SEMMELWEIS EGYETEM

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DOMOKOS ZSUZSANNA

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BEYOND THE LIMITATIONS OF CONVENTIONAL PERIODONTAL CARE: MULTIDISCIPLINARY APPROACH AND NOVEL METHODS

Ph.D. Thesis

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"Every mountain top is within reach if you just keep climbing."

Barry Finlay, mountaineer and author of Kilimanjaro and Beyond

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1. LIST OF ABBREVIATIONS

aMMP-8: active-matrix metalloproteinase-8

BOP: bleeding on probing

CAL: clinical attachment loss

CD: Crohn's diseases

CI: confidence interval

ELISA: enzyme-linked immunosorbent assay

GCF: gingival crevicular fluid

GI: gingival index

IBD: inflammatory bowel diseases

IFMA: time-resolved immunofluorometric assay

IL: interleukin

MD: mean difference

MMP-8: matrix metalloproteinase-8

OR: odds ratio

PD: periodontitis

PI: plaque index

PoC: point of care

PPD: probing pocket depth

SD: standard deviation

SE: standard error

TIMP: tissue inhibitors of MMP

tMMP-8: total-matrix metalloproteinase-8

UC: ulcerative colitis

2. STUDENT PROFILE

2.1. Vision and mission statement, specific goals

My vision is to advance interdisciplinary knowledge by exploring the association between oral health conditions and systemic diseases, with the aim of enhancing collaborative treatment approaches between dental and medical professionals.

My mission is to integrate a multidisciplinary approach into clinical practice, grounded in comprehensive scientific understanding.

My specific goals are to identify a potential risk population in periodontology, thereby contributing to the optimization of treatment strategies for patients with inflammatory bowel disease (IBD). Additionally, I aim to evaluate the reliability of a novel biomarker in periodontics, which holds promise for a significant breakthrough in the diagnosis and management of periodontitis.

2.2. Scientometrics

Number of publications:	3
Cumulative IF:	10.1
Av IF/publication:	3.36
Ranking (Sci Mago):	Q1: 3
Number of publications related to the subject of the thesis:	2
Cumulative IF:	7.3
Av IF/publication:	3.65
Ranking (Sci Mago):	Q1: 2
Number of citations on Google Scholar:	30
Number of citations on MTMT (independent):	28
H-index:	2

The detailed bibliography of the student can be found on page 74.

2.3. Future plans

I firmly believe that learning is a lifelong process, driven by continuous curiosity and the pursuit of knowledge. New research questions often arise from clinical practice. Therefore, I plan to pursue ongoing professional development, staying up-to-date with scientific advancements and promptly integrating them into patient care. By maintaining an open-minded approach in clinical practice, I aim to identify emerging questions that can be addressed through scientific inquiry.

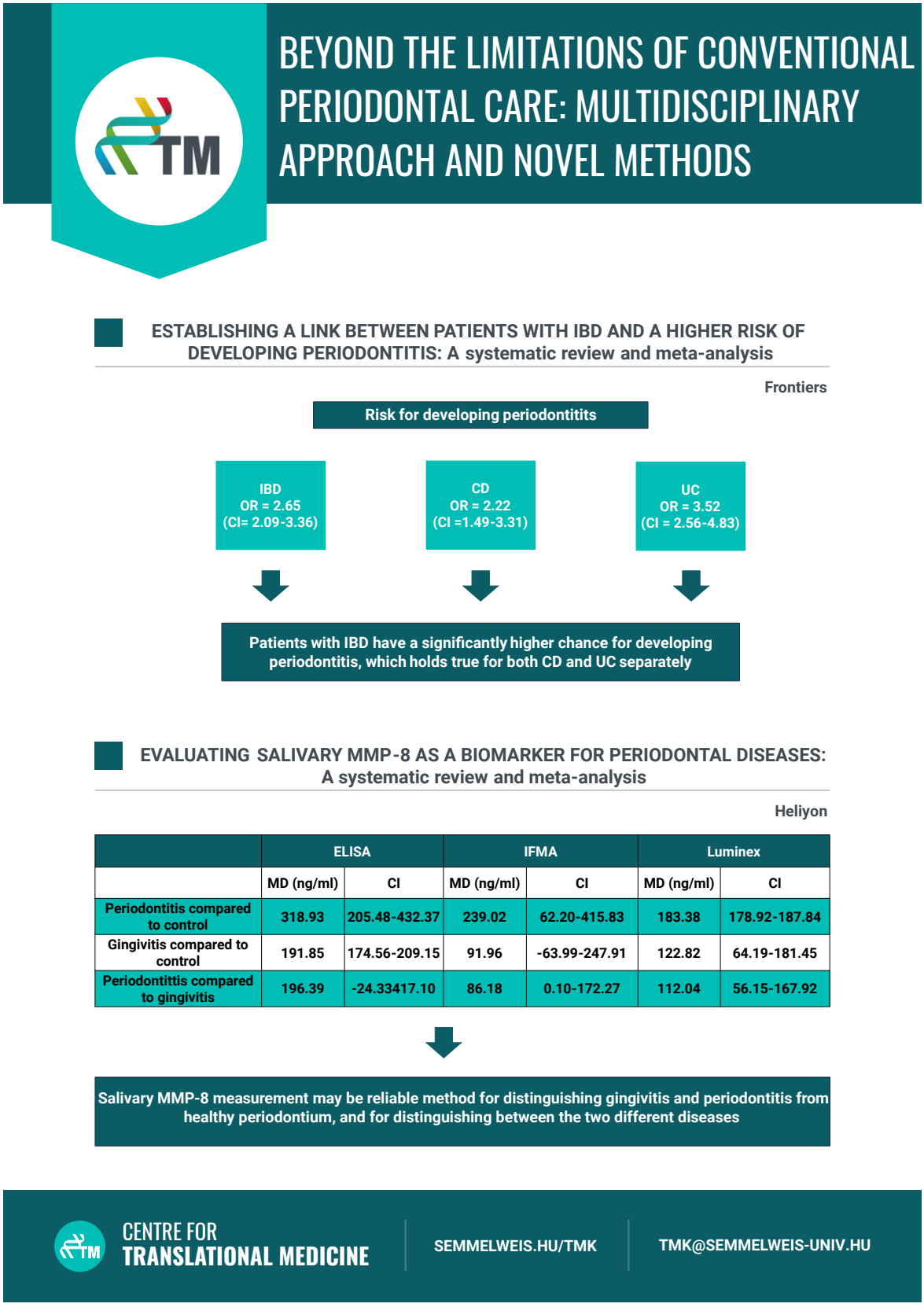
3. SUMMARY OF THE PHD

Periodontitis is a leading cause of tooth loss and edentulism among adults. It is a significant health concern affecting billions of individuals worldwide with substantial financial burdens. Beyond being a serious oral condition, it also impairs systemic health. It was linked with several systemic diseases, including cardiovascular disorders, adverse pregnancy outcomes, and diabetes mellitus. Also, recently, our research explored its potential association with IBDs. Given its irreversible damage to the tooth-supporting structures and systemic implications, the main focus of our scientific work was to investigate novel methods in periodontitis diagnostics, which could improve clinical outcomes and highlight the importance of incorporating a multidisciplinary perspective into the management and treatment of periodontitis.

Our first systematic review and meta-analysis examined the association between periodontitis and IBDs. Despite the rising prevalence of IBDs and their known extraintestinal manifestations, including oral symptoms, this relationship has received limited attention. Our analysis revealed that IBDs, including Crohn's disease (CD) and ulcerative colitis (UC), when assessed separately, are associated with periodontitis. Additionally, we explored the reverse relationship, and tendencies suggest that periodontitis may be associated with an increased risk of developing subsequent UC but not CD. These findings underline the need for interdisciplinary collaborations between dentists and gastroenterologists to enhance treatment strategies and the quality of life of affected patients.

The second study aimed to evaluate the reliability of a novel biomarker, salivary matrix metalloproteinase-8, in periodontitis diagnostics. While gingivitis remains a reversible condition affecting the periodontium, it can progress into periodontitis in susceptible individuals, making early-stage diagnostics essential. Our results showed that salivary MMP-8 levels significantly differ among patients with periodontitis, gingivitis, and a healthy periodontium, with the highest levels observed in periodontitis cases and the lowest in controls. Thus, salivary MMP-8 measurement could be a reliable tool to distinguish gingivitis and periodontitis from a healthy periodontium and differentiate between these conditions. However, further diagnostic meta-analyses are needed to establish precise cut-off values before clinical implementation.

4. GRAPHICAL ABSTRACT



5. INTRODUCTION

5.1. Overview of the topic

5.1.1. What is the topic?

The primary focus of our studies is to explore innovative methods to improve the diagnostics of periodontal diseases and to identify a potential risk population in periodontics.

5.1.2. What is the problem to solve?

Periodontitis is not solely an oral condition. The pathogenesis is characterized by altered immune-inflammatory responses, dysbiosis and it can also contribute to systemic inflammation [1]. It is known that periodontitis is associated with several systemic diseases, like diabetes mellitus, stroke, preterm birth, etc [2]. Even though the prevalence of IBDs are growing, and similar alterations to the immune-inflammatory response, and microbiome characterize their pathogenesis [3], the association between IBDs and periodontitis has not yet gained enough interest.

Furthermore, periodontitis affects 50% to 90% of the global adult population [4], and with causing irreversible damage, it is one of the leading causes of edentulism [5], thereby making it a serious health issue. Even though the development of periodontitis can be prevented. With adequate treatment, its progression and future destruction can be decelerated and halted. Therefore, the primary focus of studies should be on establishing reliable and straightforward screening methods.

5.1.3. What is the importance of the topic?

In periodontitis, the immune-inflammatory process results in the loss of periodontal tissues, which can lead to tooth loss or even edentulism [5], severely impairing chewing ability. These outcomes can contribute to additional health issues and have significant psychological consequences, potentially leading to depression [6]. Furthermore, it may adversely impact the progression and clinical outcomes of pre-existing systemic diseases or conditions.

The prevalence of IBDs are increasing, with the highest annual incidence of Crohn's disease (CD) in Europe at 12.7 per 100,000 person-years and ulcerative colitis (UC) at 24.3 per 100,000 person-years [7]. Despite advances in understanding, the current level

of knowledge indicates that IBDs remain incurable. It is of main priority that patients with life-long diseases are informed about the associated risk factors. The irreversible tissue destruction caused by periodontitis can be prevented with regular dental screenings and if proper oral hygiene is introduced in advance. Therefore, increased awareness and multidisciplinary collaboration between gastroenterologists and dentists can substantially improve the quality of life for IBD patients.

The keystone of periodontitis therapy is prevention; if prevention is not possible, regular screening and follow-up are essential [8]. It is of utmost priority to develop easy and accessible methods that both periodontists and general dentists can utilize. The feasibility of various salivary biomarkers for detecting periodontal pathology is being extensively investigated, with matrix metalloproteinase-8 (MMP-8) emerging as the most promising candidate. However, comprehensive systemic evaluations are necessary before integrating saliva MMP-8 measurement into routine practice and investing in the necessary equipment.

5.1.4. What would be the impact of our research results?

Periodontitis treatment is administered by dentists, whereas IBD treatment falls under the care of gastroenterologists. However, the bidirectional association between these conditions necessitates a multidisciplinary approach in managing IBD. Furthermore, future studies aiming to explore the reasons for this association may contribute to a deeper understanding of the pathogenesis of these diseases individually.

Conventional methods for diagnosing and monitoring periodontal disease, (visual inspection, plaque assessment, periodontal probing, radiology) [9] are time-consuming and may be inconvenient for the patient. Moreover, despite the availability of clinical and radiological diagnostic tools, diagnosing early-stage periodontal disease and monitoring its progression remains challenging [10]. Salivary MMP-8 represents a straightforward and rapid diagnostic approach that has the potential to complement or replace conventional diagnostic methods, pending systematic evaluations confirming its reliability.

