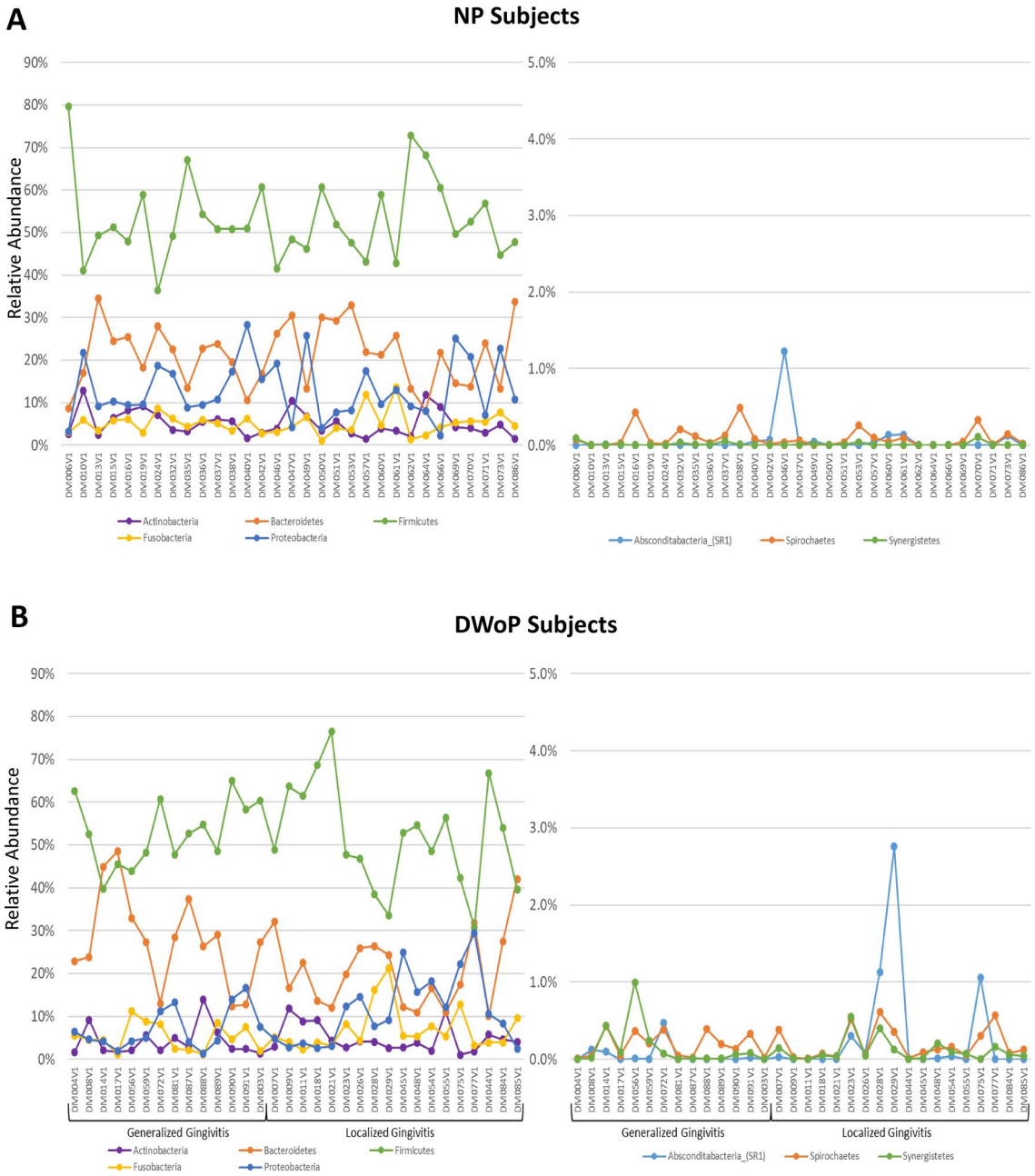

Fig. 2. Relative abundance of different phyla to total sequence readouts in individual (**A**) NP, (**B**) DWoP, or (**C**) DWP subjects identified as generalized or localized disease.

of this opportunistic pathogen in periodontitis, albeit potentially suggesting that variations in the details of the host response to this pathogen may contribute to differences in disease extent.

Salivary biomarkers, disease severity, and microbiome in diabetes

Salivary concentrations of host response analytes by study group and periodontal disease category are depicted in Fig. 4. Here, nine host biomarkers showed major concentration differences between the NP subjects and DWP individuals, both GP and LP. MMP-8, MMP-9, TIMP-1, and MIP-1α were the only markers that significantly differed between health and the subgroups of gingivitis (DWoP) patients. Resistin concentrations were significantly increased in the DWP periodontitis subgroups, while differences in adiponectin levels between disease categories were not observed.

