Journal of Clinical Laboratory Analysis

WILEY

REVIEW ARTICLE OPEN ACCESS

Levels of IL-1β, MMP-8, and MMP-9 in the Saliva of Subjects With Periodontitis: A Systematic Review and Meta-Analysis

Mario Alberto Alarcón-Sánchez[1,2] | Ruth Rodríguez-Montaño[2,3] | Seyed Ali Mosaddad[4,5,6] | Artak Heboyan[7]

[1]Doctor of Science in Molecular Biology in Medicine Program, University Center of Health Sciences, University of Guadalajara (CUCS-UdeG), Guadalajara, Jalisco, Mexico | [2]Institute of Research in Dentistry, Department of Integral Dental Clinics, University Center of Health Sciences, University of Guadalajara (CUCS-UdeG), Guadalajara, Jalisco, Mexico | [3]Department of Health and Illness as an Individual and Collective Process, University Center of Tlajomulco, University of Guadalajara (CUTLAJO-UdeG), Tlajomulco de Zuñiga, Jalisco, Mexico | [4]Department of Research Analytics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India | [5]Department of Conservative Dentistry and Bucofacial Prosthesis, Faculty of Odontology, Complutense University of Madrid, Madrid, Spain | [6]Department of Prosthodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran | [7]Department of Prosthodontics, Faculty of Stomatology, Yerevan State Medical University after Mkhitar Heratsi, Yerevan, Armenia

Correspondence: Seyed Ali Mosaddad (mosaddad.sa@gmail.com) | Mario Alberto Alarcón-Sánchez (marioaasanchez@hotmail.com)

Received: 24 January 2025 | **Revised:** 23 February 2025 | **Accepted:** 12 April 2025

Funding: The authors received no specific funding for this work.

Keywords: cytokines | interleukin 1 beta | matrix metalloproteinases | periodontitis | saliva | systematic review

ABSTRACT

Background and Objective: Proinflammatory cytokines and enzymes responsible for tissue destruction are important in the development of periodontitis. This study compared salivary concentrations of interleukin-1 beta (IL-1β), matrix metalloproteinases (MMP-8), and (MMP-9) in individuals with and without periodontitis to evaluate their diagnostic utility as potential biomarkers.

Materials and Methods: A comprehensive search was performed across PubMed, Scopus, ScienceDirect, and Google Scholar, supplemented by manual searches in relevant journals up to January 2024. Eligibility criteria focused on human studies with defined diagnostic criteria for periodontitis and saliva samples analyzed for IL-1β, MMP-8, and MMP-9. Data were extracted to compare salivary levels of these markers between periodontitis patients and healthy controls. The Joanna Briggs Institute tool was used to evaluate the risk of bias and quality of the included studies. Statistical analysis employed a random effects model to calculate standardized mean differences and assess heterogeneity and publication bias.

Results: The search yielded 122 articles, with 27 meeting the inclusion criteria. Fifteen percent of these studies presented a moderate risk of bias, while the remaining 85% exhibited a low risk of bias. The meta-analyses indicated significantly higher levels of IL-1β, MMP-8, and MMP-9 in the saliva of subjects with periodontitis compared to healthy individuals: IL-1β: Standardized Mean Difference (SMD) = 163.29 (95% CI = 104.64–221.95), $p < 0.001$; MMP-8: SMD = 282.22 (95% CI = 209.68–354.77), $p < 0.001$; MMP-9: SMD = 311.85 (95% CI = 179.64–444.05), $p < 0.001$.

Conclusion: Elevated salivary levels of IL-1β, MMP-8, and MMP-9 are linked to periodontitis.

1 | Introduction

Periodontitis is a chronic, non-transmissible infectious disease resulting from complex interactions between a dysbiotic biofilm and the host's immune response, leading to the destruction of the tissues supporting the teeth [1]. Bacteria such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, collectively known as the red complex,

possess virulence factors that can cause conditions ranging from periodontitis to gingivitis [2]. It is estimated that 62% of the global adult dentate population suffers from periodontitis, with just over 23% experiencing the most severe forms of the disease [3]. Moreover, periodontitis is linked with systemic diseases like rheumatoid arthritis [4, 5], diabetes mellitus [6, 7], obesity [8], coronary artery disease [9], and oral cancer [10], making early detection crucial [11]. Periodontal probing, involving a detailed assessment of clinical parameters such as probing depth, clinical attachment level, and bleeding/suppuration on probing, along with tooth mobility and radiographic analysis, is the standard diagnostic approach used by periodontists—nevertheless, the disease's onset, progression, and monitoring present ongoing challenges [12]. Saliva, a biofluid in direct contact with teeth and periodontal tissues, is easily collectible and serves as an ideal medium for detecting periodontitis biomarkers [13].