5.2. Periodontitis overview

According to findings from the Global Burden of Disease Study 2019, severe periodontal disease affects more than one billion individuals globally [11]. Thus, periodontal disease is acknowledged as one of the most prevalent oral conditions worldwide.

Periodontitis is a chronic inflammatory disease characterized by progressive destruction of the tooth-supporting apparatus (periodontium), including the periodontal ligament and alveolar bone [12]. It is initiated by the accumulation of a dental plaque biofilm at and beneath the gingival margin, which progressively becomes dysbiotic [13]. This dysbiotic microbiota disrupts the host immune-inflammatory response, leading to further dysbiosis and subsequent destruction of periodontal tissues [14]. Primary characteristics of periodontitis encompass gingival inflammation, clinical attachment loss (CAL), radiographic signs of alveolar bone loss, the existence of periodontal pockets, bleeding upon probing, and tooth mobility [15].

5.3. The effect of periodontitis on systemic health

Periodontitis is a multifactorial disease. Along with dental biofilm accumulation due to poor oral hygiene, the disease development is influenced by complex interactions among specific bacterial pathogens, destructive host inflammatory and immune responses, and various environmental and systemic risk factors [16].

Several systemic diseases are known to increase susceptibility to periodontitis, including diabetes mellitus, hematological disorders, and immunodeficiencies [17, 18].

Periodontitis is not only a localized oral disease but also exerts significant adverse effects on systemic health. The dissemination of periodontal bacteria from infected pockets into the bloodstream can lead to various severe health issues [15]. The pathogenesis include the translocation of periodontal pathogens and inflammatory mediators into systemic circulation. This process can trigger or exacerbate systemic inflammatory responses, leading to a bacteremia that initiates an inflammatory cascade. Consequently, this contributes to the development and progression of various systemic diseases [19]. Research has established associations between periodontitis and a range of systemic conditions, including coronary heart disease, stroke, preterm birth, respiratory disorders, rheumatoid arthritis, neurological disorders, and malignancies [20-26].

Recent studies have further explored the potential link between periodontitis and other diseases, such as IBD.

Inflammatory bowel diseases encompass chronic inflammatory conditions of the intestines, primarily including two forms: Crohn's disease and ulcerative colitis. While CD affects the entire gastrointestinal tract, UC is limited to the rectum and colon. Endoscopically, the CD is characterized by skip lesions with transmural inflammation, whereas UC presents with continuous mucosal inflammation in the affected areas [27, 28]. Additionally, IBDs exhibit extra-intestinal manifestations. These include skin conditions like erythema nodosum and pyoderma gangrenosum, joint issues such as peripheral and axial arthropathies, hepatobiliary disorders like primary sclerosing cholangitis, and eye conditions including episcleritis and uveitis [29]. Notably, IBDs can also impact the oral cavity, leading to complications such as cobblestoning, mucosal tags, aphthous ulcerations, and pyostomatitis vegetans [30, 31].

The incidence of both CD and UC is increasing, likely due to changes in dietary habits and other factors associated with modern lifestyles [32]. The pathogenesis of these diseases is multifactorial, involving factors similar to those influencing periodontitis. Genetic predisposition, abnormal immune-inflammatory responses, and dysbiosis all contribute to their development [33]. To this end, investigating the association and common processes between these diseases could enhance our understanding of their pathogenesis, leading to improved treatment options. Identifying shared pathways in their development, such as common genetic mutations, immune-inflammatory deficiencies, and common risk factors, may provide valuable insights into both conditions.

However, while IBD is not yet curable and typically involves cycles of relapse and remission throughout life, appropriate medication can significantly reduce the frequency and severity of relapses. The exact triggers of relapses remain partly unclear, making them difficult to prevent reliably. Nevertheless, it is known that systemic inflammation, such as that caused by periodontitis, may potentially trigger a relapse [14].

Our first systematic review and meta-analyses aimed to explore the potential association between two multifactorial immune-inflammatory diseases: IBD and periodontitis. While original studies suggested a correlation between these conditions, comprehensive and consistent data originating from systematic evaluations were lacking. The growing body

of literature investigating these associations underscores the importance of considering periodontitis as a significant factor in overall systemic health. It highlights the need for integrated medical and dental care approaches to effectively manage and mitigate these interconnected health risks.

5.4. Key in disease treatment: early diagnosis

Given that periodontitis leads to irreversible destruction of the supporting apparatus of the teeth, treatment strategies should prioritize prevention methods to enhance clinical outcomes. Consequently, the development of reliable diagnostic methods capable of detecting periodontal destruction at very early stages is of paramount importance. Unlike periodontitis, which causes irreversible damage, gingivitis—the inflammatory response of the gingival tissues caused by the accumulation of bacterial biofilm due to inadequate oral hygiene—is a reversible condition [17, 34]. However, without effective oral hygiene and plaque control, gingivitis can progress to periodontitis in susceptible individuals. Therefore, early detection and treatment of gingivitis are crucial in preventing the irreversible destruction associated with periodontitis. According to the new periodontal classification, clinically healthy gingiva exhibits no color alterations or bleeding on probing [35]. Nevertheless, elevated MMP-8 levels during this stage can serve as an early biomarker of gingival inflammation.

5.5. Role of MMP-8 in periodontal destruction

MMPs are Zn^{2+} and Ca^{2+} -dependent proteolytic enzymes [36]. Based on their substrate specificity and homology, they can be classified into five groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), membrane-type MMPs (MMP- 14, -15, -16, -17) and other MMPs [37-39]. MMPs cleave the components of the extracellular matrix, playing crucial roles in numerous physiological and pathological processes. They are essential in tissue remodeling, pathological inflammatory processes, physiological development, angiogenesis, apoptosis, and malignant tissue destruction, among other processes [39, 40]. The activity of MMPs is regulated by tissue inhibitors of metalloproteinases (TIMPs). An imbalance in the ratio of TIMPs to MMPs can contribute to the progression of diseases and inflammation [41].

Periodontal disease is characterized by the breakdown of extracellular matrix proteins by proteinases [42]. Periodontitis begins with the colonization of periodontopathogenic

bacteria within the dental biofilm, which subsequently triggers an inflammatory response in the gingival tissues [1]. MMP-8 is initially released in latent forms from activated immune cells, such as neutrophils, during the inflammatory process [43, 44]. The activation of MMP-8 occurs later in the inflammatory cascade [45]. MMP-8 degrades type I collagen, the primary component of the extracellular matrix in the periodontium, leading to irreversible tissue destruction and attachment loss [46].

Several MMP variants (MMP-8, MMP-9, and MMP-13) have been linked to periodontitis. However, MMP-8 predominates, contributing to 90-95% of the collagenolytic activity observed in gingival crevicular fluid (GCF) in periodontitis cases [37-39, 47-54]. This underscores MMP-8's pivotal role as an indicator of periodontitis.

5.6. Detection and measurement methods for MMP-8

The elevation of MMP-8 levels in inflamed gingival tissue manifests in samples of gingival crevicular fluid (GCF), which is a serum-like fluid rich in proteins, enzymes, and inflammatory mediators. Nevertheless, the elevation can also be detected in mouth rinse and salivary samples [37, 47, 53, 55, 56]. The use of saliva is a non-invasive approach to fluid diagnostics, and it is increasingly utilized for diagnostic purposes across various medical disciplines [57].

MMP-8 levels can be accurately measured using laboratory-based techniques or chair-side tests.

Laboratory techniques such as time-resolved immunofluorometric assay (IFMA) and enzyme-linked immunosorbent assay (ELISA) are considered the gold standard [51, 58, 59]. ELISA and similar immunodetection methods quantify total MMP-8 (tMMP-8), encompassing both latent and active forms. In contrast, IFMA specifically detects active forms of MMP-8 (aMMP-8) from neutrophils and fibroblasts [51, 58, 60-63].

Chair-side tests are also available, offering qualitative results (positive or negative) [64] or quantitative results (numeric values) [51, 65, 66]. Besides mouthrinse-based rapid tests, measurement of salivary MMP-8 levels is emerging as a promising option in periodontology, attracting increasing interest [67, 68].

6. OBJECTIVES

6.1. Study I

Our study aimed to explore the bidirectional relationship between two multifactorial diseases, periodontitis and inflammatory bowel diseases, through a systematic review and meta-analysis. Our goal was to increase the accuracy, therefore, the current level of evidence from previous meta-analyses. Accordingly, we investigated whether patients with IBD are at increased risk of developing periodontitis, and conversely, whether individuals with periodontitis are at increased risk of developing IBD.

6.2. Study II

The objective of our systematic review and meta-analysis was to evaluate whether MMP-8 can differentiate between periodontitis, gingivitis, and periodontal health. To address this question, we conducted an analysis to determine whether significant differences exist in salivary MMP-8 levels among individuals with periodontitis, gingivitis, and those with healthy periodontium.

7. METHODS

7.1. Protocol and registration

The systematic reviews and meta-analyses were reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guideline [69]. The recommendation of the Cochrane Handbook's for Systematic Reviews of Interventions Version 6.3. were followed [70]. The protocols of the reviews were registered on PROSPERO (International Prospective Register of Systematic Reviews) in advance (Study I.: CRD42021286161; Study II CRD42022362761).

7.2. Literature search

Our systematic searches were conducted in three electronic databases: MEDLINE (via PubMed), EMBASE, and the Central Cochrane Register of Controlled Trials (CENTRAL). The exact dates of the searches, and the methods are specified in the original publications [71, 72].

7.3. Eligibility criteria

7.3.1. Study I

All studies investigating the association between IBD and periodontitis were considered eligible for Study I. In order to investigate the association between the aforementioned diseases, two questions were defined and two different PECO frameworks were formed, therefore including studies using different exposed and control groups. The prevalence of periodontitis was retrieved from studies that compared the prevalence of periodontitis in patients with IBD diagnosis compared to non-IBD patients. Furthermore, the reversed association was also investigated. Accordingly, the two separate PECO frameworks were the following: PECO 1: Studies investigating clinical periodontal outcomes and the presence of periodontitis in patients with IBD and non-IBD controls:

Population (P): human subjects, regardless of age or sex (exclusion: edentulous patients);

Exposure (E): diagnosis of IBD (including CD or UC) regardless of type of IBD, treatment for IBD, or time of IBD diagnosis;

Control (C): absence of IBD;

main Outcome (O): prevalence of periodontitis; secondary outcome: any clinical

periodontal parameters examined in the study (Probing Pocket Depth (PPD), Gingival Recession (GR), Clinical Attachment Loss (CAL), Bleeding On Probing (BOP), Plaque Index (PI), Gingival Index (GI), Community Periodontal Index of Treatment Needs (CPITN), etc.)

PECO 2: Studies providing data about the presence of IBD in patients with periodontitis and patients with healthy periodontium.

P: human subjects, regardless of age or sex;

E: diagnosis of periodontitis accompanied by the definition of the disease as given by the authors;

C: absence of periodontitis;

O: prevalence of IBD (either CD or UC).

Only English language articles were screened. Case reports, case series, in vitro studies, animal studies, review articles, posters, abstracts, letters, and editorials were excluded.

7.3.2. Study II

In eligible studies for Study II, the salivary MMP-8 levels of patients with gingivitis, periodontitis and patients with healthy periodontium were compared. Also, it was evaluted that how MMP-8 level elevation correlates with the conventionally measured clinical periodontal parameters. The PECO framework was the following:

P: adult patients (exclusion: edentolous patients)

E: periodontal diseases (gingivitis, periodontitis)

C: healthy population (healthy periodontium)

O: (a)MMP-8 levels

For the second evaluation, the Population (P), Prognostic factor (F), and Outcome (O) framework was.

P: adult patients (exclusion: edentolous patients)

F: MMP-8 level

O: any clinical periodontal outcome used in the studies (CAL, BOP, PPD etc.)

We accepted any laboratory or chair-side methods for measuring salivary MMP-8 levels. Only cohort and case-control studies published in English were considered eligible, provided they included adequate data for both case and control groups.