As the oral microbiome both drives and responds to host responses in the oral cavity, it was of interest to explore the relationship of targeted salivary biomolecules to the microbiome components and how that relationship was affected by diabetes and periodontal characteristics. Figure 5A shows the frequency of significant correlations of the individual biomarker levels with the relative abundance of the 104 OTUs in the microbiome samples. These correlations were generally higher in the NP and DWP groups, with MMP-8, BAFF, adiponectin, and resistin

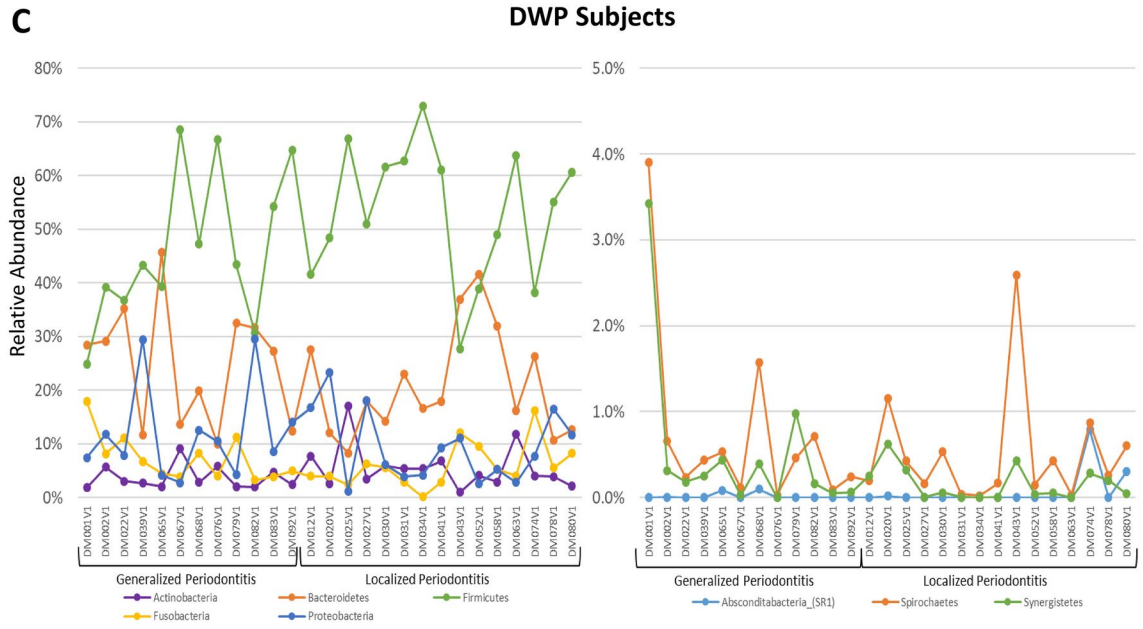

Figure 2. (continued)