Previous research has extensively investigated various salivary biomarkers in connection with periodontitis and other systemic diseases [14, 15]. Notably, the host's immunoinflammatory response begins with a polymicrobial challenge that involves, among other processes, exposure to lipopolysaccharide (LPS) from bacteria like *P. gingivalis*. This bacterium interacts with pattern recognition receptors such as Toll-like receptor 4/2 (TLR-4/TLR-2) on the surface of phagocytic cells (neutrophils and macrophages). This interaction triggers the nuclear factor kappa B (NF-κB) intracellular signaling pathway and leads to the production of proinflammatory cytokines, including interleukin 1-beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin 6 (IL-6) [16]. Mast cells are also capable of producing proinflammatory cytokines like IL-1β [17]. These mediators promote the expression of the nuclear factor receptor kappa B ligand (RANKL), which induces osteoclastogenesis, and they increase the levels of matrix metalloproteases (MMPs) that break down the extracellular matrix, including MMP-2, MMP-8, MMP-9, and MMP-13, which mainly target type I collagen, the most abundant in periodontal tissues [18]. IL-1β plays a significant role in immunity and inflammation [19]. The activation of pro-IL-1β by caspase-1's proteolytic cleavage leads to the activation of the NLRP3 inflammasome [20].

The degradation of extracellular matrix components, such as fibers (collagen, elastin, laminin, and fibronectin), proteoglycans, and polysaccharides, is facilitated by MMPs, which are vital for numerous physiological and pathological processes. MMPs, also known as “matrixins,” are capable of cleaving extracellular matrix (ECM) components like collagen fibers, elastin, laminin, and fibronectin, as well as proteoglycans, polysaccharides, adhesion proteins, growth factors, cytokines, and chemokines [21, 22].

The MMPs family in humans is categorized into six groups: membrane-type MMPs, collagenases, gelatinases, matrilysin, stromelysin, and other MMPs, totaling about 23 members [23]. Specifically, MMP-8 is primarily secreted by neutrophils as a proenzyme, but it can also be released by other cell types, including endothelial cells, epithelial cells, glial cells, chondrocytes, activated macrophages, and tumor myocytes [18, 24]. The activation of MMP-8 involves other host MMPs, proteases, and the increased oxidative stress induced by myeloperoxidase (MPO) produced by neutrophils. Notably, bacterial proteases from *T. denticola* and *P. gingivalis* (gingipain) can also activate MMPs [25]. MMP-8 is known to break down interstitial collagen types I, II, and III in periodontal tissue, leading to gingival recession and inversion [26].

Additionally, MMP-9 (also known as gelatinase B) breaks down various connective tissue proteins, including collagen types IV, V, and XI, as well as proteoglycans and elastin [27]. Cells such as polymorphonuclear leukocytes, macrophages, keratinocytes, fibroblasts, osteoclasts, eosinophils, and neutrophils are associated with the expression of the MMP-9 gene. Macrophages, upon detecting a pathogen, release MMP-9 [28] and are known to regulate IL-1, IL-6, and IL-8 during the initial phase of inflammation [29].

Previous research has measured IL-1β, MMP-8, and MMP-9 levels in the saliva of individuals with and without periodontitis [30–56] (Figure 1). Despite this, no meta-analyses exist that summarize these results. Thus, this study aims to compare the salivary levels of IL-1β, MMP-8, and MMP-9 in individuals with and without periodontitis to assess their diagnostic potential as biomarkers.

2 | Materials and Methods

The systematic review and meta-analyses adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [57] and were registered with the Open Science Framework (OSF) under the registration number 10.17605/OSF.IO/FBKQ9.

2.1 | Research Question

Are there alterations in IL-1β, MMP-8, and MMP-9 levels in the saliva of subjects with periodontitis compared to periodontally healthy subjects?

2.2 | PECO and Eligibility Criteria

- Population: Humans.
- Exposure: Individuals with periodontitis.
- Comparators: Systemically and periodontally healthy individuals.
- Outcomes: Salivary levels of IL-1β, MMP-8 and MMP-9.

