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REVIEW ARTICLE

# The Oral-Systemic Link: A Review about the strong Correlation between Diabetes mellitus, Periodontitis and COVID-19 Outcome

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**Summary**
The SARS-CoV-2 virus leads to symptoms ranging from mild flu symptoms to severe COVID-19 pneumonia requiring mechanical ventilation and even death. According to epidemiological observations, diabetes mellitus is a major risk factor for severe outcome, next to older age, s, hypertension, and other serious chronic illnesses. Recent studies have determined that the oral cavity mucosa is the main entry portal for the SARS-CoV-2 virus into the body. The viruses accumulate in the mouth at locations where the main viral receptor is highly expressed. The oral pathway of the virus into the body and the contributing factors are described in this review. The immune system of people with diabetes is generally impaired. Diabetes induces chronic systemic inflammation, which regularly manifests as periodontitis in the oral cavity. Furthermore, frequent hyperglycemia leads to additional weakening of the mucosal immune barrier. These findings provide plausible explanations for the more frequent severe courses of respiratory viral infections in diabetes patients. An oral examination helps to identify patients at elevated risk. Activated matrix metalloproteinase-8 (aMMP-8) is an established biomarker for measuring the degree of oral inflammation and is an indicator of the destruction of collagen and bone structures in the mouth. aMMP-8 point-of-care tests are readily available. We propose that the current recommendations for the prevention of SARS-CoV-2-associated severe COVID-19 disease should be extended to consider the aspects of measuring and sanitizing oral health, as well as to include preventive regular daily disinfection of the mouth and the pharynx.

## Introduction

Diabetes mellitus is one of the most frequent global diseases and affects approximately 10-12 % of the world's population [1]. It represents a major economic burden to all countries and societies [2]. When the SARS-CoV-2 pandemic spread around the world, infected people with diabetes were found to have a twofold higher risk for severe COVID-19 pneumonia and a twofold higher mortality [3-5]. A similarly increased risk for negative outcomes was already previously seen for other respiratory viral infections [6]. At the same time, it is known that people with diabetes have a higher risk for periodontitis and that periodontitis significantly contributes to developing severe COVID-19. This article aims to elucidate and explain this deleterious three-way relationship and identify preventive measures.

## The oral-systemic link

Diabetes mellitus is considered a major risk factor for periodontitis, as susceptibility to periodontitis is increased by approximately threefold in people with diabetes [7, 8]. Secondary complications of diabetes, such as macroalbuminuria, end-stage renal disease, and cardiorenal mortality (diabetic nephropathy combined with ischemic heart disease) are increased twofold and threefold, respectively, if people with diabetes suffer from severe periodontitis as compared to diabetes subjects without periodontitis. Untreated periodontitis impairs diabetes control [9], while treatment of periodontitis is associated with clinically relevant $HbA_{1c}$ improvement [10]. Therefore, it isnecessary to pay attention to the possible oral complications already in the early stages of diabetes. The IDF (International Federation of Diabetes) [11] recommends adding an annual evaluation of the oral cavity for gum disease, including bleeding during tooth brushing, or inspection for swelling during routine diabetes care visits. In times of poor glycemic control, hyperglycemia causes connective tissue damage in the oral cavity with a decreased synthesis of gingival fibroblasts, resulting in the loss of periodontal fibers and supportive alveolar bone [12]. Hyperglycemia impairs the phagocytic activity of mononuclear and polymorphonuclear cells, leading to the development of aggressive pathogenic subgingival flora. Therefore, a periodontal infection can induce systemic inflammation, which in turn builds or reinforces chronic insulin resistance. A vicious cycle consisting of hyperglycemia, periodontitis and connective tissue breakdown, inflammation (oral and systemic), and insulin resistance develops, which is virtually impossible to control without effective simultaneous interventions againstall disorders [12]. Hence, there is solid evidence to support the existence of a two-way relationship i.e., an oral-systemic link, between diabetes and periodontitis, with diabetes increasing the risk for periodontitis and periodontal inflammation negatively affecting glycemic control [8].

## Periodontitis and Respiratory Disease

Pneumonia can be caused by infection with a bacterium, virus, fungus, or parasite. Typically, the lower respiratory tract is protected from microorganisms by the cough reflex, ciliary movement of mucosal cells, and innate immune mediators able to disperse salivary bacteria aspirated during sleep or through accidental ingestion [13]. However, impairment of these defenses due to long-term smoking, diabetes, chronic obstructive pulmonary disease or immunosuppression, intubation, or prolonged postoperative hospitalization, can lead to nosocomial pneumonia [14, 15]. Cross-sectional studies have shown that in edentulous patients, poor oral hygiene and failure to attend dental appointments increase the risk of developing pneumonia, suggesting that oral pathobionts may be a potential link between oral and pulmonary disease [16]. In hospitalized individuals suffering from pneumonia, the respiratory pathogens Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae and Haemophilus parainfluenzae have been detected [17-19], while periodontal pathogens, e. g. Poryphyromonas gingivalis, Fusobacterium nucleatum, Prevotella oralis, Campylobacter gracilis, Fusobacterium necrophorum and Aggregatibacter actinomycetemcomitans, have been identified in lung aspirates from individuals with pneumonia [20-23]. Gum disease (periodontitis), even mild to moderate forms, leads to ulceration of the gingival epithelium. It can be postulated that this exposed, ulcerated surface increases the risk of invasion by pathogens such as SARS-CoV-2 in the same way as has already been shown for Human Immunodeficiency Virus (HIV) transmission [24, 25].