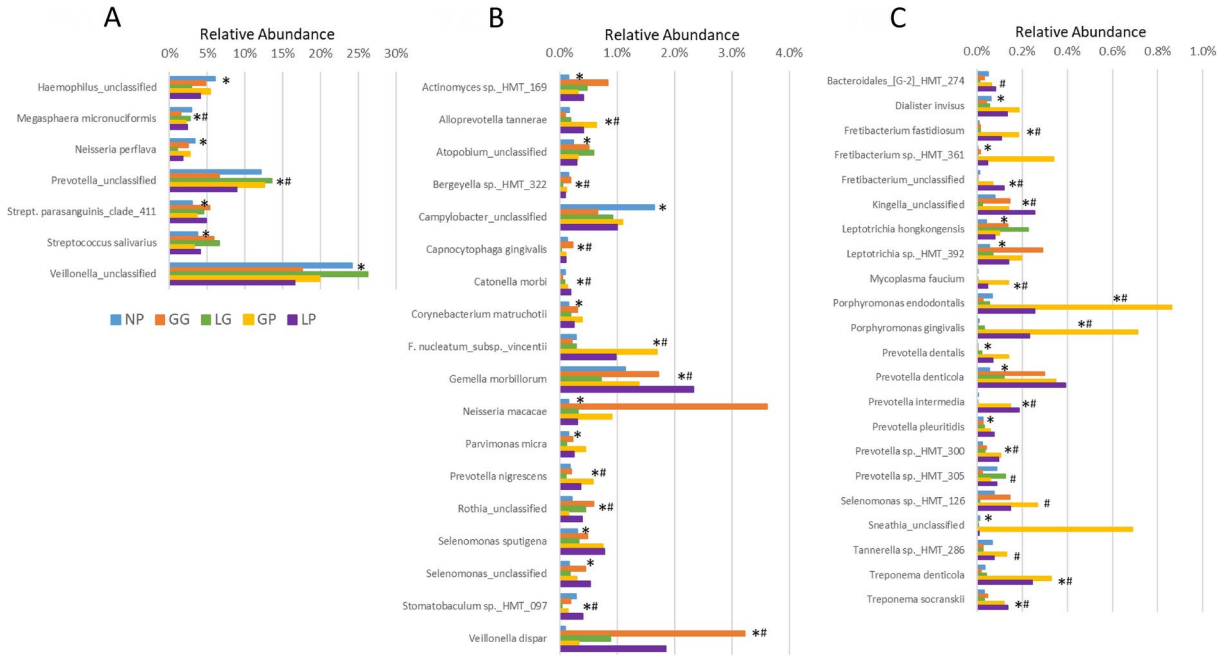

Fig. 3. Relative abundance of OTUs (eg. species, genera) in to total sequence readouts of NP, DWoP (GG, LG), and DWP (GP, LP) in salivary microbiome samples. Panels A, B and C organize the microbes based upon general relative abundance across the samples. Bars denote group means with asterisk (*) signifying a significant difference between NP and disease groups, and hashtag (#) identifying one or more disease subsets that differs in OTU (eg. species, genera) abundance from the other groups. OTUs were only included if a significant difference at $p < 0.01$ was detected for any comparison as determined by ANOVA.

showing the greatest frequency of positive correlations within the 3 subject groups. In contrast, there were only a limited number of negative correlations with any of the host molecules and the array of microbial taxa identified. Figure 5B summarizes the details of the bacterial species that dominated these correlations with the 11 salivary biomarkers. As was expected few correlations with individual bacterial species were noted in the DWoP samples. This observation of a general decrease in significant correlations across the host factors and with individual microbiome members in the DWoP patients could be explained by the individual variation in the dynamics of

GG vs. LG	GP vs. LP	GG vs. GP	LG vs. LP
*Prevotella*_unclassified *M. micronuciformis* *Prevotella* sp._HMT_305 *Bergeyella* sp._HMT_322	*P. gingivalis*	*F. nucleatum_subsp._vincentii* *Prevotella nigrescens* *Rothia_unclassified* *Alloprevotella tannerae* *P. gingivalis* *Catonella morbi* *Fretibacterium fastidiosum*	*Veillonella_unclassified* *Gemella morbillorum* *Prevotella nigrescens* *Stomatobaculum sp._HMT_097* *Capnocytophaga gingivalis* *P. endodontalis* *Treponema socranskii* *Selenomonas sp._HMT_126* *Prevotella intermedia* *Porphyromonas gingivalis* *Treponema denticola* *Fretibacterium fastidiosum* *Tannerella sp._HMT_286* *Fretibacterium_unclassified* *Kingella_unclassified* *Bacteroidales_[G-2]_HMT_274* *Prevotella sp._HMT_300* *Mycoplasma faucium*

Table 1. Microbiome relative abundance differences between generalized and localized disease in T2DM patients. These taxa are significantly different between the groups determined by a *t test* (see Fig. 2). GG: Generalized gingivitis, GP: Generalized periodontitis, LG: Localized gingivitis, LP: Localized periodontitis.

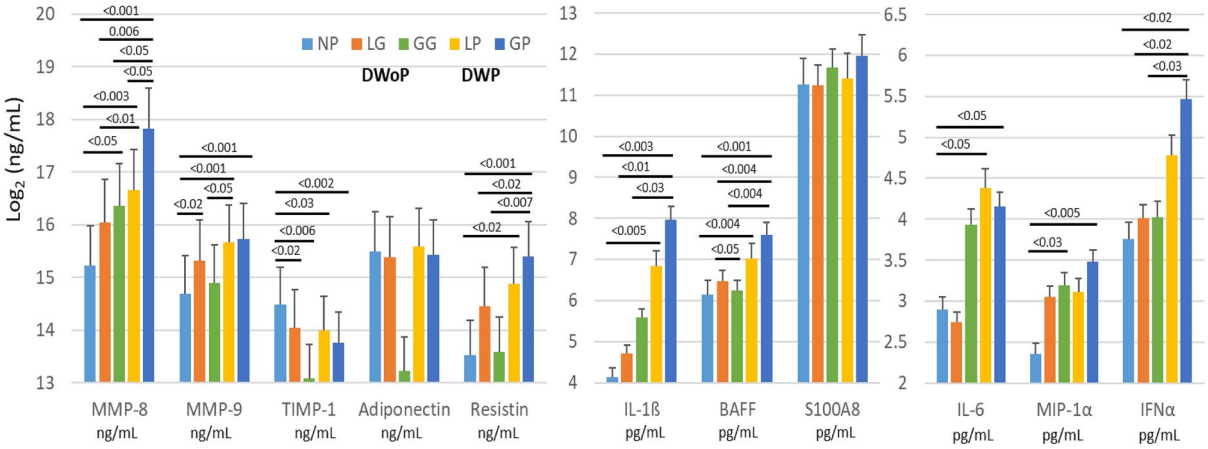

Fig. 4. Levels of salivary analytes in subsets of DWoP and DWP T2DM patients with localized (LG, LP) or generalized (GG, GP) disease compared to control subjects (NP). Bars denote group means and vertical brackets signify 1 SEM. Significance is indicated compared to the NP group, as well as between the various disease strata as determined by ANOVA.

the host response to the evolving microbiome that occurs early in the transition from health to overt periodontal lesions. The findings depicts 27 of the OTUs with extensive correlations to the salivary analytes, with 17 in the DWP patients and 10 in the NP subjects. The identified OTUs encompassed members of the *Bacteroidetes, Firmicutes (Bacillota), Fusobacterium, Spirochaetes,* and *Synergistetes* phyla.