The inclusion criteria encompassed cross-sectional studies and clinical trials involving human subjects, individuals with periodontitis defined by probing depth greater than 3 mm, clinical attachment loss greater than 2 mm, and/or evidence of bone resorption, saliva samples (either stimulated or unstimulated) used for the immunodetection of IL-1β, MMP-8, and MMP-9,

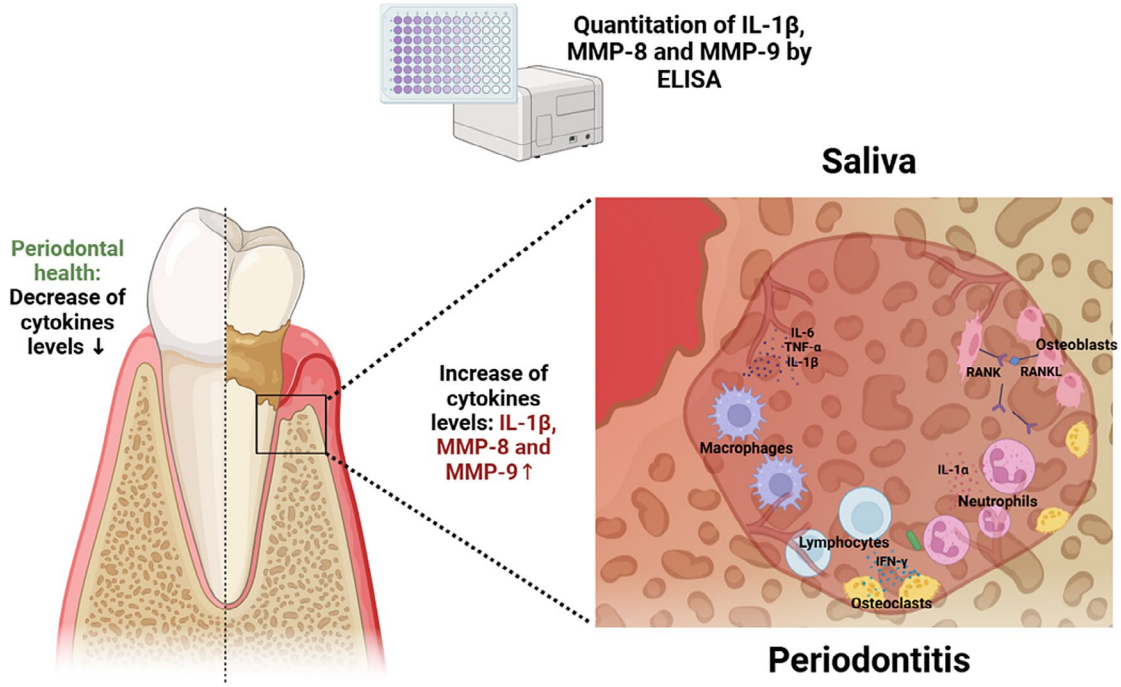

FIGURE 1 | Increased levels of IL-1β, MMP-8, and MMP-9 in saliva of subjects with periodontitis.

and studies presenting numerical values of these markers expressed in ng/ml or pg/mL. The exclusion criteria included studies published in languages other than English, studies published before 1990, and research involving cell lines or animal models, case reports and case series, and meta-analyses, narrative, systematic, or scoping reviews.

2.3 | Search Strategy and Screening Process

A comprehensive electronic search was conducted across four databases: PubMed, Scopus ScienceDirect, and Google Scholar, spanning from February 10, 2006, to January 15, 2024. The search strategy for PubMed involved the following terms: ((((“Interleukin-1beta”[Mesh]) OR “Matrix Metalloproteinase 8”[Mesh]) OR “Matrix Metalloproteinase 9”[Mesh]) AND “Saliva”[Mesh]) AND “Chronic Periodontitis”[Mesh]. For the other databases, the keywords “Interleukin 1 beta”, “Matrix Metalloproteinase 8”, “Matrix Metalloproteinase 9”, “Saliva,” “Biomarkers,” and “Periodontitis” were utilized, in conjunction with the Boolean operators “AND” and “OR.” Additionally, a manual search was executed in the following journals: “Periodontology 2000”, “International Journal of Periodontics & Restorative Dentistry,” “Journal of Clinical Periodontology,” “Journal of Periodontal and Implant Science,” “Journal of Periodontology” and “Journal of Periodontal Research”.

Subsequent to the retrieval of reports via the search strategy, two investigators (M.A.A.S and A.H) independently reviewed the titles and abstracts of all articles. Articles unrelated to the research interest were excluded. A thorough full-text analysis of the remaining potentially relevant articles was then conducted, adhering to predefined inclusion and exclusion criteria. In case of disagreement, a third investigator (R.R.M) was consulted to reach a consensus through discussion.