7.4. Study selection

Endnote X9 was used for the selection process (Clarivate Analytics, Philadelphia, PA, USA). Duplicates were removed both automatically and manually. Then, the articles were selected manually by two independent investigators in a stepwise manner based on their title, abstract, and full-text contents. Cohen's kappa coefficient was calculated to measure interrater reliability during the selection process. Disagreements were resolved by a third author.

7.5. Data collection

Data were extracted independently in a pre-defined Excel (Microsoft Corporation, Redmond, Washington, United States) data sheet by two review authors. Disagreements were solved by consensus. In the case of missing data, we contacted the corresponding author. In the case of overlapping populations, the studies with more participants were included.

7.5.1. Study I

In the case of Study I the following data were extracted: data regarding the article (first author, year of publication, DOI, study design, country, study size), data regarding participants (demographics and subject characteristics: sample size, mean age, type of IBD, extra data that might affect outcome results: e.g.: smoking habit, comorbidities, drugs used to treat IBD), and the applied definition of periodontitis.

As primary outcome the total number of patients and those with the event of interest was extracted from each study. If this not being possible, the reported OR values were utilized instead. As secondary outcomes, all data regarding periodontal screening methods (CAL, PPD, BOP, PI, GI, etc.) values were collected.

7.5.2. Study II

The following data were extracted from eligible studies for Study II: data regarding the article (first author, year of publication, DOI, study design, country, study size), data regarding participants (demographics and subject characteristics: sample size, female:

male ratio, mean age of participants, smoking habit), definition of periodontitis, gingivitis, device used for measurement.

Data regarding outcomes the level of salivary MMP-8 was extracted from each population separately. Furthermore, the correlation coefficient (r) with the statistical results between MMP-8 and conventionally measured clinical periodontal parameters was extracted.

7.6. Quality assessment

The Newcastle-Ottawa Scale was used to assess the quality of the studies included in the meta-analyses. Studies were evaluated based on (1) the selection of study groups, (2) the comparability of groups, (3) and the ascertainment of the exposure [73]. Two review authors carried out the risk of bias assessment. If discrepancies occurred, they were solved by a third author.

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework was used to evaluate the level of certainty of evidence for the outcomes examined in the meta-analyses [74]. On the basis of the assessed domains, the overall quality of evidence was classified for each outcome as high, moderate, low, or very low.

7.7. Data synthesis and analysis

7.7.1. Study I

All statistical analyses were made with R [v4.1.1] using the meta [5.0.0] package.

For continuous outcomes, the difference between the mean of the IBD and the healthy population was used for the effect size measure. To calculate the pooled difference, the sample size, mean, and corresponding standard deviation were extracted from each study.

For categorical outcomes, the odds ratio (OR) with a 95% confidence interval (CI) was used for the effect size measure. To calculate the OR, the total number of patients and those with the event of interest (in each group separately for OR) was extracted from each study. If available, the OR values were used if the patient quantity with the event of interest could not be extricated.

As we anticipated considerable between-study heterogeneity, a random-effects model was used to pool effect sizes. Pooled OR was calculated using the Mantel-Haenszel method [75-77]. The exact Mantel-Haenszel method (without continuity correction) was

used to handle zero cell counts, and the inverse variance weighting method was used to calculate the pooled mean difference [78, 79]. For more robust results, a Hartung-Knapp adjustment was applied for the calculations, where the study number was over 5 [80, 81]. To estimate the heterogeneity, the variance measure τ^2 was used. For continuous outcomes the restricted maximum-likelihood estimator was applied, and for OR measures the Paule-Mandel method [82] recommended by Veroniki et al. [83]was applied to estimate the variance with the Q profile method for the confidence interval. Additionally, between-study heterogeneity was described by means of Cochrane's Q test and Higgins & Thompson's I^2 statistics [84].

Forest plots were used to graphically summarize the results. For mean difference, the effect size measuring the confidence interval of an individual study was calculated based on the t-distribution. Where applicable, we reported the prediction intervals (i.e., the expected range of effects of future studies) of results following the recommendations of IntHout et al. [80]. Outlier and influence analyses were carried out following the recommendations of Harrer et al. [85] and Viechtbauer and Cheung [86]. For analysis with at least 10 studies, funnel plots and Egger's test with a 10% significance level were used to check for potential publication bias [87].

7.7.2. Study II

Throughout the analyses, the recommendations of Harrer, M., Cuijpers, P., Furukawa, T., & Ebert, D. were followed [85]. All statistical analyses were performed with open-source R software [88], supplemented with the packages meta [89], dmetar [90] and metafor [91]. For continuous outcomes, the difference between the mean of exposed and the control population was used for the effect size measure.

Forest plots were used to summarize the results graphically by this analysis as well. If applicable, that is, if the number of studies in a given analysis was larger than 4, then prediction intervals of results were also included on the forest plots.

Mean differences (MD) were calculated with 95% confidence intervals (CIs) to compare MMP-8 levels among patient groups using a random effect model with inverse variance weighting. The sample size, mean, and standard deviation (SD) were used for the pooled effect calculation. In those cases where the mean and the SD were not given in the articles, they were estimated using the following measures: standard error (SE), the five main quartiles (minimum, 1st quartile median, 3rd quartile and maximum), limits of the 95%

confidence intervals (CI). If the studies provided data only separately for subgroups, their results were combined. Subgroup analyses were performed using mixed-effects models to compare the different salivary MMP-8 measurement methods.

Due to the lack of data and heterogeneity, the correlation coefficient results between MMP-8 values and other clinical parameters were narratively summarized.

8. RESULTS

8.1. Study I - Investigating the association between IBD and periodontitis

8.1.1. Search and selection, characteristics of the included studies

A total of 1,715 records were initially identified by our search query, which was narrowed down to 1,411 records after removing duplicates. These 1,411 studies underwent screening based on their titles and abstracts. Subsequently, the full texts of 23 articles were screened. Additionally, a manual search of reference lists from previous review articles was conducted, resulting in five additional articles, of which two met our inclusion criteria. Ultimately, fourteen articles were included in the qualitative synthesis [92-105], eight in the quantitative synthesis [92, 94, 95, 99, 102-105], and six of these were utilized to address the main outcome for PECO 1 [92, 95, 99, 102-104]. Based on PECO 2, two eligible studies were identified [96, 98]. Some studies were excluded due to specific reasons, such as representing only CD or UC patients, rather than both conditions, or because prevalence data could not be obtained. The flowchart of the selection is shown in Figure 1. Characteristics of the identified case-control and cohort studies for our systematic review and meta-analysis are detailed in Table 1.

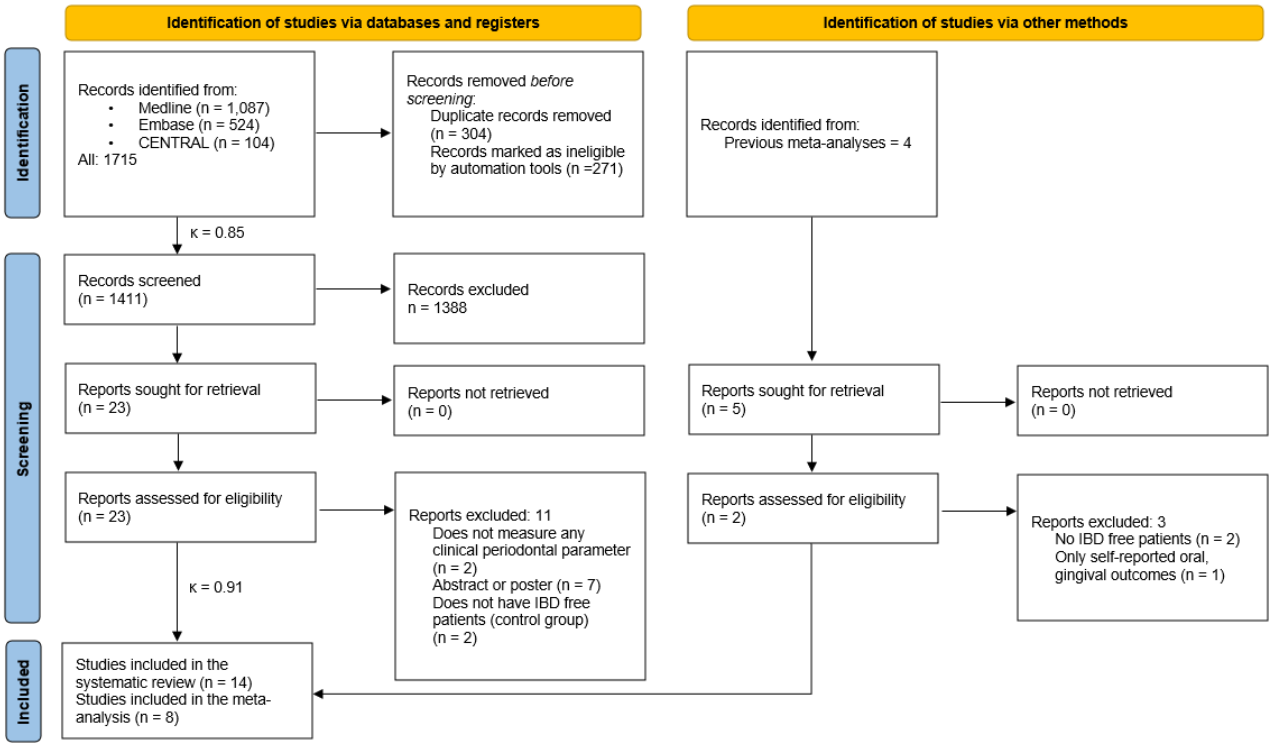


Figure 1 - PRISMA Flow Diagram of the screening and selection process for Study I

Table 1 - Main characteristics of the included studies for Study I

Study (year)	Study design	Country	Patients (mean age)	Smoking habitual (smoker; non-smoker; former smoker)	Drugs used for IBD	Comorbidities	Definition of periodontitis
Grössner-Screiber et al. 2006	case-control	Germany	IBD: 62 CD: 46 UC: 16 Control: 59	IBD: 25 (40%); 34 (55%); 3 (5%) CD: 24; x; x UC: 1; x; x Control: 24 (41%); 29 (49%); 6 (10%)	CS (n=20) IS (eg. AZA, MTX) (n=24) ASA (n=39) Anti-TNF (n=13) AB (n=12) Mono- or combined therapy	no data	Not defined
Zervou et al. 2007	case-control	Greece	IBD: 30 (40) CD: 15 UC: 15 Control: 47 (43)	no data	Mesalazine (n=22) AZA (n=2)	no data	Not defined
Brito et al. 2008	case-control	Brazil	IBD: 179 CD: 99 (39) UC: 80 (43) Control: 74 (40)	IBD: 19; 101; 59 CD: 12 (12.1%); 63 (63.6%); 24 (24.3%) UC: 7 (8.7%); 38 (47.5%); 35 (43.8%) Control: 9 (12.2%); 57 (77.0%); 8 (10.8%)	No medication (n=9) ASA (n=78) IM (n=25) ASA+IM (n=26) ASA+CS (n=17) IM+CS (n=11) ASA+IM+CS (n=13) Anti-TNF (n=10)	IBD: HT, DM, EIM Control: no data	Having CAL >=3mm in at least four sites in different teeth.