Supplemental Fig. 2A–D summarizes features of the correlations between individual OTUs of the 104 dominant members and levels of the individual salivary analytes. In the DWoP LG group, several significant correlations were observed, with the majority of positive correlations occurring with resistin and TIMP-1 (Supplemental Fig. 2A). In the DWoP GG patients, IL-1ß and adiponectin showed parallel patterns of positive correlations (Supplemental Fig. 2B). Additionally, correlations with MMP-8 and IL-6 were all positive but with different OTUs. All significant correlations with MIP-1α were negative in this patient subset. Supplemental Fig. 2C summarizes the values for the DWP LP group that showed IFNα, TIMP-1, and adiponectin positively correlated with multiple OTUs. Finally, the largest frequency of correlations was noted in the DWP GP (Supplemental Fig. 2D) subjects. Here IL-1β, IL-6, IFNα, BAFF, adiponectin, and resistin were the predominant biomarkers that correlated with specific OTU, and frequently the same OTU.

These various relationships allowed us to explore whether a targeted array of microbes and salivary biomarkers might be predictive of generalized and localized disease in T2DM patients. Odds Ratios (OR) were determined comparing levels of bacteria and host response biomolecules in periodontitis versus gingivitis subjects (Fig. 6A). Here we observed OR of > 2.5 for most of the host biomarkers in the GP group, as well as increased ORs in the LP group. A similar number of oral bacteria exhibited ORs > 2 in LP or GP when compared to the gingivitis group. However, striking was an array of the bacterial species that displayed substantially lower ORs in the GP group compared to GG group. Figure 6B provides a similar analysis for generalized versus localized inflammation or disease. Over 60% of the targeted host analytes showed ORs > 2 in LP compared with GP. Additionally, multiple