A recently published long-term study examined the association between oral health and the incidence of pneumonia in 98,800 people in Taiwan over 12 years. The authors concluded that patients who received periodontal treatment had an average 31% reduced risk of pneumonia and that those who received even more intensive periodontal therapy had as much as a 66% reduced risk of pneumonia compared to the control

group. The study also found that patients with other chronic diseases, including diabetes, had a significantly increased risk of pneumonia. Diabetic patients had a 78% increased risk of developing pneumonia compared to the control group. This study demonstrates the value of oral care for the primary prevention of pneumonia through oral care, especially in people with diabetes [26]. In a systematic review from 2017, dental and/or periodontal disease was found to be a clear significant risk factor for community-acquired pneumonia with an adjusted odds ratio of 2.78 [27]. It is further known that bacterial colonization of dental plaques with respiratory problem bacteria such as Staphylococcus. aureus or Pseudomonas aeroginosa can be detected in ventilated patients [28, 29]. Regular oral care resulted in a 7-12% lower absolute risk of pneumonia in hospitalized patients and in residents of nursing homes compared to omitting this preventive measure [30], as also shown by other studies. In ventilator patients, regular oral care with chlorhexidine results in a significant 18-24% reduction in ventilator-associated pneumonia (systematic review of 28 randomized controlled trials) [31-33].

Researchers have identified angiotensin-converting enzyme II (ACE2) as the likely receptor by which SARS-CoV-2 infects human cells [34]. Recent evidence now suggests that ACE2 is highly expressed in the oral cavity and that it may be even more prevalent in the oral cavity than in the lung, which has been commonly considered the primary route of infection of SARS-CoV-2 [35, 36]. Furthermore, detectable viral concentrations are found in saliva in severe COVID-19 disease, so it can be assumed that a viral load is also present in the oral mucosa and periodontal pockets [37-39]. These findings suggest that the initial entry of the virus into the body may indeed be more likely through the oral mucosa, from where it then spreads to the rest of the body, as has been described for other viruses [40, 41]. A symptom frequently reported by COVID-19 patients, temporary loss of sense of taste can be taken as further evidence of the specific focus of the SARS-CoV-2 virus on the mouth and its mucous membranes as a portal of entry [38, 42]. It could, therefore, possibly increase the sensitivity of the polymerase chain reaction tests (PCR tests) if the pharyngeal cavity and the sulcus or the saliva-filled fold of the mouth were included in the smear.

**The oral immune barrier**

Oral pathogens can cause respiratory disease when (i) oral bacteria or respiratory pathogens are aspirated from oral reservoirs into the lower airways, (ii) salivary enzymes released during chronic periodontal disease or smoking modify the oral mucosa and lead to increased adhesion of airway pathogens and/or (iii) circulating pro-inflammatory cytokines, released as a result of periodontal inflammation, modify the oral mucosa and weaken the protective barrier [43, 44].

Salivary proteins (mucins and immune-globulins) play an important role in natural oral immunity to prevent bacterial colonization of the oral cavity. This is especially true for respiratory pathogens commonly found in the mouth, such as Staphylococcus. aureus and Pseudomonas aeruginosa. Normally, salivary mucins bind to respiratory pathogens to remove them as they swim around in planktonic form. However, when Staphylococcus aureus forms a biofilm due to poor oral hygiene, salivary defense proteins are unable to bind as well, which increases bacterial colonization [45]. Salivary mucins and amylase are produced more during periodontitis as the body reacts to the disease and tries to get rid of the bacteria [46, 47].

There are several hypotheses about how salivary enzymes promote the colonization of respiratory pathogens in the oral cavity in people with and without diabetes [47-50]:

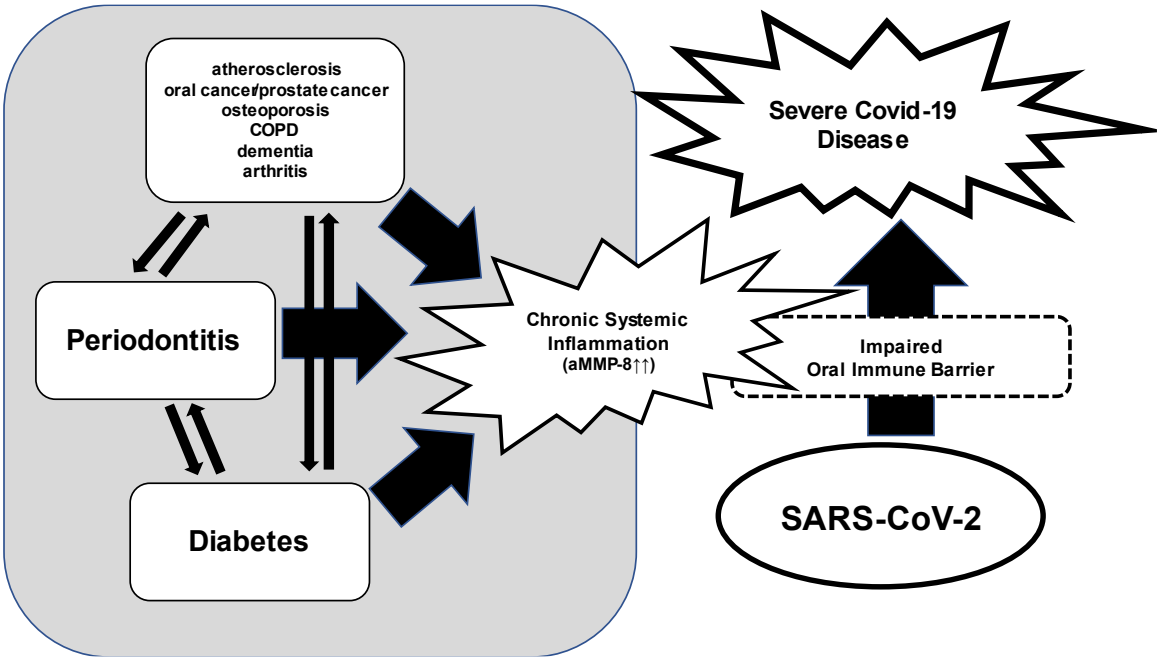
1. salivary enzymes associated with periodontal disease may alter mucosal surfaces along the airways, facilitating colonization by pathogens. Possible mechanisms of mucosal surface modification leading to increased bacterial adhesion include (a) modification of the mucosal epithelium due to high concentrations of proteolytic periodontal bacteria and their specific enzymes such as mannosidase, fucosidase, hexosaminidase, and sialidase; (b) loss of surface fibronectin, the protein that covers the mucosa, resulting in the unmasking of surface receptors; (c) removal of surface fibronectin by hydrolytic enzymes; and (d) release of cytokines.