					Metronidazol (n=5) Ciprofloxacin (n=4)		
Habashneh et al. 2011	case-control	Jordan	IBD: 160 CD: 59 UC: 101 Control: 100	IBD: 48; 78; 34 CD: 31 (52.5%); 23 (39%); 5 (8.5%) UC: 17 (16.8%); 55 (54.5%); 29 (28.7%) Control: 49 (49%); 44 (44%); 7 (7%)	no data	HT	Presence of four or more teeth with one or more sites with probing pocket depth >=4mm and clinical attachment level >=3mm.
Slebioda et al. 2011	case-control	Poland	IBD: 95 CD: 70 (37,4) UC: 25 (37,2) Control: 70 (31,6)	no data	no data	no data	no data
Vavricka et al. 2013	case-control	Germany	IBD: 13 (40.6) CD: 69 (39.6) UC: 44 (42.3) Control: 113 (41.7)	IBD: 23 (20.4%); 48 (42.5%); 42 (37.2%) CD: 21 (30.4%); 25 (36.2%); 3 (4.3%) UC: 2 (4.5%); 23 (52.3%); 19 (43.2%) Control: 21 (18.6%); 71 (63.8%); 21 (18.6%)	CS (n=24) AS (n=37) Thiopurines (AZA & 6-MP; n=25) MTX (n=5) Cyclosporine, Tacrolimus (n=3, 3) Anti-TNF (IFX, ADA, CZP n=45) Probiotics (n=8)	IBD: EIM, alcohol Control: alcohol	Defined as LA-PPD >=5mm and/or BOP

					NSAID (n=3)		
Koutschristou et al. 2015	case-control	Greece	IBD: 55 (12.27) CD: 36 UC: 19 Control: 55 (all <18)	no data	AS CS Anti-TNF IM (n=28) Combination of 2 or 3 drugs (n=55)	no data	no data
Chi et al. 2018	cohort study	Taiwan	IBD: 6,657 CD: 6,657 UC: 0 Control: 26,628	no data	ASA (n=162) AZA (n=24) Other IS (n=136) CS (n=5766) Non IBD related medications Control group takes medication too	IBD: HT, HL, CHD, DM, HF, RD Control: HT, HL, CHD, DM, HF, RD	ICD-9-CM code: 523.3 or 523.4 (bi-annual checkups periodontal examination, probing of the sulcus and radiographs a depth of 3 mm or greater is considered to support a diagnosis of peri-odontal disease)
Schmidt et al. 2018	cross-sectional study, case-control	Germany	IBD: 59 (49.8) CD: 30 (49.6) UC: 39 (50.0) Control: 59 (51.3)	IBD: 0 CD: 14 (48%) UC: 0 Control: 0	AS CS Anti-TNF IM single or combination	no data	no data
Yu et al. 2018	retrospective cohort study	Taiwan	IBD: 27 (38) CD: 7	no data	no data	no data	ICD-9-CM diagnosis code (ICD-9-CM codes 523.3,

			UC: 20 Control: 108 (36,3)				523.4, and 523.5). ICD-9 procedure code 9,654, 2,431, and 2,439 were also defined
Zhang et al. 2020	case-control	China	IBD: 389 CD: 265 (29) UC: 124 (39) Control: 265 (26)	IBD: 35; 295; 59 CD: 21 (7.9%); 208 (78.5%); 36 (13.6%) UC: 14 (11.3%); 87 (70.2%); 23 (18.5%) Control: 22 (8.3%); 226 (85.3%); 17 (6.4%)	ASA (n=94) CS (n=28) IS (n=133) Biologicial therapy (n=113) Untreated (n=21)	no data	≥ 2 interproximal sites with CAL ≥3 mm, and ≥ 2 interproximal sites with PD ≥4 mm (not on the same tooth), or ≥ 1 site with PD ≥ 5 mm
Tan et al. 2021	case-control	Netherlands	IBD: 229 (51) CD: 148 UC: 80 +1 Undetermined Control: 229 (51)	IBD: 53 CD: UC: Control: 72	CS (n=36) Biologicials (n=27) IS (n=25) ASA (n=59)	IBD: DM, alcohol Control: DM, alcohol	no data

1. Equation Abbreviations: inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), corticosteroids (CS), aminosalicilate (ASA), immunosuppressants (IS), immunmodulators (IM), azathioprine (AZA), methotrexate (MTX), 6-mercaptopurine (6-MP), anti-tumor necrosis factor (anti-TNF), antibiotics (AB), infliximab (IFX), adalimumab (ADA), certolizumab pegol (CZP), nonsteroidal anti-inflammatory drugs (NSAID), hypertension (HT), diabetes mellitus (DM), extraintestinal manifestations of IBD (EIM), hyperlipidaemia (HL), coronary heart disease (CHD), heart failure (HF), renal disease (RD), clinical attachment loss (CAL), largest-periodontal probing depth (LA-PPD), bleeding on probing (BOP), international classification of diseases, ninth version, clinical modification (ICD-9-CM), pocket depth (PD)

8.1.2. Results of the quantitative analysis

8.1.2.1. Association between IBD and periodontitis

Data from six articles [92, 95, 99, 102-104], encompassing a total of 1,605 patients—898 diagnosed with IBD (either CD or UC) and 707 healthy controls—were analyzed to evaluate the association between IBD and periodontitis (PD). On average, the odds ratio (OR) (pooled effect size) of having PD was 2.65 (95% CI: 2.09-3.36), indicating a statistically significant difference between the investigated groups (Figure 2). The odds of having PD are higher in the population with IBD compared to the healthy population. The between-study heterogeneity, quantified by an I^2 value, was 0% (95% CI: 0% - 0.75%). The variance of true effects (τ^2) was 0, and the standard deviation of true effects (τ) was also 0. The prediction interval ranged from 1.87 to 3.75.

These results indicate that patients with IBD have a higher chance of developing periodontitis compared to individuals without IBD.

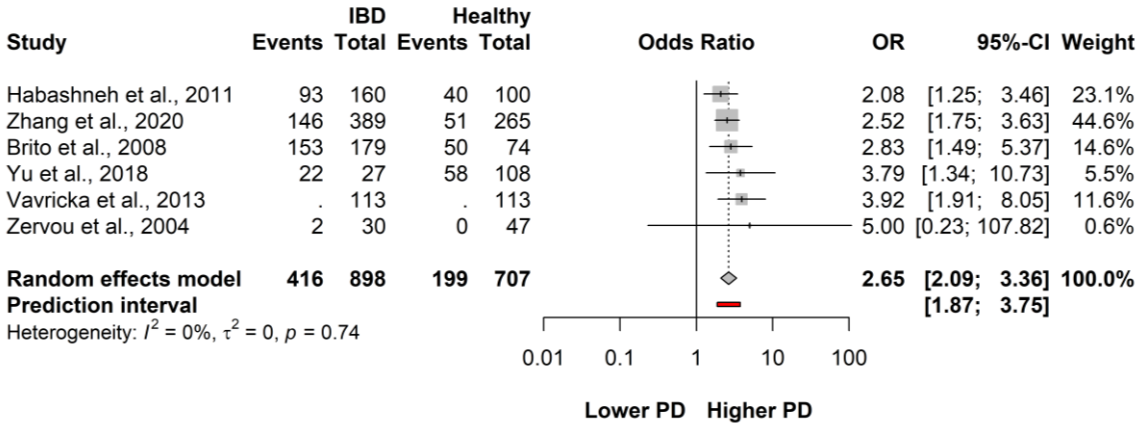


Figure 2 - Forest plot showing the odds of developing periodontitis in inflammatory bowel disease (IBD) and IBD-free group
odds ratio (OR), confidence interval (CI), and periodontitis (PD)

Through subgroup analysis, both IBD types – CD and UC patients – were evaluated separately.

8.1.2.2. Association between CD and periodontitis

From the six studies [92, 95, 99, 102-104], covering altogether 1,605 patients, 514 were

classified CD.

On average, the OR (the pooled effect size) for the presence of periodontitis (PD) was 2.22 (95% CI: 1.49-3.31). The between-study heterogeneity, indicated by an I^2 value, was 0.05% (95% CI: 0% - 0.76%). These findings lead to the conclusion that the odds of developing PD are higher in the CD population compared to the healthy population (Figure 3).

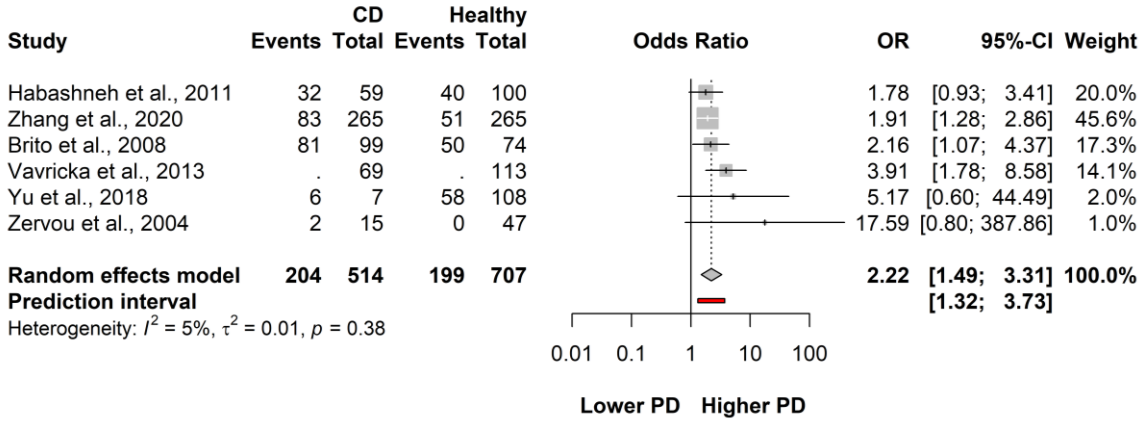


Figure 3 - Forest plot showing the association between Crohn's disease (CD) and periodontitis
odds ratio (OR), confidence interval (CI), and periodontitis (PD)

8.1.2.3. Association between UC and periodontitis

From the investigated exposed population [92, 95, 99, 102-104], 384 participants had UC diagnosis. On average, the OR (the pooled effect size) of having PD was 3.52 (CI: 2.56-4.83). The between-study heterogeneity represented by an I^2 value was 0 (95% CI: 0 - 0.75). It can be concluded that the odds of having PD in the UC population are higher than those in the healthy population (Figure 4). Notably, the OR is highest in the UC population compared to the controls.

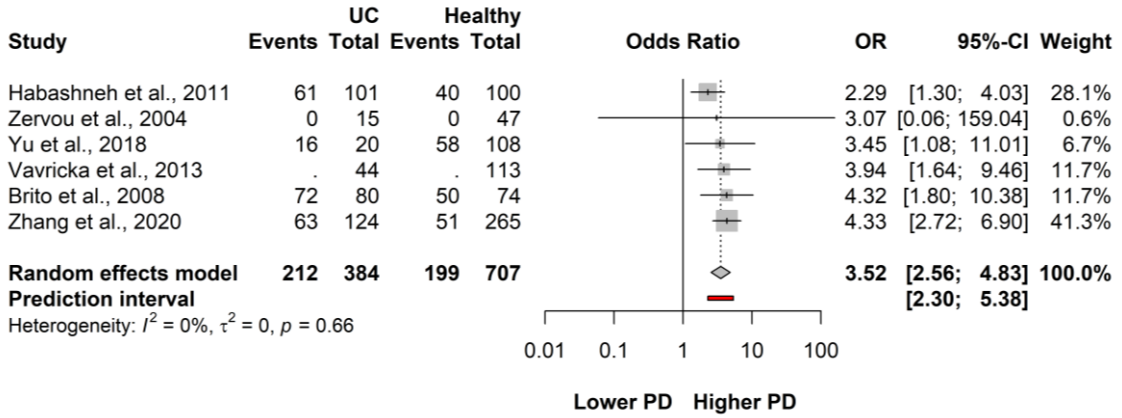


Figure 4 - Forest plot showing the association between ulcerative colitis (UC) and periodontitis. odds ratio (OR), confidence interval (CI), and periodontitis (PD)

Based on our findings, both CD and UC individually show a significant association with the development of periodontitis. Further evaluation and funnel plots of the aforementioned evaluations are detailed in the Supplementary Material of the original publication.

8.1.2.4. Risk of developing IBD in patients with periodontitis compared to patients with a healthy periodontium

Two studies met our defined PECO 2 framework [96, 98]. These studies utilized the databases of insurance companies and identified 6,646 CD and 6,108 UC patients. The control population comprises 10,085,738 healthy controls. Despite the enormous database, the results are derived only from two studies, therefore only tendencies could be exmined from the statistical evaluation as yet.