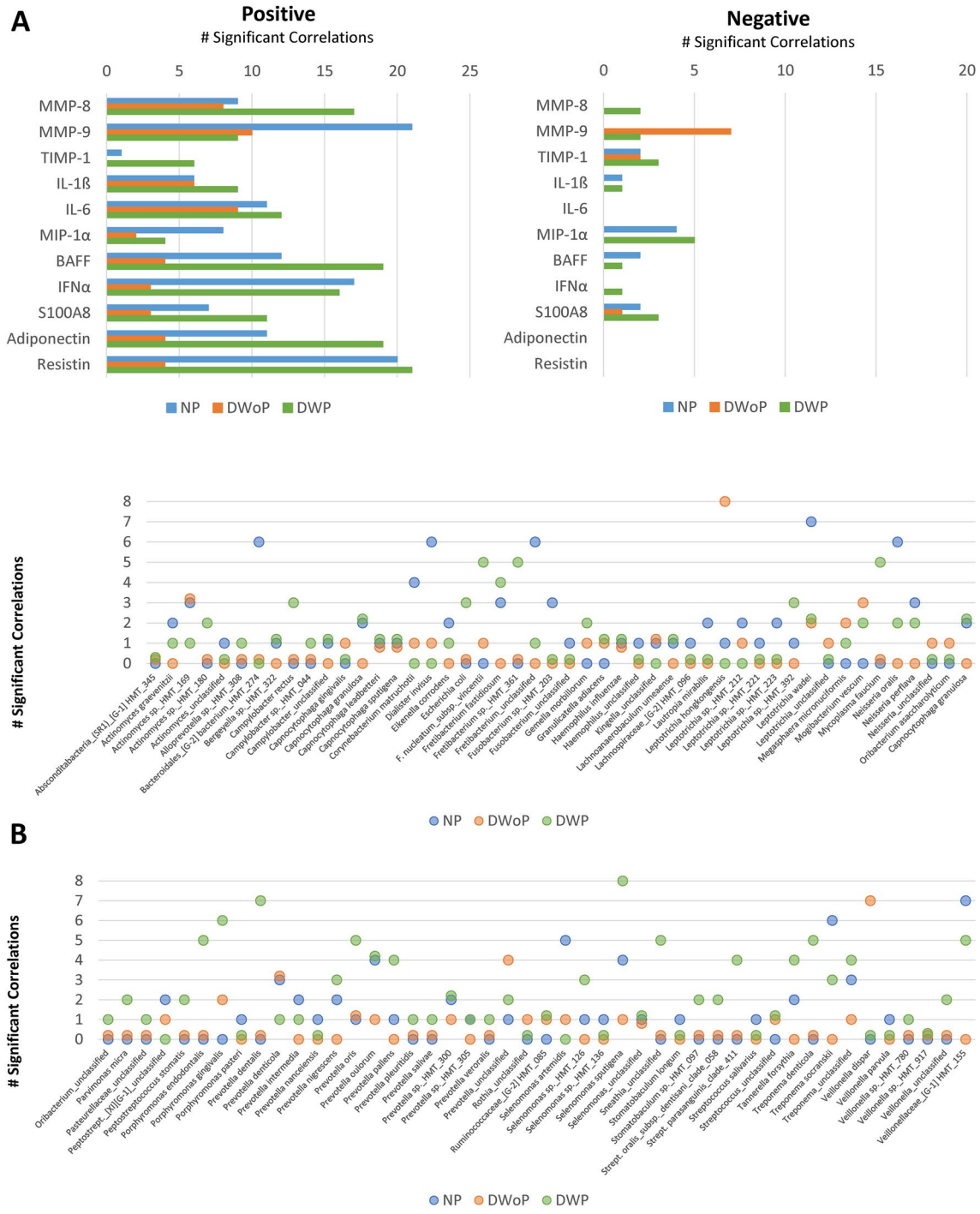

Fig. 5. Frequency of OTUs with significant correlations with levels of the individual salivary analytes. (**A**) Bars denote the number of positive or negative correlations for each analyte within the groups. (**B**) Frequency of significant correlations across the 11 salivary analytes with the individual OTUs. Each dot represents the number of significant ($p < 0.05$) correlations for a specific subject group. Only OTUs were included where there was any significant correlation in one or more patient groups.

bacterial species were specifically elevated in samples in the GP patients (ORs > 2). Similarly, multiple species of bacteria were specifically increased in the GG versus LG patients that were frequently different from those elevated in GP (eg. *R. mucilaginosa, Prevotella* sp. HMT305, *V. dispar*). Also, a limited number of host response markers varied in the GG compared to the LG group (i.e., IL-6, MMP-8, adiponectin).