2.4 | Data Extraction

From the previously selected articles, two reviewers (M.A.A.S and A.H) extracted the following data independently in predefined tables with Word software (Microsoft): First author's name and year of publication, country, sex, age, individuals with periodontitis (cases) and control subjects, detection methodology, values of IL-1β, MMP-8, and MMP-9 with statistical significance (*p*-value).

Quantitative variables were represented with mean ± standard deviation, while qualitative data were represented with absolute and relative frequency *n* (%). In the absence of data, the investigators were contacted for further details, if necessary.

2.5 | Quality Assessment

The quality was independently evaluated by two investigators (M.A.A.S and R.R.M) using the Joanna Briggs Institute tool for cross-sectional studies and controlled clinical trials [58]. The final score determined the quality of the assessment: 0%–49% indicated a low quality; 50%–69% indicated a moderate quality; and a score above 70% indicated a high quality. A third investigator (A.H) confirmed the scores, and any disagreements were settled through group discussion.

2.6 | Statistical Analysis

The STATA 15V software (StataCorp, College Station, TX, USA) was utilized for quantitative analysis of IL-1β, MMP-8, and MMP-9 levels, comparing the periodontitis group with the control group. The standardized mean difference (SMD) along with 95% confidence intervals (CI) was determined using a random effects model, guided by the heterogeneity value's

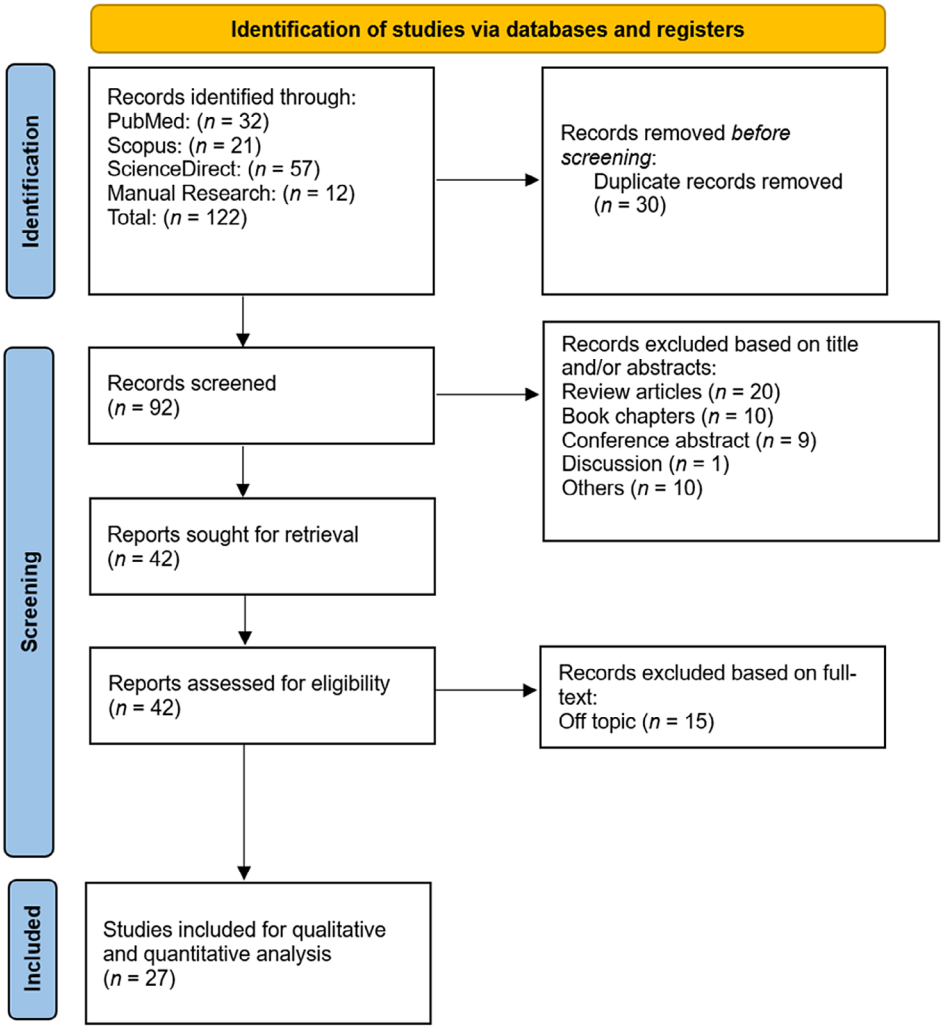

FIGURE 2 | PRISMA flow diagram of the study selection process. PRISMA Preferred Reporting Items for Systematic and Meta-Analyses.

significance (> 50% = high), estimated via the Q statistic and quantified by the I^2 statistic. A p-value of less than 0.05 was deemed statistically significant. Forest plots were generated to illustrate the estimates with 95% CI, and both funnel plot and Egger linear regression were employed to evaluate publication bias.