Nevertheless, both studies concluded, that periodontitis was significantly associated with the risk of subsequent UC, but not with subsequent CD (Figure 5, Figure 6). However, additional studies are required for a reliable statistical analysis, and these results should be treated cautiously.

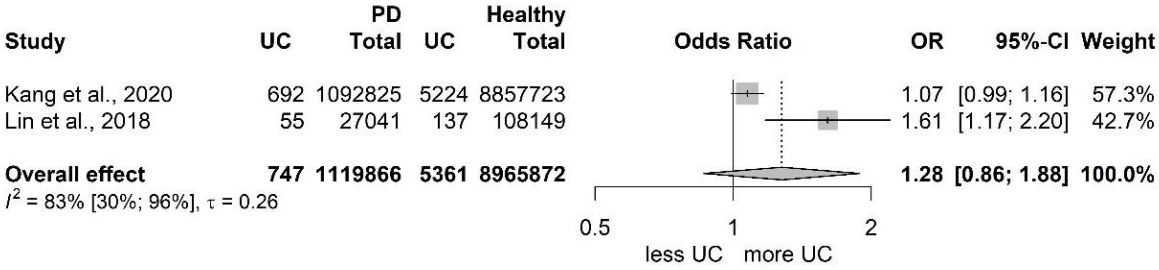


Figure 5 - Risk for developing ulcerative colitis (UC) in patients with periodontitis Periodontitis (PD), odds ratio (OR), and confidence interval (CI)

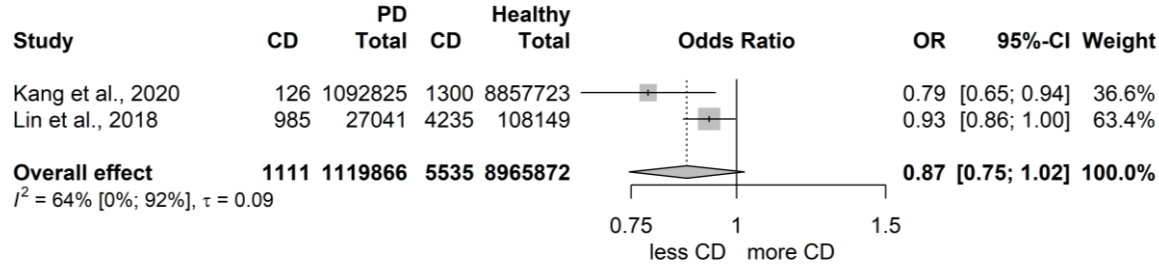


Figure 6 - Risk for developing Crohn's disease (CD) in patients with periodontitis Periodontitis (PD), odds ratio (OR), and confidence interval (CI)

8.1.3. Results of qualitative analysis, additional analyses

As secondary outcomes, we compared various clinical periodontal parameters (PPD, CAL, GI, BOP, PI, etc.) of patients with IBD compared to healthy population. The results indicated that the mean PPD was statistically significantly higher in the IBD group, compared to the IBD-free groups. Although the CAL results were also higher in the IBD group, they did not reach statistical significance. However, the substantial heterogeneity in the measurement methods across the studies and the low number of studies limited the reliability of this evaluation. Additionally, the observed differences between the exposed and control groups were clinically negligible. The detailed analysis can be seen in the

Supplementary part of the original publication.

8.1.4. Quality assessment

Based on the results of the risk of bias assessment using the Newcastle-Ottawa Scale, no study achieved less than 6 stars out of 9. Therefore, all were considered high or moderate-quality studies. The selection of controls contributed most to the bias. The formal assessment of reporting bias was not feasible due to the small number of studies. The assessment of the quality of evidence across all outcomes revealed a moderate level. The primary reason for not classifying them as high certainty was the study design, as randomized controlled studies were not available, since they were not suitable due to the study question. No additional factors were found to downgrade the quality of evidence.

Detailed results of the risk of bias assessment and quality assessment can be found in the Supplementary Materials of the publications.

8.2. Study II – Investigating the role of MMP-8 in periodontal diseases

8.2.1. Search and selection, characteristics of the included studies

The initial search query identified 4,806 records. Following the removal of duplicates, 3,104 records remained. A subsequent screening of titles and abstracts narrowed these to 110 studies for full-text review. Ultimately, 20 studies met the criteria for inclusion in the quantitative synthesis [44, 59, 61, 106-122]. The full selection process is on Figure 7.

Table 2 summarizes the basic characteristics of the included studies.

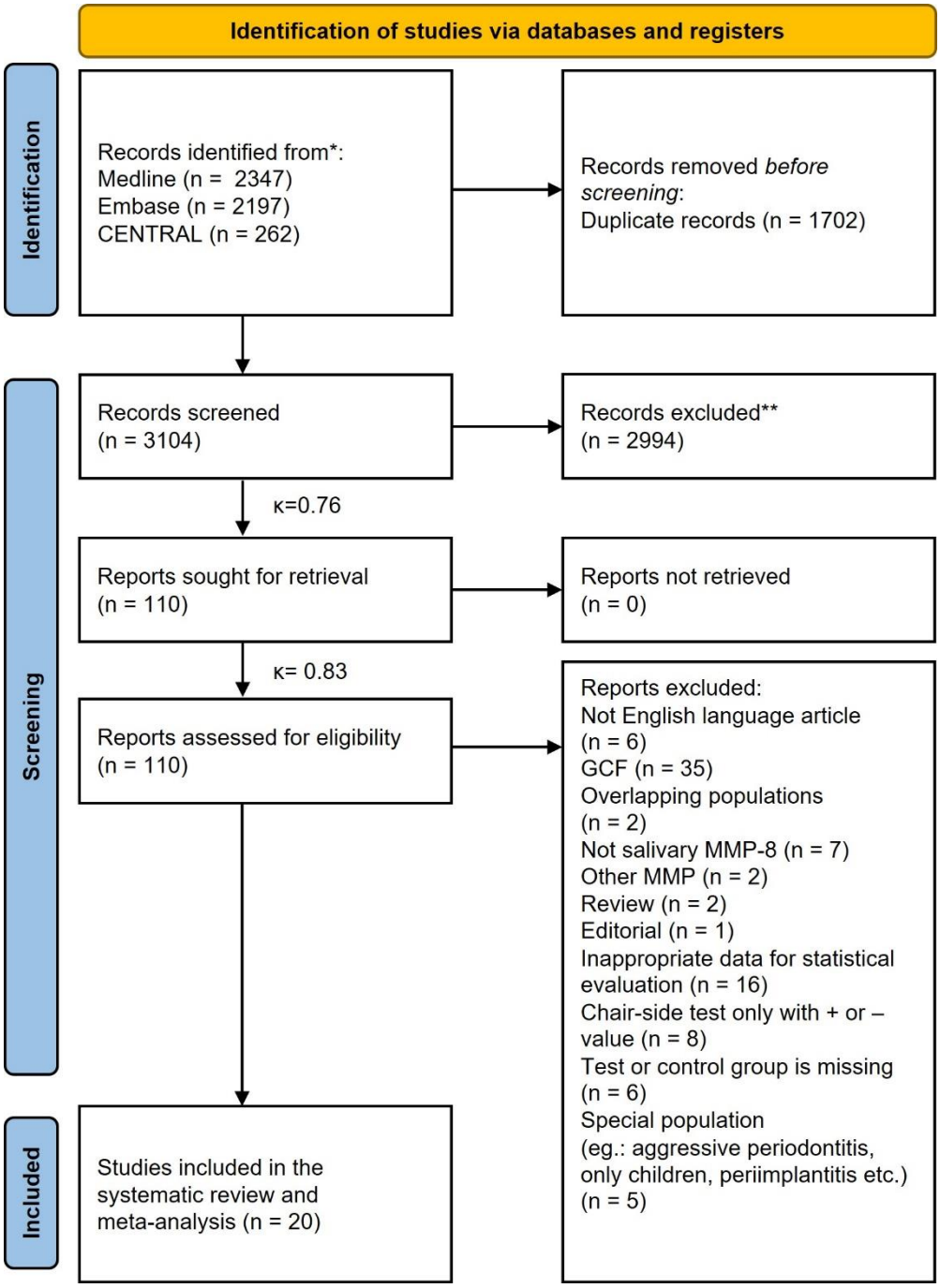


Figure 7 - PRISMA Flow Diagram of the screening and selection process for Study II

Table 2 - Main characteristics of the included studies for Study II

Study	Country	Sample size (P/G/H)	Mean age	Female:male ratio	Smokers / non-smokers	Method for diagnosing MMP-8
Akbari et al. 2013 [106]	India	100/ 100/ 50	30-39 years	ND	mixed cohort	ELISA
Bostanci et al. 2021 [107]	Turkey	60/ 31/ 36	P: 39.6±5.7 G:33.1±5.9 H:33.7±6.7	female:male - 71:56	non-smoker	ELISA
Christodoulides et al. 2007 [108]	United States of America	28/ - / 28	≥18 years of age	ND	not defined	ELISA
Ebersole et al. 2013 [110]	United States of America	50/ - / 30	P: age: 43.0±10.8 H: age: 31.4±6.8	P: female: 28% H: female: 46,7%	mixed cohort	ELISA
Gupta et al. 2015 [111]	India	40/ - / 20	age range of 35–55 years Group I: 43.30±8.64 Group II: 42.80±8.02 Group III: 44.20±7.40	Group I: 10 male: 10 female Group II: 11 male: 9 female Group III: 14 male: 6 female	mixed cohort	ELISA
Gursoy et al. 2010 [44]	Finland	84/ - / 81	periodontitis smoker: 48.6±5.3	periodontitis smoker: 52.3% men	mixed cohort	ELISA, IFMA

			periodontitis non-smoker: 50.7±4.9 control smoker: 44.4±4.3 Control non-smoker: 48.6±5.7	periodontitis non-smoker: 67.5% men control smoker: 42.9% men Control non-smoker: 33.3% men		
Lee et al. 2020 [123]	South Korea	93/ - / 28	H: 30.04±8.79 P- PS-I: 35.00±15.10 PS-II:49.21±16.92 PS-III: 58.17±14.40 PS-IV: 61.41±11.35	38 male (30.4%) and 87 female (69.6%)	mixed cohort	ELISA
Miller et al. 2006 [115]	United States of America	28/ - / 29	H: age 43.1±7.2 P: age 45.4±8.5	H: 41.4% male P: 42.9% male	mixed cohort	ELISA
Rai et al. 2008 [118]	India	20/ 18/ 15	P: 35.3±9.6 G: 36.1±9.3 H: 35.1±8.7	ND	-	ELISA
Ramseier et al. 2009 [119]	United States of America	49/ 32/ 18	Group A (healthy): 45 years, Group B (gingivitis): 42 years, Group C (mild chronic periodontitis): 53 years,	Group A (healthy): 56% (males %) Group B (gingivitis): 41%	mixed cohort	ELISA