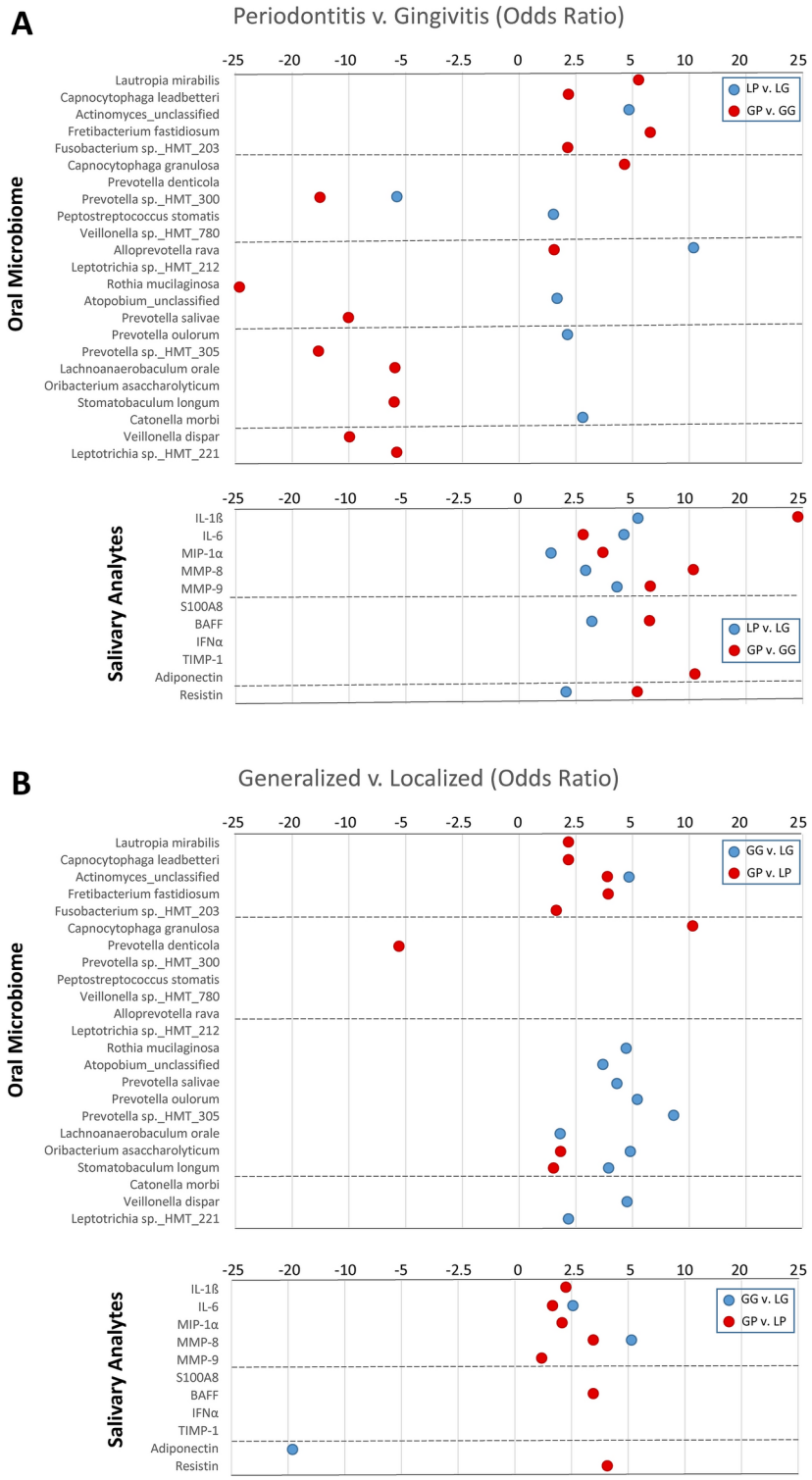

Fig. 6. Display of Odd Ratios (OR) for various microbes (TOP panel) and host factors (BOTTOM panel) comparing (**A**) localized gingivitis (LG) to localized periodontitis (LP) and generalized gingivitis (GG) to generalized periodontitis (GP), and (**B**) LG to GG and LP to GP in the T2DM groups. Each point denotes an OR value for the specific variable (microbe, host factor). Positive OR denotes elevated level in generalized disease compared to localized disease or in gingivitis vs. periodontitis. A negative OR denotes elevated level in LG, LP, or gingivitis compared to generalized disease or periodontitis, respectively.

Discussion

Epidemiological studies confirm that diabetes is a significant risk factor for periodontitis, and periodontitis increases the risk of poor diabetes management and sequelae in affected individuals[16,29–32]. Thus, this interplay between these chronic inflammatory conditions reflects the difficulty in controlling glucose levels and the initiation and progression of periodontitis[33–39]. The related clinical features reflect dysregulated inflammatory responses that are linked to tissue changes in T2DM, a microbiome contribution from periodontitis, and genetic regulation of the inflammatory status of the individual[33,40–45]. Extensive reports identify the bidirectional linkage of diabetes and periodontitis, and studies that identify biological factors that contribute to the various stages of periodontal disease are not well characterized with these comorbidities. Hence, we sought to further characterize the salivary microbiome and associated host responses in T2DM patients (i.e., T2DM patients with periodontitis [DWP] or with gingivitis [DWoP]), with the goal of better understanding the panel of biological factors involved in the different extent and categories of periodontal disease compared to an NP healthy control group. There are numerous publications examining features of the oral microbiome, salivary and serum/plasma analytes and metabolites related to oral health. However, these generally segregate into studies of periodontitis in systemically healthy subjects (ie. controls, gingivitis, periodontitis)[46], or investigations of T2DM that incorporate T2DM patients with periodontitis compared to orally and systemically healthy controls[47–51], or periodontitis patients with and without T2DM[52,53]. Very limited studies are available where comparisons are made across the range of oral and systemic health/disease. However, attempting to integrate the available literature indicates some oral microbial and host response differences between orally healthy controls and T2DM patients. Major differences are reported between systemically healthy controls and T2DM patients with periodontitis. Moreover, limited literature reports differences in periodontitis and biological parameters in well versus poorly-controlled T2DM, and compared to non-diabetic subjects. Importantly, these reported comparisons or oral health and periodontitis with/without T2DM were mostly mutually exclusive. Thus, this study provides direct comparisons of the microbiome and host response differences among the 3 cohorts is rather unique. This report described differences in the microbiomes at the individual patient level regarding the phyla distribution, and significant differences between localized and generalized gingivitis and periodontitis. Of the 104 dominant OTUs, approximately ¼ of these bacterial identifiers showed significant differences between health and the various disease presentation of localized and generalized periodontal diseases. These findings are consistent with the pathogenic microbiome in periodontitis, and particularly with more generalized disease.

Considerable variation in inflammatory biomolecules concentrations have been observed in gingival crevicular fluid, saliva, and blood of patients with diabetes and periodontal disease[54–58]. Other studies have attempted to associate these mediators with periodontitis and the transition from health to metabolic syndrome and even T2DM[59]. Substantial evidence attributes pathogenic mechanisms linking periodontitis and diabetes to dysregulated inflammation[60]. We have shown previously in this population differences in salivary biomarkers between normal subjects and patients with T2DM, with or without periodontitis[61] that was consistent with the extant literature. While this literature includes reports demonstrating a relationship between HbA1c levels and periodontitis[62], as well as the impact of treatment for improving periodontal health that lowers the HbA1c levels[63], we did not observe a relationship in our gingivitis or periodontitis group. However, as our population showed a mean HbA1c level from 7.1 to 7.3, the relatively high glycemic control in this group may have precluded detection of correlations with host responses and/or oral microbiome components. However, in spite of the large number of studies on inflammatory mechanisms in periodontitis and diabetes, few have documented oral microbiome features that relate with specific host responses in the presence of these comorbidities. Various classic periodontal pathogens have been identified in diabetic and non-diabetic periodontitis patients, with *P. gingivalis* appearing in higher levels in both T2DM and T1DM patients[64–68]. A growing body of evidence suggests that diabetes may alter the local periodontal pocket environment favoring certain bacterial species to emerge. However, existing studies suggest some subtle differences in the oral microbiomes in diabetic patients, but clear clinical relevance of these differences have not been discerned. In the present study, significant differences were observed in various salivary analytes, especially MMP-8, MMP-9, TIMP-1, IL-1β, BAFF, and resistin primarily in the DWP versus both DWoP and NP subjects. Exploration of the relationship between the salivary analytes and individual members of the oral microbiome showed a greater number of significant, generally positive, correlations with MMP-8, BAFF, IFNα, adiponectin, and resistin in the DWP individuals. As the relationship between the chronic diseases of T2DM and periodontitis have emphasized a dysregulated host response, these findings support alterations in the host-microbe interactions when these diseases are coincident.