3 | Results

3.1 | Study Selection and Demographic, Clinical, and Immunological Features of Included Studies

Initially, 260 articles were identified across four electronic databases: PubMed ($n = 32$), Scopus ($n = 21$), ScienceDirect ($n = 57$), and Google Scholar ($n = 150$). An additional 12 articles were located through manual search, totaling 272 articles. During the identification phase, 35 duplicates were removed. Subsequently, 24 articles were excluded due to inaccessible abstracts. Of the 213 studies reviewed based on title and abstract, 186 records were discarded after applying eligibility criteria (Off-topic $n = 114$; reviews $n = 20$; book chapters $n = 10$; short communications $n = 10$; letters to the editor $n = 1$; conference abstracts $n = 31$). Following full-text analysis, 27 articles were selected for inclusion in the qualitative and quantitative synthesis of this review. The study selection process is depicted in Figure 2.

This study reviewed 27 articles, including 23 cross-sectional studies [30–36, 38, 40–43, 45–50, 52–56] and 4 clinical trials [37, 39, 44, 51]. A total of 1838 subjects were examined, with 1028 in the case group (subjects with periodontitis) and 810 in the control group (periodontally healthy subjects). The patient ages ranged from 18 to 75 years, with an average age of 41.58 ± 7.24 years; 44% were male, 50% female, and 6% did not specify gender [31, 45]. The majority of the articles (22 or 81.48%) were published post-2011 [30–51], with the oldest from 2006 [56] and the latest from 2024 [30]. These 27 studies originated from 15 countries [30–56], with five (18.52%) from the USA [43, 47, 53, 54, 56], four (14.81%) from India [31, 45, 51, 55], three (11.11%) from Turkey [33, 35, 44], two (7.41%) each from Taiwan [37, 38], Switzerland [39, 40], and Finland [48, 52], and the remaining (3.70%) from Iran [30], Morocco [32], China [34], Korea [36], Germany [41], Brazil [42], Poland [46], Colombia [49], and Russia [50] (Table 1).

Enzyme-linked immunosorbent assay (ELISA) was the predominant method (92.59%) for detecting IL-1β, MMP-8, and MMP-9 levels in the saliva of subjects with periodontitis [30–42, 44–46, 49–56], followed by Luminex (7.40%) [43, 47] and IFMA (2%) [48] (Table 1).

TABLE 1 | Demographic, clinical, and immunological characteristics of included studies.