			Group D (moderate to severe chronic periodontitis): 50 years	Group C (mild chronic periodontitis): 39% Group D (moderate to severe chronic periodontitis): 38 %		
Rangbulla et al. 2017 [120]	India	30/ - / 20	aged 18 to 45 years	ND	non-smokers	ELISA
Umeizudike et al. 2022 [121]	United Kingdom	67/ 63/ 59	40.4 ± 11.7 years (range 18–62 years)	Females (n = 118) represented 62.4% of the total study population	non-smokers	ELISA, IFMA
Zhang et al. 2021 [122]	China	31/ 24/ 25	P: 42.58±3.39 G: 26.32±4.02 H: 24.68±3.52	P: 17/14 (M/F) G: 11/13 H: 12/13	non-smokers	ELISA
Keles et al. 2020 [112]	Turkey	40/ 20/ 23	aged 25-50 years mean age: 37.16 ± 5.96 years	41 females and 42 males	non-smokers	IFMA
Mauramo et al. 2021 [114]	Switzerland	116/ - /86	Mean age: P: 48.2 (29–56) H:42.9 (25–57)	Male/Female (%/%) P:55/61 (47.4/52.6) H:27/59 (31.4/68.6)	mixed cohort	IFMA
Nizam et al. 2014 [116]	Turkey	18 (chronic	P: GCP age 50.0* (45.0–54.0)	P: GCP: 10 males and 8 females;	mixed cohort	IFMA

		periodontitis) / - / 18	H: age 44.50* (39.50–52.50)	H: 11 males and 7 females		
Noack et al. 2017 [117]	Germany	20/ 20/ 19	H: 24.3 (23.6-26.4) G: 24.6 (23.2-25.3) P: 50.3 (39.1-57.6)	H: 3 (15.8) Male (%) G: 11 (55.0) P: 12 (60.0)	mixed cohort	IFMA
Ozturk et al. 2021 [61]	Turkey	37/ 21/ 22	PSIV: 41 ± 7.8 PSIII:44.7 ± 9.6 G: 30 ± 9.0 H: 31 ± 6.4	PSIII: 11/8 (Females/Males) PSIV: 8/10 G: 11/10 H: 10/12	non-smokers	IFMA
Ebersole et al. 2015 [124]	United States of America	101/ 43/ 65	Age (years; mean ± SD) H: 28.2 ± 5.9 G:27.8 ± 4.5 P:42.0 ± 10.4	H: 60.0 (Female %) G: 48.8 P: 32.7	mixed cohort	LUMINEX
Johnson et al. 2016 [125]	United States of America	31/ - / 10	P: 44.9 ± 14.3 H: 31.5 ± 5.2	ND	mixed cohort	LUMINEX

2. Equation Abbreviations: P: periodontitis, G: gingivitis, H: Healthy population, ND: Not defined

8.2.2. Results of the quantitative analysis

8.2.2.1. MMP-8 level elevation in periodontitis

20 studies [44, 59, 61, 106-122], encompassing 1,725 participants, were selected for the statistical analysis. The level of MMP-8 was specified in ng/ml. The overall and subgroup differences of patients with healthy periodontium and patients with periodontitis are summarized on Figure 8. The different subgroups represent different laboratory measurement methods (IFMA, ELISA, LUMINEX). Two studies measured the MMP-8 level with both ELISA and IFMA [44, 121], and for the analysis the IFMA method was used. The analysis that used the ELISA methods can be seen in the Supplementary Material of the original publication.

Salivary MMP-8 levels were significantly higher in patients with periodontitis. (MD=273.26, CI: 194.42;352.10). Among the different measurement methods, MMP-8 level measures with ELISA showed the greatest difference (MD=318.92, CI: 205.48;432.37), which utilizes tMMP-8 levels. This was followed by IFMA, which measures aMMP-8 levels (MD=239.02, CI: 62.20;415.83), then by LUMINEX (MD=183.38, CI: 178.92;187.84).

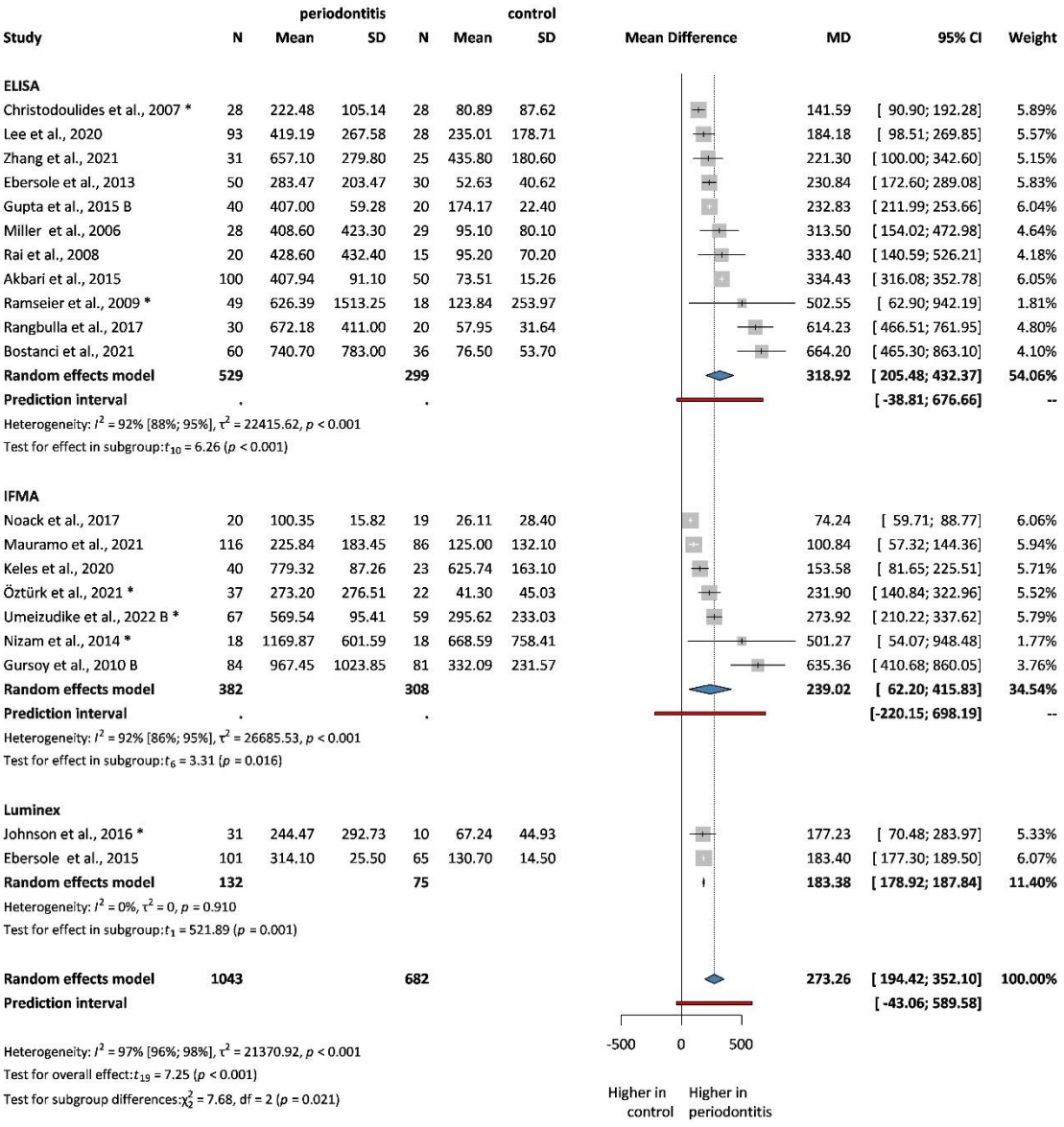


Figure 8 - Mean difference of MMP-8 level results in periodontitis patients compared to healthy population

8.2.2.2. MMP-8 level elevation in gingivitis

Our comparison of gingivitis versus healthy populations is derived from data encompassing 704 patients originating from 10 studies (Figure 9). Patients diagnosed with gingivitis presented elevated salivary MMP-8 levels compared to those in healthy

individuals (MD=122.82, CI: 64.19;181.45). The highest mean difference was measured with ELISA (tMMP-8) (MD=191.85, CI: 174.56; 209.15), followed by IFMA (aMMP-8) (MD=91.96, CI: -63.99;247.91) and Luminex (MD=68.30, CI: 58.92;77.68). While ELISA represents statistically significant difference, IFMA does not. Where studies used both ELISA, and IFMA, IFMA was represented on the evaluations, and the ELISA method can be seen in the Supplementary Material of the original publication.

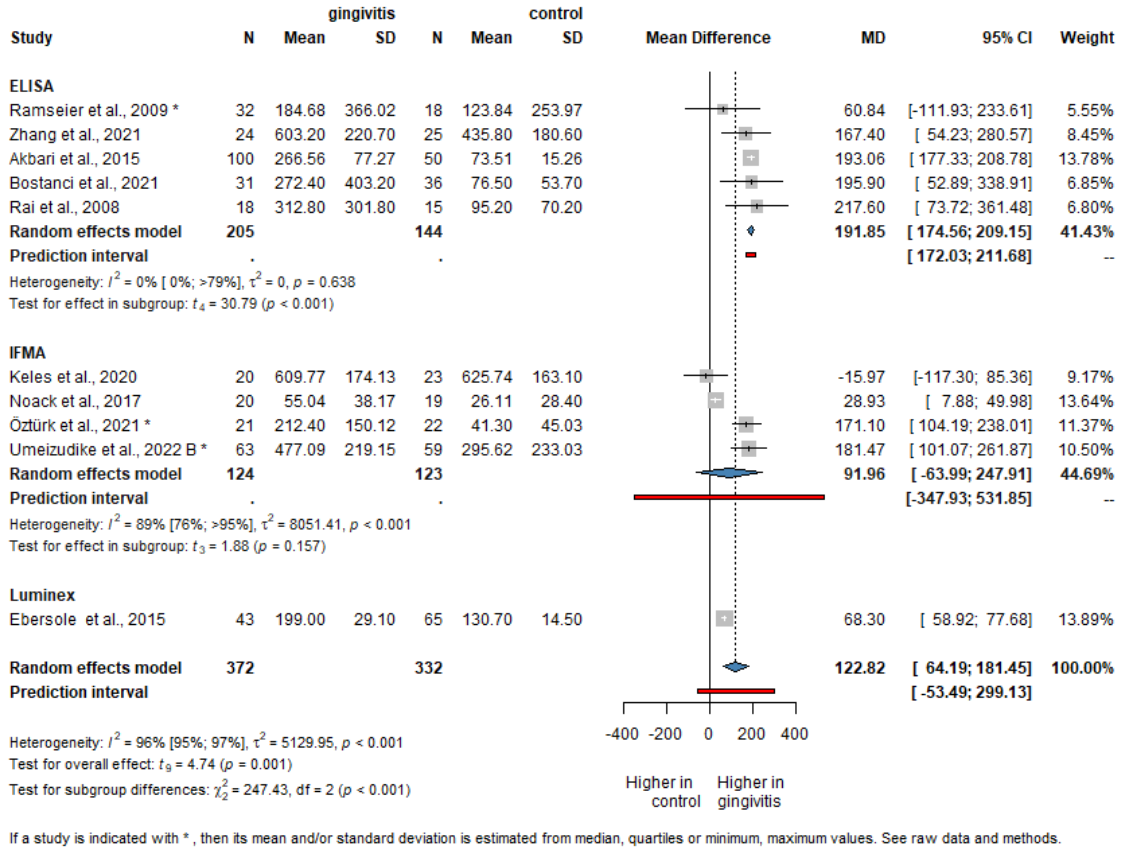


Figure 9 - Mean difference of MMP-8 level results in gingivitis patients compared to healthy population

8.2.2.3. MMP-8 level elevation in gingivitis compared to periodontitis

In addition, the salivary MMP-8 levels of the two exposed groups, gingivitis and

peridontitis cases was compared. MMP-8 level is significantly higher in the saliva of patients with periodontitis compared to patients with gingivitis. (MD=112.04, CI: 56.15;167.92) (Figure 10). The highest difference was observed by ELISA (tMMP-8) (MD=196.39, CI: -24.33;417.10). By IFMA (aMMP-8) the results were (MD=86.18, CI: 0.10;172.27), and by Luminex one study found (MD=115.10 CI:105.08;125.12). ELISA method can be seen in the Supplementary Material of the original publication.