2. hydrolytic enzymes resulting from periodontal disease can destroy salivary films, making it more difficult for bacteria to be removed and promoting the possibility of these pathogens being aspirated into the lungs.

3. inflammatory molecules and peripheral mononuclear cells present in saliva may modify the respiratory epithelium and promote colonization by respiratory pathogens [47-50].

The pathophysiological relationship between oral health and lung disease is illustrated in Figure 1.

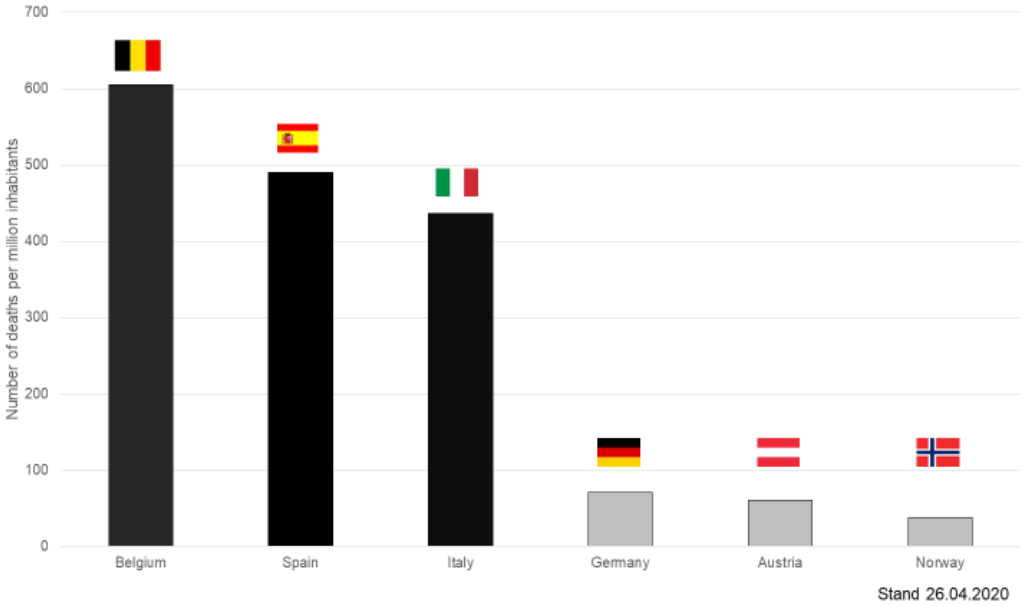
**Figure 1:** The oral-systemic link negatively impacts chronic systemic inflammation in the body and in the oral cavity and impairs the oral immune barrier. Consecutively, there is an elevated risk that a SARS-CoV-2 infection leads to severe COVID-19 disease and increased mortality. Elevated aMMP-8 levels (>20 ng/mL) in the gingival crevicular fluid or in saliva indicate the ongoing destructive periodontitis process leading to this situation.




**Epidemiological considerations**

Thus, patients with pre-existing gum disease, which is often accompanied by oral inflammation with a consequent reduction in the protective function of the mucosa, are currently likely to be at increased risk of COVID-19 infection, in addition to the other systemic complications that can be expected. Current population-based analyses of severe COVID-19 courses in China [51] show clear parallels with regard to age distribution to the increasing prevalence of periodontitis in comparable studies, e.g. in the USA [52]. Such high-risk patients with compromised oral mucosa are usually unaware of their increased risk, as the oral symptoms of chronic inflammation are often subclinical. Another indication can be found in the COVID-19 mortality statistics at the beginning of the pandemic. European countries without regular and government-supported oral hygiene consultations and treatments (such as Belgium, Italy and Spain) had significantly higher death rates compared to countries with better-established oral hygiene programs, but with otherwise comparable infrastructure and standard of living (such as Germany, Austria or Norway, Fig 2) [7].

**Figure 2:** COVID-19 mortality at the beginning of the SARS-Cov-2 pandemic (end of April 2020) in countries with a lower level of governmentally supported dental care (black) and countries with intensive and regular dental care efforts.




The multimorbid patient with periodontitis as a comorbidity has a frighteningly high risk of pneumonia even without other underlying respiratory diseases (e.g. COPD). This is due to the large surface area of the ulcerated periodontal pocket, which poses a "triple" risk for virus penetration, which is often neglected. This exposed ulcerated surface area in periodontitis has been estimated to be about 44 $cm^2$ (= half the palm of an adult hand) [45]. In addition, some periodontopathogenic bacteria associated with periodontitis, including Porphyromonas gingivalis, are able to further affect the integrity of the mucosa by blocking the natural immune response of the oral epithelium and epithelial barrier function (e.g., tight junctions and adherens junctions) [53]. The dentist should, therefore, advise patients to undergo regular oral examinations and dental hygiene treatments [54, 55].