Our previous report described differences in the oral microbiomes of normal subjects, T2DM patients without periodontitis (DWoP), and T2DM patients with periodontitis (DWP)[28]. In this analysis, we also identified several differences in the microbiomes within the DWoP and DWP groups that were stratified based upon localized or generalized clinical presentation of inflammation and destructive disease. While members of the *Firmicutes (Bacillota)* phyla were the dominant group of bacteria in both DWP and DWoP patients, there were clear differences in the GG (DWoP) and GP (DWP) subgroups showing elevated abundance of *Bacteroidetes, Fusobacteria, Spirochaetes,* and *Synergistetes* compared to the localized disease group. Thus, for the first time we demonstrated within the T2DM stratification, microbiomic variations appeared to relate to the magnitude (extent, severity) of oral inflammation and disease. Further studies will be needed to better understand not just a diagnostic grouping of the patients, but underlying biological features/differences that contribute to these microbial and host variations.

For oral health in systemically healthy subjects or T2DM patients, there is a critical balance between tissue homeostasis and a transition into the microbial driven disease of periodontitis. Both genetic and environmental features regulate features of an individual's response to the burden and quality of the oral microbiome[32,69–71]. As we have shown previously, there are distinctive differences in targeted host response biomolecules in saliva of these T2DM patients, as well as the characteristics of the salivary microbiome patterns coincident with these

altered host response profiles[72]. We now identified clear differences in both the microbiome and salivary analytes related to disease extent in T2DM patients. Additionally, correlations between selected salivary analytes and specific members of the microbiome appeared to vary with disease extent, with the T2DM patients who had GP showing the greatest number of significant relationships. This type of analysis does not provide any cause-and-effect understanding of the processes that occur to reach this disease susceptible milieu in the oral environment. However, the results appear to reflect certain bacterial genera/species including *Actinomyces*_unclassified, *Pr. dentalis, F. nucleatum*_ssp._*vincentii, Leptotrichia* sp._HMT_218, *P. endodontalis, Sneathia*_unclassified, *T. denticola*, and *M. faucium* that relate more directly to specific salivary analytes, such as IL-6, adiponectin, BAFF, and resistin. Additionally, an interesting finding regarding correlations among the salivary analytes was identified in this cohort. An elevated frequency of positive correlations were observed in the NP and DWP patients, with low levels (NP) or high levels (DWP) across the analyte profiles. However, generally the DWoP (gingivitis) patients demonstrated a rather consistently decreased number of significant correlations. While we recognize that these are only associations, an interpretation is that with the transition to gingivitis the dynamics of response changes show considerable patient-specific features in the kinetics of the individual analyte responses. Consideration of these host and microbial biomarkers could be useful in portending the likelihood of more severe disease and aid in improved clinical decision-making on patient specific therapy.

Limitations of this study include an overall number of subjects that were stratified based upon periodontal disease clinical characteristics of health or extent of oral disease that reduced the individual subgroup size. As such, the findings observed from these small subgroups may not be biologically generalizable to the broader T2DM or non-T2DM population. Also, poorly controlled diabetics were not included and may manifest distinctive host and microbiome traits[40,73]. This study, as most oral microbiome reports, describes the microbial ecology based upon a discrete sample thus lacking a general assessment of the permanency or transitory nature of the salivary microbiome in individual subjects. Finally, although saliva reflected many microbiome patterns apparent in the subgingival environment, saliva may not reflect all aspects important for understanding the biology of periodontitis.

Methods

Study and participants

This cohort clinical study, approved by the Institutional Review Board of the University of Kentucky (UK) #17-0439-F6A in accordance with the Helsinki Declaration, was performed from June 2017 to July 2019. The participants were recruited from the patient population attending the University of Kentucky College of Dentistry seeking general or specialized dental services. Inclusion criteria were: 35–80 years, a minimum of 16 teeth (excluding 3rd molars), in good systemic health (excluding the case definition), or diagnosed with T2DM based on the criteria of the American Diabetes Association[74]. We have reported previously that T2DM was confirmed by the patient's physician with glycated hemoglobin A1c (HbA1c) (mean = 7.3), body mass index (BMI), and complete head, neck, and oral examination were recorded[61]. All subjects provided signed informed consent reviewed and accepted by the University of Kentucky Institutional Review Board.