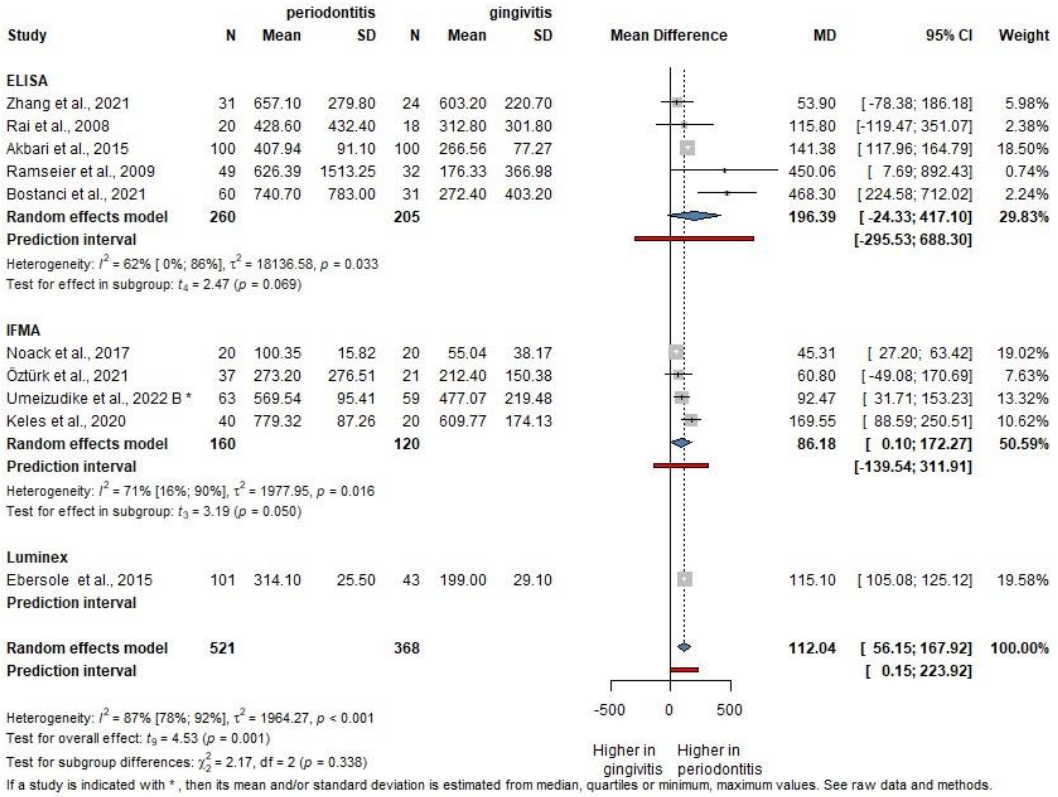


Figure 10 - Mean difference in MMP-8 level results in periodontitis population compared to gingivitis patients

8.2.3. Results of qualitative analysis, additional analyses

The correlation between clinical periodontal parameters and salivary MMP-8 level was investigated. All included studies observed a positive association between PPD and MMP-8 levels, although the strength of this association varied widely (from r = 0.319 to r = 0.95). Numerous studies investigated the relationship between CAL or BOP and

MMP-8 levels, similarly finding positive associations with broad ranges (mean CAL: from r = 0.507 to r = 0.94; BOP: from r = 0.241 to r = 0.58).

In addition to the previously mentioned parameters, the association between salivary MMP-8 and other clinical periodontal parameters was examined. The correlation results retrieved from these studies are further detailed in Table 3.

Table 3 - Correlation coeffitients reported in included studies

Study, year	**Salivary MMP-8 level correlated with x**	**Correlation type**	**Corr.**	**p value**	**Method for diagnosing MMP-8**
Noack et al. 2017	PI	Spearmann	0.651	p≤0.01	IFMA
	GI	Spearmann	0.688	p≤0.01	
	mean PPD	Spearmann	0.677	p≤0.01	
	number of sites with PPD≥ 4mm	Spearmann	0.669	p≤0.01	
	number of sites with PPD≥6mm	Spearmann	0.682	p≤0.01	
	smoking	Spearmann	0.39	p≤0.01	
Gupta et al. 2015	GI	Pearson	0.65	p<0.001	ELISA
	PI	Pearson	0.93	p<0.001	
	PPD	Pearson	0.95	p<0.001	
	CAL	Pearson	0.94	p<0.001	
Miller et al. 2006	BOP	Pearson	0.58	p=0.001	ELISA
	CAL>2mm	Pearson	0.39	p=0.003	
	Sites with PPD>4mm	Pearson	0.62	p<0.001	
	Sites with PPD>5 mm	Pearson	0.62	p<0.001	
Nizam et al. 2014	Chronic periodontitis group: PPD	Spearmann	0.61	p=0.007	IFMA
	Chronic periodontitis group: PI	Spearmann	0.634	p=0.005	
	Chronic periodontitis group: BOP	Spearmann	0.47	p=0.049	
	Healthy group: PI	Spearmann	-0.289	p=0.274	
	Healthy group: age	Spearmann	0.439	p=0.09	
Zhang et al. 2021	PPD	Spearmann	0.319	p=0.008	ELISA
	BOP	Spearmann	0.377	p=0.002	
	Age	Spearmann	0.161	p=0.187	
Gursoy et al. 2013 (same population as Gursoy 2010) [46]	MMP-9	Spearmann	0.344	p<0.001	IFMA
	number of teeth with PPD ≥4mm	Spearmann	0.359	p<0.001	
	total bone loss	Spearmann	0.192	p=0.004	
	PPD	Spearmann	0.469	p≤0.1	IFMA

Keles et al. 2020	CAL	Spearmann	0.507	p≤0.1	
	PI	Spearmann	0.382	p≤0.1	
	BOP	Spearmann	0.241	p≤0.5	
Ebersole et al. 2015	Total population: BOP%>0	Pearson	0.484	p≤0.5	Luminex
	Total population: PPD>4mm	Pearson	0.450	p≤0.5	
	Total population: PPD>5mm	Pearson	0.459	p≤0.5	
	Total population: Mean PPD	Pearson	0.463	p≤0.5	
	Periodontitis group: BOP%>0	Pearson	0.383	p≤0.5	
	Periodontitis group: PPD>4mm	Pearson	0.361	p≤0.5	
	Periodontitis group: PPD>5mm	Pearson	0.375	p≤0.5	
	Periodontitis group: Mean PPD	Pearson	0.386	p≤0.5	
	Gingivitis group: BOP%>0	Pearson	0.258		
	Gingivitis group: PPD>4mm	Pearson	-0.008		
	Gingivitis group: PPD>5mm	Pearson	-0.140		
	Gingivitis group: Mean PPD	Pearson	-0.060		
	Healthy group: BOP%>0	Pearson	0.166		
	Healthy group: PPD>4mm	Pearson	-0.125		
	Healthy group: PPD>5mm	Pearson	-0.118		
	Healthy group: Mean PPD	Pearson	0.007		

All data used for statistical evaluation and further evaluations can be found in the original publications (results and discussion) and their Supplementary Material.

8.2.4. Quality assessment

The Newcastle-Ottawa Scale was used as well to assess the risk of bias. All studies exhibited a low to moderate risk of bias, categorizing them as moderate to high quality. Egger's test and funnel plot analysis indicated that the detection of publication bias was not warranted. Similarly, the selection of controls contributed most to the bias.

The quality assessment across all three outcomes revealed a moderate level. However, the main reason for the downgrading was the lack of RCTs. Nevertheless, these study designs were not meaningful due to the study question.

Comprehensive results from the risk of bias and quality assessments are provided in the Supplementary Materials accompanying the publications.

9. DISCUSSION

9.1. **Summary of findings, international** comparisons

The object of the first systematic-review and meta-analysis was to investigate a special association between two inflammatory conditions, IBD (separately CD, UC and both) and periodontitis. Our aim was to overcome the limitations identified in previous meta-analyses and to determine if a two-way association existed, therefore, we uniquely investigated the relationship from both directions for the first time.

Our results confirmed our hypothesis, that patients with IBD have a significantly higher likelihood of developing periodontitis (OR: 2.65). This association holds true for both CD (OR: 2.22) and UC (OR: 3.52), which were analyzed separately. Therefore, it can be declared that both types of IBD are associated with an increased risk of periodontitis.

Previous meta-analyses investigating the link between IBD and periodontitis also confirmed a positive association [126-129], which was verified by our results. Compared to the first-meta-analysis in the topic [127] - conducted with the highest quality - our results indicate a slightly weaker correlation. However, our analysis included new studies that have been published since that meta-analysis. Additionally, in comparison to the most recent meta-analysis preceding our study, we found a stronger association [129].

To determine if periodontitis is a risk factor for IBD, we were able to include only two eligible studies, which prohibited to draw definitive conclusions. However, the findings suggest a tendency that periodontitis is associated with increased risk of UC, but not CD.

Although this was the first systematic evaluation to explore the association from this perspective, a subsequent meta-analysis, by Wang et al., has since been conducted examining this direction. They included only longitudinal studies with a minimum follow-up of three years duration and incorporated two additional studies published after our manuscript. Their results confirmed our observation, that periodontitis elevated the risk of UC, but not CD [130].

The reason for the difference could be due to the subtly different pathogenesis of the two forms of IBDs [131]. As an example, in the pathogenesis of CD, T helper 1 (Th1) cells predominate, and an excessive production of interferon-γ (IFN-γ) and IL-12 can be examined. In contrast, UC is characterized by the role of T helper 2 (Th2) cells, and IL-

13 elevation [132]. These observations underline the importance of investigating the immune-inflammatory pathways that play a key role in IBD pathogenesis. Moreover, some similarities could be discovered in the pathogenesis of IBDs and periodontitis, and by investigating the common pathways, the knowledge can contribute to knowing and treating the diseases separately. Among others, common immune-inflammatory pathways were identified. Both the innate and adaptive immunity is affected in the pathogenesis of both diseases, leading to dysfunctional immune-inflammatory response. The occurring imbalance of the protective and inflammatory response perpetuates the chronic inflammation. For instance, the coexistence of IBD and periodontitis was associated with higher levels of prostaglandin E2, aMMP8, IL-18 and S100A12, IL-17A and INF-γ, compared to individuals, without the presence of both diseases [133, 134]. Additionally, these diseases exhibit similar expression patterns of inflammatory cytokines [134]. It was also observed, that steroids used to treat IBD have a protective effect against periodontitis, leading to the conclusion that IBD and periodontitis might share common immune-inflammatory alterations [93].

The inflammatory response to different bacteria is critical in the pathogenesis of both diseases. Therefore, another potential link between the diseases is a similar shift in the microbiome. Microbiome alterations, known as dysbiosis, are a significant element in the pathogenesis [134]. The development of periodontitis is characterized by a shift in the subgingival microbial communities, which triggers an uncontrolled immune-inflammatory response, ultimately resulting in tissue destruction [135]. One of the principal pathogens in periodontitis is Porphyromonas gingivalis, which, when ingested, can induce changes in the gut microbiota. It increases gut epithelial permeability and contributes to endotoxemia, subsequently leading to systemic inflammation [1].

Although the specific genetic variations responsible for periodontitis and IBD have not yet been identified, genetic predisposition is present for both conditions and should be considered in their management [136, 137].

Common behavioral and environmental factors may also contribute to the observed positive association between periodontitis and IBD. Smoking, one of the most important risk factor for periodontitis, has been shown to increase the risk of CD as well as periodontitis. However, this association does not extend to UC, which may be due to the

distinct pathogenesis of the two inflammatory bowel diseases [138].

Another major risk factor for periodontitis is inadequate oral hygiene [139]. IBD can present with oral manifestations such as ulcers, aphthous stomatitis, cobblestoning, tag-like lesions, and mucogingivitis, which are often painful and can impair the patient's ability to maintain proper oral hygiene [140]. Consequently, these oral complications contribute to inadequate oral hygiene practices, which can lead to the development of periodontitis.

These several similarities underline the importance of investigating common patterns, which can contribute in further understanding their pathogenesis, therefore, improving treatment methods and patient outcomes.

The **second** systematic review and meta-analysis aimed to investigate the MMP-8 level in the saliva of patients with periodontitis, gingivitis and those with healthy periodontium. Our results indicate that salivary MMP-8 levels vary significantly among patients with periodontitis, gingivitis, and a healthy periodontium, with the highest levels observed in periodontitis cases and the lowest in healthy controls. Therefore, measuring salivary MMP-8 levels may serve as a reliable method for distinguishing gingivitis and periodontitis from a healthy periodontium, as well as for differentiating between these two conditions. However, precise cut-off values need to be determined to effectively differentiate between the study groups, which will require a future diagnostic meta-analysis.