**Diagnostic Aspects**

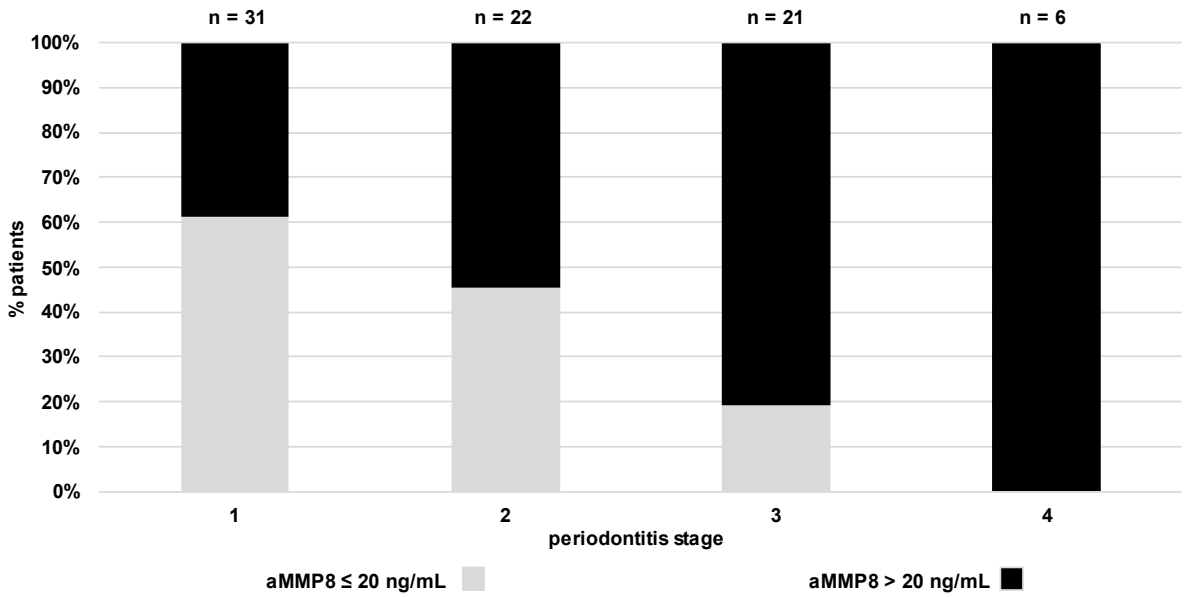
To identify people with and without diabetes who are at elevated risk for severe COVID-19 disease due to the presence of periodontitis, it is of uppermost importance to employ regular and valid screening measures for the detection of gum disease. Traditionally, the presence and severity of periodontitis are assessed by clinical indices and radiographic parameters, which primarily reflect periodontal tissue destruction. [56]. A new case definition of periodontitis combines the traditional approach with serum and oral fluid biomarkers to improve diagnostic accuracy in the early detection of the risk of periodontal breakdown [57]. The new periodontitis classification system includes grading parameters to assess the future risk of periodontitis progression. They are associated with periodontitis and increase the likelihood of future periodontal breakdown, but they cannot be considered to be reliable enough to indicate when periodontitis is in its active phase. This applies to even the grade modifiers smoking and diabetes, which are well-known risk factors for periodontitis [58-60]. The current grading parameters are mainly able to predict that it is likely that the periodontal breakdown will occur in the future, but not the exact time when it is occurring. A noninvasive point-of-care assay for an inflammatory biomarker (active matrix metalloproteinase-8; aMMP-8) has proven to be an invaluable adjunctive measure to periodontal pocketing of the index teeth and to increase the probability of detecting periodontal attachment tissue loss in a recent study [61-63].

Activated matrixmetalloproteinase-8 (aMMP-8, collagenase 2) is a collagenolytic enzyme, which induces the destruction and digestion of the most dominant (type I) collagen in the periodontal tissue [64-66]. Elevated aMMP-8 levels have been observed in many chronic systemic diseases, such as atherosclerosis, diabetes mellitus, inflammatory bowel disease, asthma, and oral

cancer [64, 66]. The enzyme plays a major role in both physiological collagen turnover activity and pathologic tissue destruction [65, 67-70]. It exists in both inactive and active form, and the active form has been demonstrated to have a strong association with periodontal status and better diagnostic accuracy for detection of periodontal disease [61, 65, 71]. An oral rinse Point-of-care (POC) immunoassay detecting aMMP-8 has been developed and has been used to evaluate the importance of aMMP-8 in detecting the progression of periodontal disease and attachment loss by numerous prospective studies in several countries [61, 62, 65, 70-77]. In a most recent investigation, this lateral-flow-based oral mouth rinse test was employed in addition to the new periodontitis staging for the identification of active periodontal disease in COVID-19 patients as well as individuals spared from the pandemic. Compromised periodontal status, as indicated by elevated aMMP-8 levels, significantly correlated with admission to intensive care units among patients with COVID-19 [78, 79]. Furthermore, patients with severe periodontitis defined by elevated aMMP-8 levels had a much higher risk for hospital admission and need for ventilation, developing COVID-19 pneumonia, and mortality. These findings strongly support the aMMP-8 point-of-care test as a screening tool for periodontitis in COVID-19 patients.

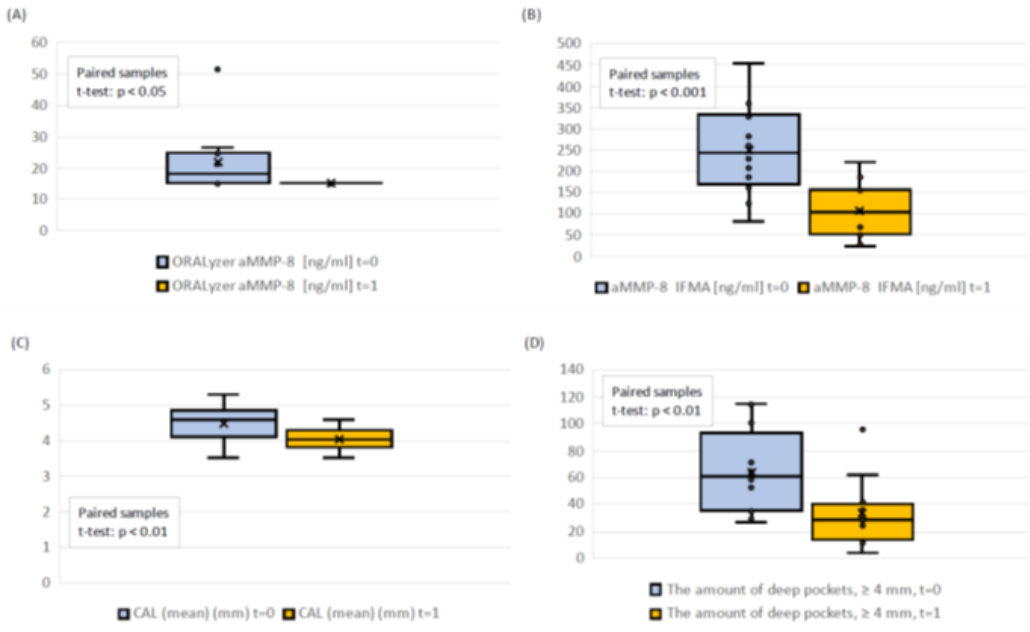
The aMMP-8 point-of-care test can be utilized to to quantitatively screen patients in a timely fashion [65-70]. In a most recent investigation in Germany, 80 healthy subjects without signs or with only very minor symptoms of periodontal disease (stages 1 and 2 according to the international classification [57]) were additionally screened for elevated aMMP-8 levels in the oral cavity. A substantial amount of 39 % in the stage 1 cohort and 55 % in the stage 2 cohort, respectively, had an active ongoing destructive periodontitis process as indicated by the biomarker (see Figure 3; Pfützner Science & Health Institute, data on file).