A full-mouth periodontal examination was conducted by a single calibrated dental examiner. Periodontal health measures included bleeding on probing (BOP), pocket depth (PD) and clinical attachment loss (CAL) at six sites per tooth[75]. Three cohorts were enrolled: (a) T2DM subjects with chronic periodontitis (DWP; n = 29), (b) T2DM subjects without chronic periodontitis (DWoP; n = 32) and (c) a cohort of healthier participants designated as 'Not-Periodontitis' (NP) (n = 31). Periodontitis was defined as having at least four teeth in two quadrants with a probing depth (PD) of ≥ 5 mm, clinical attachment loss (AL) of ≥ 2 mm and bleeding upon probing (BOP)[76,77]. Participants in the NP group were defined as (a) no diabetes, (b) < 20% proximal probing sites with a bleeding score of > 1 (pinpoint bleeding on gentle probing), (c) < 5% of sites with PD of ≥ 4 mm (d) no PD ≥ 5 mm, (e) < 2% of sites with CAL of > 2 mm with concurrent BOP and PD of ≥ 4 mm. The three groups were balanced for age (~ 60 years) and sex (30–50% male).

The extent of gingivitis and periodontitis in each patient was evaluated and used to categorize the patients as displaying localized/generalized gingivitis (LG, GG) or localized/generalized periodontitis (LP, GP). The DWoP group demonstrated gingivitis (i.e., BOP at ≤ 20% of sites [LG] or > 20% BOP sites [GG] coupled with all having less than five sites with ≥ 4 mm periodontal pockets). The DWP group demonstrated either LP (4–87% BOP; ≤ 10% sites with ≥ 4 mm pockets) or GP (21–97% BOP; > 10% sites with ≥ 4 mm pockets).

As reported previously[72], medical and dental histories were obtained and subjects were excluded with alcoholism; liver, kidney or salivary gland dysfunction; inflammatory bowel disease; granulomatous diseases; immunosuppressive or cancer therapy. Additionally, acute illness (i.e., fever, sore throat, body aches and diarrhea), pregnancy or lactation, use of antibiotics within the last 6 months, need for antibiotics for dental procedures, or the presence of an oral mucosal inflammatory conditions (e.g., aphthous, lichen planus, leukoplakia, and oral cancer) and current smoker were exclusion criteria.

Biologic samples and analysis

Unstimulated whole saliva samples was obtained from each participant, managed, stored, and concentrations of interleukin (IL)-1β, interleukin-6 (IL-6), MIP-1α (macrophage inflammatory protein 1 alpha), BAFF (B-cell activating factor; TNFSF13b), IFNa (interferon alpha), adiponectin, and resistin were measured in duplicate using Luminex technology with human cytokine/chemokine multiplex kits (Millipore, St. Charles, MO, USA). Salivary concentrations of matrix metalloprotease (MMP)-8, MMP-9 and TIMP-1 (tissue inhibitor of matrix matellaoproteinases-1) were determined in duplicate for each subject using human Quantikine enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA, as we have previously described[61,78,79].

Salivary microbiome characteristics were determined in each subject's samples using 16S rRNA sequencing as we have previously described[80–82]. The saliva microbiome provides a "patient-level" prespective of the

oral microbiome, which has been shown to reflect microbial changes that occur in sites of the periodontium transitioning from health to disease[83–85]. Sequences were assigned to their respective taxonomic classification using the Human Oral Microbiome Database (HOMD V13) (http://www.homd.org/index.php?name=seqDownload&file&type=R). The raw data are deposited at BioProject ID PRJNA516659 through the NIH NCBI.

Statistical analysis

Descriptive statistics were performed for the clinical features, Operational Taxonomic Unit (OTU) microbiome differences, and log transformed salivary analyte comparisons through ANOVA or two sample t-test. Tukey' honest significant difference method (HSD) was used for pairwise comparisons of the variables among groups of patients, if an ANOVA test was significant. The statistical software package (SAS 9.4, Cary, NC; IBM Inc., 2020) was used for the analysis and the statistical significance level was set at 0.05. Correlation analyses were evaluated using a Pearson correlation coefficient with a significant level of 0.05. The data for gene expression have been uploaded into GEO accession GSE180588 (https://www.ncbi.nlm.nih.gov/gds).

Data availability

The microbiome data are deposited at BioProject ID PRJNA516659 through the NIH NCBI. The data for gene expression have been uploaded into GEO accession GSE180588 (https://www.ncbi.nlm.nih.gov/gds).

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Author contributions

Author contributions include JLE and CSM contributed to conception, design, data acquisition and interpretation, drafted and critically revised the manuscript. SSK contributed to data acquisition, analysis, and interpretation, and critically reviewed the manuscript. DDIII contributed to obtaining and interpreting the clinical data and critically reviewed the manuscript. XDZ provided biostatistical evaluation for data analysis, interpretation, and critical revision of the manuscript. All authors gave their final approval and agreement to be accountable for all aspects of the work. The authors state no conflict with any information provided in the report.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.L.E.

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