The increasing number of publications investigating the applicability and reliability of MMP-8 level measurement, particularly in recent years, highlights the significance of the topic. Despite the substantial volume of original research, our study is the first to systematically examine salivary MMP-8 levels both in gingivitis and periodontitis, and healthy controls.

A previous meta-analysis by Zhang et al. [54] evaluated the diagnostic value of salivary MMP-8 in periodontitis, but focusing solely on periodontitis patients and not including those with gingivitis. Furthermore, their statistical methods differed slightly from ours. Nonetheless, they also found that salivary MMP-8 levels were significantly elevated in periodontitis patients. Notably, our analysis includes data from a greater number of

eligible studies, thereby enhancing the statistical power and robustness of our findings.

The number of studies utilizing the chair-side method for quantitative measurement was limited. Consequently, despite our protocol, a quantitative assessment was not feasible.

However, point-of-care (PoC) chair-side tests, based on aMMP-8 immunoassay, have demonstrated significant efficacy in periodontology. They are capable of detecting periodontal tissue destruction [59, 60, 141-148] and monitoring the stage and treatment of periodontitis. Additionally, PoC chair-side tests can be utilized to predict disease progression and CAL [51, 149-155]. They also have benefits, compared to laboratory-based techniques, which are being cheaper as not needing specially trained staff, special storage, being more accessible and quicker. Although numerous studies have investigated the efficacy of chair-side tests, yet there is insufficient data to conduct a meta-analysis assessing their reliability.

Besides diagnostic purposes, the clinical applicability of MMP-8 in periodontitis prevention and treatment follow-up was also investigated. Numerous studies have concluded that MMP-8 levels increase before the clinical signs of periodontal disease [149]. Consequently, patients can receive necessary interventions earlier, which is a primary objective in periodontitis treatment. This early intervention can prevent irreversible tissue destruction and tooth loss. Other studies have demonstrated that non-surgical periodontal therapy, which effectively reduces periodontal pocket depth, clinical attachment loss and full mouth bleeding score, also results in a decrease in MMP-8 levels [49, 156, 157]. A study observed higher levels of MMP-8, both in GCF and saliva, were associated with greater PPD and BOP [158]. Following treatment, MMP-8 levels can return to levels comparable to those observed in healthy individuals [47, 66, 106, 156, 159, 160]. This post-treatment reduction in enzyme levels suggests that MMP-8 can serve as a reliable biomarker for measuring the efficacy of periodontal therapy [37].

Furthermore, studies have demonstrated that elevated MMP-8 levels are associated with peri-implant tissue breakdown [37, 51, 60, 67, 141, 161-164]. Thus, MMP-8 measurement can be effectively implemented in the detection and treatment of peri-implantitis [164].

The clinical applicability of MMP-8 holds significant potential; however, there is still insufficient data to implement new protocols. Therefore, further research on this topic is

recommended, along with allocating funds to support such studies.

9.2. Strengths

Both systematic reviews and meta-analyses were conducted with rigorous methodology, adhering to the Cochrane Handbook and PRISMA Guidelines. Protocols were registered in advance, and subsequently followed. The risk of bias assessment revealed that none of the studies included in the statistical evaluations were of low methodological quality. Both meta-analyses concentrated on highly investigated and novel topics, as evidenced by the vast number of recent publications. In Study I we overcame the identified limitations of previous systemic investigations in the topic. Namely, this study was the first systematic evaluation of both directions of association between IBD and periodontitis simultaneously. One previous meta-analysis combined studies that investigated the association from the one direction and the opposite, thereby confusing the exposed and control groups and causing significant bias, which was separately handled by our analysis. Furthermore, we were able to incorporate recent studies that were not available in previous meta-analyses.

Study II was the first systematic analysis to evaluate not only periodontitis, but gingivitis patients' salivary MMP-8 results compared to those with healthy gums. The different laboratory methods were evaluated separately allowing for the distinction between the different isoforms of MMP-8 (activated and total), avoiding serious bias.

9.3. Limitations

A common limitation among the studies was the variation in the definitions of periodontitis and gingivitis used. This highlights the need for accurate and standardized terminology in study protocols. Additionally, in Study I, the use of various periodontal screening methods for PPD, CAL, gingival, and plaque indices prevented reliable statistical analyses. Although the studies provided data on the medication used by enrolled patients to treat IBD, the medication varied widely. Also, no subgroup analyses were carried out based on the medication type. Therefore, the potential effect of IBD drugs on periodontitis could not be excluded. Moreover, in Study I, only two studies met the PECO 2 criteria, that suggested a potential tendency for patients with periodontitis to have a higher likelihood of receiving an IBD diagnosis. However, definitive conclusions cannot yet be drawn. It should also be noted that while the analyses for PECO 1 proved a

link between IBD and periodontitis, the results are not derived from randomized controlled trials, thus a causal relationship cannot be established.

The biggest limitation of Study II was the significant heterogeneity among the included studies, which could not be substantially reduced even with subgroup analysis. The diversity observed in the study populations may be attributed to the heterogeneity. It is known that smoking has a significant influence on the periodontium, thereby potentially impacting salivary MMP-8 levels. Therefore, reliable data on salivary MMP-8 results should ideally derive from non-smoking individuals. Although are results confirmed that the salivary MMP-8 level of patients with periodontitis, gingivitis and healthy periodontium is significantly different, future diagnostic meta-analyses are necessary to define exact cut-off values that can be implemented in clinical practice. In addition, the intended assessment of chair-side salivary MMP-8 level measurement was not applicable due to the limited number of studies available.

10. CONCLUSIONS

10.1. Study I

Our systematic review and meta-analysis confirmed that patients with IBD have an increased risk of developing periodontitis, which holds true for CD and UC separately as well. Although the findings suggest a tendency for periodontitis to be associated with an increased risk of UC, but not CD, more studies are needed to establish a definitive bidirectional association. Nevertheless, patients with IBD should be regarded as a risk population in dental care.

10.2. Study II

According to our statistical evaluation, patients with periodontitis or gingivitis exhibited significantly higher salivary MMP-8 levels compared to those with a healthy periodontium. Additionally, there was a significant difference between the two affected groups, with the periodontitis group showing higher MMP-8 levels than the gingivitis group. Consequently, measuring salivary MMP-8 levels may serve as a reliable method for distinguishing between periodontal health and periodontal disease, as well as for differentiating between gingivitis and periodontitis. Nonetheless, further studies are necessary to verify its reliability, particularly concerning chair-side methods.

11. IMPLEMENTATION FOR PRACTICE

The management of IBD is predominantly a gastroenterological concern, but patients often remain unaware of their increased risk for other health issues, such as periodontitis, which exacerbate systemic inflammation [15]. Systemic inflammation can exacerbate disease severity, potentially triggering flare-ups, and reduce medication effectiveness. Therefore, preventing periodontal inflammation through regular dental check-ups and proper oral hygiene is vital. A multidisciplinary approach, involving both gastroenterologists and dentists, is essential. Gastroenterologists should refer patients for regular dental evaluations, while dentists should routinely inquire about IBD status to implement preventive periodontal measures. In addition, patient education should be emphasized. IBD patients should be informed about the importance of oral health and its impact on their systemic condition. Educating patients to maintain proper oral hygiene and the need for regular dental check-ups can empower them to take proactive steps in managing their health. Integrating dental care into IBD management can significantly improve patient outcomes and quality of life.

Salivary MMP-8 level measurement can eliminate several limitations of conventional periodontal diagnostic methods, such as being time-consuming and inconvenient, thereby enhancing patient care. Moreover, it was also examined, that salivary MMP-8 levels elevate before the signs of clinical inflammation [149], and can be reduced with non-surgical periodontal therapy [49, 156, 157]. Therefore, salivary MMP-8 level measurement has the potential to be the essential part in both the prevention and treatment of periodontitis.

12. IMPLEMENTATION FOR RESEARCH

Both periodontitis and IBD are multifactorial, and their complete pathogenesis contains some unanswered questions. They share some common immune-inflammatory processes, common behavioral and environmental factors, common bacterial changes and also genetic predisposition can contribute to their development. The numerous potential shared links between these diseases highlight the necessity for future research to investigate their association. Such studies are likely to expand our understanding of the pathology of each disease independently as well, which will contribute to better patient care.

Although laboratory-based techniques are considered the gold standard, chair-side rapid are also available and gaining attention. These tests offer a simpler, quicker, and more cost-effective alternative that does not require specially trained personnel. Therefore, it is recommended that standardized, high-quality studies be conducted on chair-side tests. This will allow for systematic evaluations to be performed prior to their implementation in routine clinical practice.

In addition to its various applications (diagnostics, prevention, therapy follow-up, peri-implant diseases), the limitations of MMP-8 measurement should be thoroughly explored as well before clinical implementation. Systemic diseases and inflammatory conditions can alter MMP-8 levels, potentially masking or amplifying results [118, 165-167]. Therefore, it is recommended to establish studies that investigate these specific patient groups, where MMP-8 levels may not provide reliable information.

13. IMPLEMENTATION FOR POLICYMAKERS

As detailed above, dentists should be integral members of the healthcare team managing IBD patients. This multidisciplinary approach should be standard practice, with all gastroenterologists being aware of the association between IBD and periodontal disease and referring their patients for periodontal screening accordingly. In addition, the costs associated with dental care, particularly periodontal therapy, can be substantial. Therefore, it is recommended that patients with IBD receive financial support for dental care, contingent upon their regular attendance at screenings.

Moreover, establishing future studies in this field, which investigate either patient outcomes or common pathological factors (such as genetics, immune-inflammatory response, and dysbiosis), is of significant importance, although these studies can be very expensive. Nevertheless, policymakers are encouraged to support research efforts that can directly enhance patient well-being.

Furthermore, the establishment of systemic databases, such as an international registry containing standardized data on oral complications associated with IBD, is of utmost importance. Such databases would be fundamental in understanding the association, drawing reliable conclusions, and facilitating the sharing of knowledge globally.

Studies have demonstrated that MMP-8 elevation can be observed in the very early stages of periodontal disease and can also assess disease progression. Therefore, MMP-8 measurement has the potential to be an effective method in periodontitis prevention. Given that periodontitis is widespread and causes serious health deficits as well as significant financial concerns in healthcare, prevention is of paramount importance. Policymakers are strongly advised to support studies focused on preventing the consequences of periodontitis, as these efforts can reduce the financial burden associated with its treatment and improve clinical outcomes for patients.

14. FUTURE PERSPECTIVES

Although meta-analyses represent a high level of evidence, they also generate new questions based on synthesized knowledge. Furthermore, meta-analyses highlight gaps in existing knowledge and help identify methodological errors in previous studies. Therefore, a deliberate, precise, and comprehensive clinical study, grounded in the results of our systematic evaluation, should be established.

My vision regarding the topic, would be implementing knowledge derived from the meta-analyses and answer emerging questions through a clinical study. Given the nature of the topic, a cohort study with a long duration would be adequate. Patients with IBD should participate in regular periodontal screenings, with their IBD status continuously monitored, including disease activity, medication, and quality of life. Participants should consistently receive the necessary periodontal treatment. Furthermore, a rapid chair-side MMP-8 test should be performed at each visit. It was shown, that MMP-8 levels increase in colorectal cancer patients [167], however, there is no data regarding IBD patients. A study utilizing this methodology could facilitate the monitoring of the impact of periodontal status on IBD and provide insights into the applicability of MMP-8 testing for IBD patients. However, ideally, future research should involve international collaborations to aggregate data from multiple IBD centers, thereby accelerating direct benefits for patients.

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16. BIBLIOGRAPHY

16.1. Publications related to thesis

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16.2. Publications not related to thesis

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Q1, IF: 2.8

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