**Figure 3:** Prevalence of elevated aMMP-8 levels in German patients with different degrees of periodontitis as classified by the international 2018 staging [57].




For effective prevention of severe COVID-19 disease, it is therefore recommended to even screen apparently healthy individuals for the presence of periodontal inflammation. In addition, the parameter can be used to monitor treatment success. Figure 4 shows the aMMP-8 levels, clinical attachment level (CAL), and the number of deep dental pockets in 12 Finish patients prior to intervention and four weeks later. aMMP-8 was measured in parallel with a point-of-care test (Oralyzer, dentognostics GmbH, Solingen, Germany) and with a reference method (immunoflurometric assay, IFMA).

**Figure 4:** Treatment effect of anti-infective treatment/scaling and root planing (SRP) in 12 Finnish adult patients with periodontitis (A) ORALyzer (point-of-care test) aMMP-8 (ng/ml), (B) aMMP-8 immunoflourometric assay (IFMA, ng/ml), (C) clinical attachment level (CAL, mean, mm), and (D) the number of pockets with a probing depth ≥4 mm (PPD) at t0 = base level and t1 = a recall visit (4 weeks) (data on file, Department of Oral and Maxillofacial Diseases, Helsinki University)




According to a recent systematic review, aMMP-8/MMP-8 is currently the most accurate diagnostic biomarker in gingival crevicular fluid and saliva for periodontitis in systemically healthy patients [82-84]. In consideration of the above mentioned pathophysiological information related to COVID-19 entry into the body via the ACE2 receptors in the oral mucosa, it is of note that salivary levels of ACE2 increase with the severity and complexity of periodontitis and correlate positively with alveolar bone loss and salivary MMP8 [85].

## Conclusions

There is a strong correlation between diabetes mellitus, periodontitis, and a severe course of COVID-19 disease. Therefore, in addition to the typical recommendations of official health organizations, targeted oral prevention measures based on the high susceptibility of the oral cavity to SARS-CoV-2 binding appears to be particularly important. The goal of targeted oral prevention must be to reduce the individual risk of COVID-19 disease for patients with chronic oral inflammatory activity present and medically vulnerable patients with an elevated oral risk profile, such as people with diabetes mellitus. The measurement of aMMP-8 in a mouth rinse sample appears to be the most effective diagnostic tool for this purpose.

**References**


1. Lovic D, Piperidou A, Zografou I, Grassos H, Pittaras A, Manolis A. The Growing Epidemic of Diabetes Mellitus. Curr Vasc Pharmacol. 2020;18(2):104-109. doi: 10.2174/1570161117666190405165911. PMID: 30961501.
2. Bommer C, Heesemann E, Sagalova V, Manne-Goehler J, Atun R, Bärnighausen T, Vollmer S. The global economic burden of diabetes in adults aged 20-79 years: a cost-of-illness study. Lancet Diabetes Endocrinol. 2017 Jun;5(6):423-430. doi: 10.1016/S2213-8587(17)30097-9. Epub 2017 Apr 26. PMID: 28456416.
3. Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. Diabetes Metab Syndr. 2020 Jul-Aug;14(4):535-545. doi: 10.1016/j.dsx.2020.04.044. Epub 2020 May 6. PMID: 32408118; PMCID: PMC7200339
4. Miller LE, Bhattacharyya R, Miller AL. Diabetes mellitus increases the risk of hospital mortality in patients with Covid-19: Systematic review with meta-analysis. Medicine (Baltimore). 2020 Oct 2;99(40):e22439. doi: 10.1097/MD.0000000000022439. PMID: 33019426; PMCID: PMC7535849.
5. Pfützner A, Lazzara M, Jantz J. Why Do People With Diabetes Have a High Risk for Severe COVID-19 Disease?-A Dental Hypothesis and Possible Prevention Strategy. J Diabetes Sci Technol. 2020 Jul;14(4):769-771. doi: 10.1177/1932296820930287. Epub 2020 Jun 7. PMID: 32506937; PMCID: PMC7673189.
6. Li S, Wang J, Zhang B, Li X, Liu Y. Diabetes Mellitus and Cause-Specific Mortality: A Population-Based Study. Diabetes Metab J. 2019 Jun;43(3):319-341. doi: 10.4093/dmj.2018.0060. PMID: 31210036; PMCID: PMC6581547.
7. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. Periodontol 2000. 2007;44:127–153. doi: 10.1111/j.1600-0757.2006.00193.x.
8. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012 Jan;55(1):21-31. doi: 10.1007/s00125-011-2342-y. Epub 2011 Nov 6. PMID: 22057194; PMCID: PMC3228943.
9. Tsai C, Hayes C, Taylor GW. Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. Community Dent Oral Epidemiol. 2002;30:182–192. doi: 10.1034/j.1600-0528.2002.300304.x.
10. Teeuw WJ, Gerdes VEA, Loos BG. Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. Diabetes Care. 2010;33:421–427. doi: 10.2337/dc09-1378
11. Skamagas M, Breen TL, LeRoith D. Update on diabetes mellitus: Prevention, treatment, and association with oral diseases. Oral Dis 2008; 14:105–14.
12. Janket SJ, Jones JA, Meurman JH, Baird AE, Van Dyke TE. Oral infection, hyperglycemia, and endothelial dysfunction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008; 105:173–9.
13. Levison ME. Pneumonia, including necrotizing pulmonary infections (lung abscess). In Harrison's Principles of Internal Medicine, 13th edn, ed. KJ Isselbacher, E Braunwald, JD Wilson et al., pp. 1197–1206. McGraw Hill, New York, 1994
14. Sinclair DG, Evans TW. Nosocomial pneumonia in the intensive care unit. Br J Hosp Med 1994; 51:177– 180.
15. Toews GB. Nosocomial pneumonia. Am J Med Sci 1986; 291:355– 367.
16. Terpenning M, Bretz W, Lopatin D, Langmore S, Dominguez B, Loesche W. Bacterial colonization of saliva and plaque in the elderly. Clin Infect Dis 1993; 16(Suppl 4):S314– S316.
17. Scannapieco FA, Stewart EM, Mylotte JM. Colonization of dental plaque by respiratory pathogens in medical intensive care patients. Crit Care Med 1992; 20:740– 745.
18. Scannapieco FA. Pneumonia in nonambulatory patients. The role of oral bacteria and oral hygiene. J Am Dent Assoc 2006; 137(Suppl):21S–25S.
19. Russell SL, Boylan RJ, Kaslick RS, Scannapieco FA, Katz RV. Respiratory pathogen colonization of the dental plaque

of institutionalized elders. Spec Care Dentist 1999; 19:128–134.

20. Lorenz KA, Weiss PJ. Capnocytophagal pneumonia in a healthy man. West J Med 1994; 160:79– 80.
21. Zijlstra EE, Swart GR, Godfroy FJ, Degener JE. Pericarditis, pneumonia and brain abscess due to a combined Actinomyces–Actinobacillus actinomycetemcomitans infection. J Infect 1992; 25:83–87.
22. Yuan A, Yang PC, Lee LN, Chang DB, Kuo SH, Luh KT. Actinobacillus actinomycetemcomitans pneumonia with chest wall involvement and rib destruction. Chest 1992; 101:1450– 1452.
23. Shinzato T, Saito A. The Streptococcus milleri group as a cause of pulmonary infections. Clin Infect Dis 1995; 21(Suppl 3):S238– S243.
24. Wood LF, Chahroudi A, Chen HL, Jaspan HB, Sodora DL. The oral mucosa immune environment and oral transmission of HIV/SIV. Immunol. Rev. 254:10.1111/imr.12078, 2013
25. Embree JE, Njenga S, Datta P, Nagelkerke NJ, Ndinya-Achola JO, Mohammed Z, Ramdahin S, Bwayo JJ, Plummer FA. Risk factors for postnatal mother-child tranmission of HIV-1, AIDS 10:2535-2541, 2000
26. Yang LC, Suen YJ, Wang YH, Lin TC, Yu HC, Chang YC. The Association of Periodontal Treatment and Decreased Pneumonia: A Nationwide Population-Based Cohort Study. Int. J.. Environ. Res. Public Health. 17:3356, 2020 https://doi.org/10.3390/ijerph17010356
27. Almirall J, Serra-Prat M, Bolibar I, Balasso V. Risk factors for community-accquired pneumonia in adults: a systematic review of observational studies. Respiration 94:299-311, 2017
28. Sands KM, Wilson MJ, Lewis MAO, Wise MP, Palmer N, Hayes AJ, Barnes RA, Williams DW, Respiratory pathogen colonization of dental plaque, the lower airways, and endotracheal tube biofilms during mechanical ventilation. J. Critical Care, 37:45-49, 2017
29. El-Sohl AA, Pietrantoni C, Bhat A, Okada M, Zambon J, Aquilina A, Berbary E. Colonization of dental plaques. Chest 126:1575-1582, 2004
30. Sjörgren P, Nilsson E, Forsell M, Johansson O, Hoogstraate J. A Systematic Review of the Preventive Effect of Oral Hygiene on Pneumonia and Respiratory Tract Infection in Elderly People in Hospitals and Nursing Homes: Effect Estimates and Methodological Quality of Randomized Controlled Trials. J. Am. Geriatric Soc. 56:2124-2130, 2008
31. Hua F, Xie H, Worthington HV, Furness S, Zhang Q, Li C. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. Cochrane Database of Sytem. Rev. 10:CD008367, 2016
32. Scannapieco FA, Binkley CJ. Modest reduction in risk for ventilator-associated pneumonia in critically ill patients receiving mechanical ventilation following topical oral chlorhexidine. J Evid Based Dent Pract 2012; 12:15– 17.
33. Yoneyama T, Hashimoto K, Fukuda H, Ishida M, Arai H, Sekizawa K, Yamaya M, Sasaki H. Oral hygiene reduces respiratory infections in elderly bed-bound nursing home patients. Arch Gerontol Geriatr 1996; 22:11– 19.
34. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579:270-273.
35. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020; 12:8.
36. Wu C, Zheng M. Single-cell RNA expression profiling shows that ACE2, the putative receptor of COVID-2019, has significant expression in nasal and mouth tissue, and is co-expressed with TMPRSS2 and not co-expressed with SLC6A19 in the tissues. BMC Infectious Diseses (under review), https://www.researchsquare.com/article/rs-16992/v1
37. To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, Lung DC, Yip CCY, Cai JP, Chan JMC, Chik TSH, Lau DPL, Choi CYC, Chen LL, Chan WM, Chan KH, Ip JD, Ng ACK, Poon RWS, Luo CT, Cheng VCC, Chan JFW, Hung IFN, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis (published online).

https://doi.org/10.1016/S1473-3099(20)30196-1

38. Chen L, Zhao J, Peng J, Li X, Deng X, Geng Z, Shen Z, Guo F, Zhang Q, Jin Y, Wang L, Wang S. 1/ 24Detection of 2019-nCoV in Saliva and Characterization of Oral Symptoms in COVID-19 Patients. SSRN (published online) https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3557140
39. To KKW, Tsang OTY, Yip CCY, Chan KH, Wu TC, Chan JMC, Leung WS, Chik TSH, Choi CYC, Kandamby DH, Lung DC, Tam AR, Poon RWS, Fung AYF, Hung IFN, Cheng VCC, Chan JFW, Yuen KY. Consistent Detection of 2019 Novel Coronavirus in Saliva. Clin Infect Dis. (epub ahead of print, 2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108139/
40. Santosh ABR, Muddana K. Viral infections of oral cavity. J. Family Med Prim Care 9:36-42, 2020
41. Ortiz AP, Gonzalez D, Vivaldi-Olivier J, Castaneda M, Rivera V, Diaz E, Centeno H, Munoz C, Palefsky J, Joshipura K, Perez CM. Periodontitis and oral human papillomavirus infection among Hispanic adults. Papillomavirus Res. 5:128-133, 2018
42. Giacomelli A, Pezzati L, Conti F, Bernacchia D, Siano M, Oreni L, Rusconi S, Gervasoni C, Ridolfo AL, Rizzardini G, Antinori A, Galli M. Self-reported Olfactory and Taste Disorders in Patients With Severe Acute Respiratory Coronavirus 2 Infection: A Cross-sectional Study Cin. Infect. Dis. 2020. https://doi.org/10.1093/cid/ciaa330
43. Paju S, Scannapieco FA. Oral biofilms, periodontitis, and pulmonary infections. Oral Dis 2007; 13:508– 512.
44. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev 2007; 13:547– 558.
45. Heo SM, Choi KS, Kazim LA, Reddy MS, Haase EM, Scannapieco FA, Ruhl S. Host defense proteins derived from human saliva bind to staphylococcus aureus. Infection and Immunity, 81:1364-1373, 2020. https://iai.asm.org/content/iai/81/4/1364.full.pdf
46. Biesbrock AR, Reddy MS, Levine MJ. Interaction of a Salivary mucin-secretory immunoglobulin A complex with mucosal pathogens. Infection and Immunity 59:3492-3497, 1991
47. Sanchez GA, Miozza V, Delgado A, Busch L. Determination of salivary levels of mucin and amylase in chronic periodontitis patients. J. Periodontal Res. 46:221-227
48. Gomez-Filho IS, Passos JS, Seixas da Cruz S. Respiratory disease and the role of bacteria. J. Oral Microbiol 2:10.3402/jom.v2i0.5811. doi: 10.3402/jom.v2i0.5811
49. Chan HH, Rahim ZHA, Jessie K, Hashim OH, Taiyeb-Ali TB. Salivary Proteins Associated with Periodontitis in Patients with Type 2 Diabetes Mellitus. Int. J. Mol. Sci. 13:4642-4654
50. Gaeckle NT, Pragman AA, Pendleton KM, Baldomaro AK, Criner GA. The Oral-Lung Axis: The Impact of Oral Health on Lung Health. Respiratory Care (paper in print, epub ahead of print) 2020, doi: 10.4187/respcare.07332
51. Sun K, Chen J, Viboud C. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study Lancet Digital Health 2020; 2:e201-208
52. Thornton-Evans G, Eke P, Wie L, Palmer A, Moeti R, Hutchins S, Borrell LN. Periodontitis Among Adults Aged ≥30 Years — United States, 2009–2010, Morbidity and Mortality Weekly Report (MMWR) 2013, 62:129-135
53. Groeger S, Meyle J. Oral Mucosal Epithelial Cells. Front Immunol 2019;10:208. doi: 10.3389/fimmu.2019.00208
54. Chi AC, Neville BW, Krayer JW, Gonsalves WC. Oral manifestations of systemic disease. Am Fam Physician. 2010; 82:1381–8.
55. Kiran M, Arpak N, Unsal E, Erdoğan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. J Clin Periodontol. 2005; 32:266–72.
56. Kinane DF, Stathopoulou PG, Papapanou PN. (2017). Periodontal diseases. Nature Reviews. Diseases Primers, 22(3), 17038.
57. Tonetti MS, Greenwell H, Kornman KS. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of Clinical Periodontology,

45(Suppl. 20), 149–161. https://doi.org/10.1111/jcpe.12945

58. Leite FRM, Nascimento GG, Scheutz F, López R. Effect of Smoking on Periodontitis: A Systematic Review and Meta-regression. Am. J. Prev. Med. 2018;54:831–841. doi: 10.1016/j.amepre.2018.02.014.
59. Cervino G, Terranova A, Briguglio F, De Stefano R, Famà F, D'Amico C, Amoroso G, Marino S, Gorassini F, Mastroieni R, et al. Diabetes: Oral Health Related Quality of Life and Oral Alterations. Biomed. Res. Int. 2019;2019:5907195. doi: 10.1155/2019/5907195.
60. Nascimento GG, Leite FRM, Vestergaard P, Scheutz F, López R. Does diabetes increase the risk of periodontitis? A systematic review and meta-regression analysis of longitudinal prospective studies. Acta Diabetol. 2018;55:653–667. doi: 10.1007/s00592-018-1120-4.
61. Gul SS, Abdulkareem AA, Sha AM, Rawlinson A. (2020). Diagnostic accuracy of oral fluids biomarker profile to determine the current and future status of periodontal and peri-implant diseases. Diagnostics (Basel), 10(10), 838.
62. Leppilahti JM, Sorsa T, Kallio MA, Tervahartiala T, Emingil G, Han B, Mäntylä P. (2015). The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site- level clinical treatment outcome. Journal of Periodontology, 86, 777–787. https://doi.org/10.1902/jop.2015.140421
63. Pussinen PJ , Paju S , Viikari J, Salminen A, Taittonen L, Laitinen T, Burgner D, Kähönen M, Lehtimäki T, Hutri-Kähönen N, Raitakari O, Juonala M. (2020). Childhood oral infections associate with adulthood metabolic syndrome: A longitudinal cohort study. Journal of Dental Research, 99(10), 1165–1173.
64. Kuula H, Salo T, Pirilä E, Tuomainen AM, Jauhiainen M, Uitto VJ, Tjäderhane L, Pussinen PJ, Sorsa T. (2009). Local and systemic responses in matrix metalloproteinase 8-deficient mice during porphyromonas gingivalis-induced periodontitis. Infection and Immunity, 77(2), 850–859.
65. Sorsa T, Gursoy UK, Nwhator S, Hernandez M, Tervahartiala T, Leppilahti J, Gursoy M, Könönen E, Emingil G, Pussinen PJ, Mäntylä, P. (2016). Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontology 2000, 70(1), 142–163.
66. Umeizudike K, Räisänen I, Gupta S, Nwhator S, Grigoriadis A, Sakellari D, Sorsa T. Active matrix metalloproteinase-8: A potential biomarker of oral systemic link. Clin Exp Dent Res. 2022 Feb;8(1):359-365. doi: 10.1002/cre2.516. Epub 2021 Nov 19. PMID: 34800007; PMCID: PMC8874056.
67. Herr AE, Hatch AV, Throckmorton DJ, Tran HM, Brennan JS, Giannobile WV, Singh A. (2007). Microfluidic immunoassays as rapid saliva-based clinical diagnostics. Proceedings of the National Academy of Sciences of the United States of America, 104(13), 5268–5273.
68. Nwhator SO, Ayanbadejo PO, Umeizudike KA, Opeodu OI, Agbelusi GA, Olamijulo JA, Arowojolu MO, Sorsa T, Babajide BS, Opedun, D. O. (2014). Clinical correlates of a lateral-flow immunoassay oral risk indicator. Journal of Periodontology, 85(1), 188–194.
69. Sorsa T, Suomalainen K, Uitto, V. J. (1990). The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal diseases. Archives of Oral Biology, suppl 35, S193–S196.
70. Sorsa T, Alassiri S, Grigoriadis A, Räisänen IT, Pärnänen P, Nwhator SO, Gieselmann DR, Sakellari, D. (2020). Active MMP-8 (aMMP-8) as a grading and staging biomarker in the periodontitis classification. Diagnostics, 10, 61. https://doi.org/10.3390/diagnostics10020061
71. Lee W, Aitken S, Sodek J, McCulloch CA. (1995). Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: Role of active enzyme in human periodontitis. Journal of Periodontal Research, 30, 23–33. https://doi.org/10.1111/j.1600-0765.1995.tb01249.x
72. Alassiri S, Pärnänen P, Rathnayake N, Johannsen G, Heikkinen A-M , Lazzara R, van der Schoor P, van der Schoor JG, Tervahartiala T, Gieselmann DR, Sorsa T. (2018). The ability of quantitative, specific, and sensitive point-of-care/chair-side oral

fluid immunotests for aMMP-8 to detect periodontal and peri-implant diseases. Disease Markers, 2018, 1306396.

73. Keskin M, Lähteenmäki H, Rathnayake N, Räisänen IT, Tervahartiala T, Pärnänen P, Şenışık AM, Karaçetin D, Yentek Balkanay A, Heikkilä P, Hagström J, Rautava J, Haglund C, Gursoy UK, Silbereisen A, Bostanci N, Sorsa T. (2020). Active matrix metalloproteinase-8 and interleukin-6 detect periodontal degeneration caused by radiotherapy of head and neck cancer: a pilot study. Expert Review of Proteomics, 17, 777–784. https://doi.org/10.1080/14789450.2020.1858056

74. Gupta S, Sahni V, Räisänen IT, Grigoriadis A, Sakellari D, Gieselmann DR, Sorsa T. (2021). Linking oral microbial proteolysis to aMMP-8 PoC diagnostics along with the stage and grade of periodontitis: A cross-sectional study. Oral Diseases. https://doi.org/10.1111/odi.14008

75. Mancini S, Romanelli R, Laschinger CA, Overall CM, Sodek J, McCulloch C. A. (1999). Assessment of a novel screening test for neutrophil collagenase activity in the diagnosis of periodontal diseases. Journal of Periodontology, 70, 1292–1302. https://doi.org/10.1902/jop.1999.70.11.1292

76. Räisänen IT, Lähteenmäki H, Gupta S, Grigoriadis A, Sahni V, Sorsa T. (2021). An aMMP-8 point-of-care and questionnaire based real-time diagnostic toolkit for medical practitioners. Diagnostics, 11, 711. https://doi.org/10.3390/diagnostics11040711

77. Romanelli R, Mancini S, Laschinger C, Overall CM, Sodek J, McCulloch CA. (1999). Activation of neutrophil collagenase in periodontitis. Infection and Immunity, 67, 2319–2326. https://doi.org/10.1128/IAI.67.5.2319-2326.1999

78. Gupta S, Mohindra R, Singla M, et al. The clinical association between periodontitis and COVID-19. Clin Oral Investig 2021; 27: 1–14

79. Anand PS, Jadhav P, Kamath KP, Kumar SR, Vijayalaxmi S, Anil S. A case-control study on the association between periodontitis and coronavirus disease (COVID-19). J Periodontol 2021; published online Aug 4. https://doi.org/10.1002/JPER.21-0272.

80. Gupta S, Saarikko M, Pfützner A, Raisanen IT, Sorsa T. Compromised periodontal status could increase mortality for patients with COVID-19. The Lancet (Infect. Dis.) 22:314, 2022

81. Gupta S, Mohindra R, Singla M, Khera S, Kumar A, Rathnayake N, Sorsa T, Pfützner A, Raisanen IT, Soni RK, Kanta P, Jain A, Gauba K, Goyal K, Singh MP, Ghosh A, Kajal K, Mahajan V, Suri V, Bhalla A. Validation of a noninvasive aMMP-8 point-of care diagnostic methodology in COVID-19 patients with periodontal disease. Clin. Exp. Dent. Res. 2022; 1-4; DOI: 10.1002/cre2.58935.

82. Arias-Bujanda N., Regueira-Iglesias A., Balsa-Castro C., Nibali L., Donos N., Tomás I. Accuracy of single molecular biomarkers in gingival crevicular fluid for the diagnosis of periodontitis: A systematic review and meta-analysis. J. Clin. Periodontol. 2019;46:1166–1182. doi: 10.1111/jcpe.13188.

83. Arias-Bujanda N., Regueira-Iglesias A., Balsa-Castro C., Nibali L., Donos N., Tomás I. Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: A systematic review and meta-analysis. J. Clin. Periodontol. 2020;47:2–18. doi: 10.1111/jcpe.13202

84. Sukriti K.C., Wang X.Z., Gallagher J.E. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: Systematic review. J. Clin. Periodontol. 2019 doi: 10.1111/jcpe.13218.

85. Zhao D, Cheng T, Koohi-Moghadam M, Wu MZ, Yu SY, Ding X, Pelekos G, You KH, Jin L. Salivary ACE2 and TMPRSS2 link to periodontal status and metabolic parameters. Clin. Transt. Disc. 2022:e37, https://doi.org/10.1002/ctd2.37