Author's/year	Country of origin	Gender F[e]/M[a]	Age (Me/Ra)	*n* (CG/PG)	*n* (total)	Marker/methods	Value PG	Value CG	*p* PG versus CG
Tavakoli et al. 2024 [30]	Iran	30/30	50.4	30/30	60	MMP-8/ELISA	380.63 (18.079)	205 (14.431)	<0.05*
Balaji et al. 2022 [31]	India	NR	25–65	17/17	34	IL-1β, MMP-8/ELISA	28.84 (4.28) 147.16 (15.25)	15.67 (3.39) 85.83 (22.32)	<0.001*
Reddahi et al. 2022 [32]	Morocco	30/10	26.5	10/30	40	IL-1β, MMP-8/ELISA	11.25 1150	0.01 431.50	<0.001* <0.05*
Bostanci et al. 2021 [33]	Turkey	53/43	36.6	36/60	96	IL-1β, MMP-8, 9/ELISA	1147 740.7 423.4	344 76.5 57	<0.001*
Zhang et al. 2021 [34]	China	27/29	33.5	25/31	56	IL-1β, MMP-8/ELISA	162.2 (55.9) 657.1 (279)	92.2 (31.9) 435.8 (180.6)	<0.001*
Yucel et al. 2020 [35]	Turkey	31/32	37.1	23/40	63	MMP-8/ELISA	779 (87.2)	625.7 (163.1)	<0.001*
Hyuna et al. 2020 [36]	Korea	80/69	48.9	50/99	149	MMP-9/ELISA	370.73 (368.23)	191.88 (260.36)	<0.001*
Lee et al. 2020 [37]	Taiwan	28/26	46.1	20/34	54	IL-1β, MMP-8, 9/ELISA	306.1; 203.7; 186.7	112.1; 36.8; 93.8	NR
Wu et al. 2017 [38]	Taiwan	31/26	42.4	27/30	57	IL-1β, MMP-8, 9/ELISA	228.7; 173.58; 263,219	71.5; 36.733; 65,000	<0.05*
Martinez et al. 2017 [39]	Sweden	20/16	43.6	7/29	36	MMP-8/ELISA	333 (66.7)	66.7 (33.3)	<0.05*
Virtanen et al. 2017 [40]	Sweden	42/48	59.6	39/51	90	MMP-8, 9/ELISA	318.25 (87.9) 189.87 (112.21)	301.84 (101.07) 153.75 (105.10)	NS
Noack et al. 2017 [41]	Germany	24/15	37.3	19/20	39	MMP-8/ELISA	100.35 (15.82)	26.11 (28.40)	<0.001*
Moura et al. 2017 [42]	Brazil	28/19	39.1	23/24	47	IL-1β/*ELISA*	57.4 (47.3)	46.1 (30.2)	>0.05
Johnson et al. 2016 [43]	USA	22/19	38.2	10/31	41	MMP-8/Luminex	129.8 (891.9)	51.9 (102.4)	<0.05*
Ozcan et al. 2016 [44]	Turkey	17/15	42.7	15/17	32	IL-1β/ELISA	366 (177)	176 (82.5)	<0.05*
Gupta et al. 2015 [45]	India	NR	30–55	20/20	40	MMP-8/ELISA	348.76 (202.1)	190.91 (143.89)	<0.001*
Malgorzata et al. 2014 [46]	Poland	27/18	36.9	13/32	45	MMP-9/ELISA	418.97 (414.58)	221.15 (156.60)	<0.05*
Ebersole et al. 2013 [47]	USA	28/52	37.2	30/50	80	IL-1β/*Luminex* MMP-8/ELISA	90.94 (85.22) 283.47 (203.47)	7.24 (7.69) 52.63 (40.62)	<0.0001*

(Continues)

TABLE 1 | (Continued)

Author's/year	Country of origin	Gender F^e/M^a	Age (Me/Ra)	*n* (CG/PG)	*n* (total)	Marker/methods	Value PG	Value CG	*p* PG versus CG
Gursoy et al. 2013 [48]	Finland	86/79	49	81/84	165	MMP-8, 9/IFMA	888.6 (990.1) 90.2 (301.1)	304 (183.4) 69.1 (198)	<0.05*
Isaza-Guzmán et al. 2011 [49]	Colombia	77/46	21–75	54/69	123	MMP-9/ELISA	140.5	19.76	<0.001*
Kushlinskii et al. 2011 [50]	Russia	49/26	18–52	63/12	75	MMP-8, 9/ELISA	249 (181) 950 (855)	191 (170) 541 (489)	NR
Kaushik et al. 2011 [51]	India	35/17	34.3	24/28	52	IL-1β/*ELISA*	1312.75 (691.22)	161.51 (149.6)	<0.0001*
Gursoy et al. 2010 [52]	Finland	49/57	49.6	66/40	106	MMP-8/ELISA	96.7	75.6	<0.05*
Ramseier et al. 2009 [53]	USA	27/21	47.5	18/28	48	IL-1β, MMP-8, 9/ELISA	462.2 203.8 780.8	158.6 23.6 106.4	>0.05 <0.001* <0.01*
Teles et al. 2009 [54]	USA	45/73	40.5	44/74	118	IL-1β/*ELISA*	673 (69)	633 (91)	>0.05
Rai et al. 2008 [55]	India	NR	35.2	15/20	35	MMP-8/ELISA	428.6 (432.4)	95.2 (70.2)	<0.001*
Miller et al. 2006 [56]	USA	33/24	44.3	29/28	57	IL-1β, *MMP-8/ELISA*	753.7 (1022.4) 408.6(423.3)	212.8 (167.4) 95.1 (80.1)	<0.01* <0.001*

Abbreviations: CG, control group; DNS, data not shown; IFMA, immunofluorometric assay; Me, median; NR, not reported; PG, periodontitis group; Ra, range; F^e, female; M^a, male; *significant.

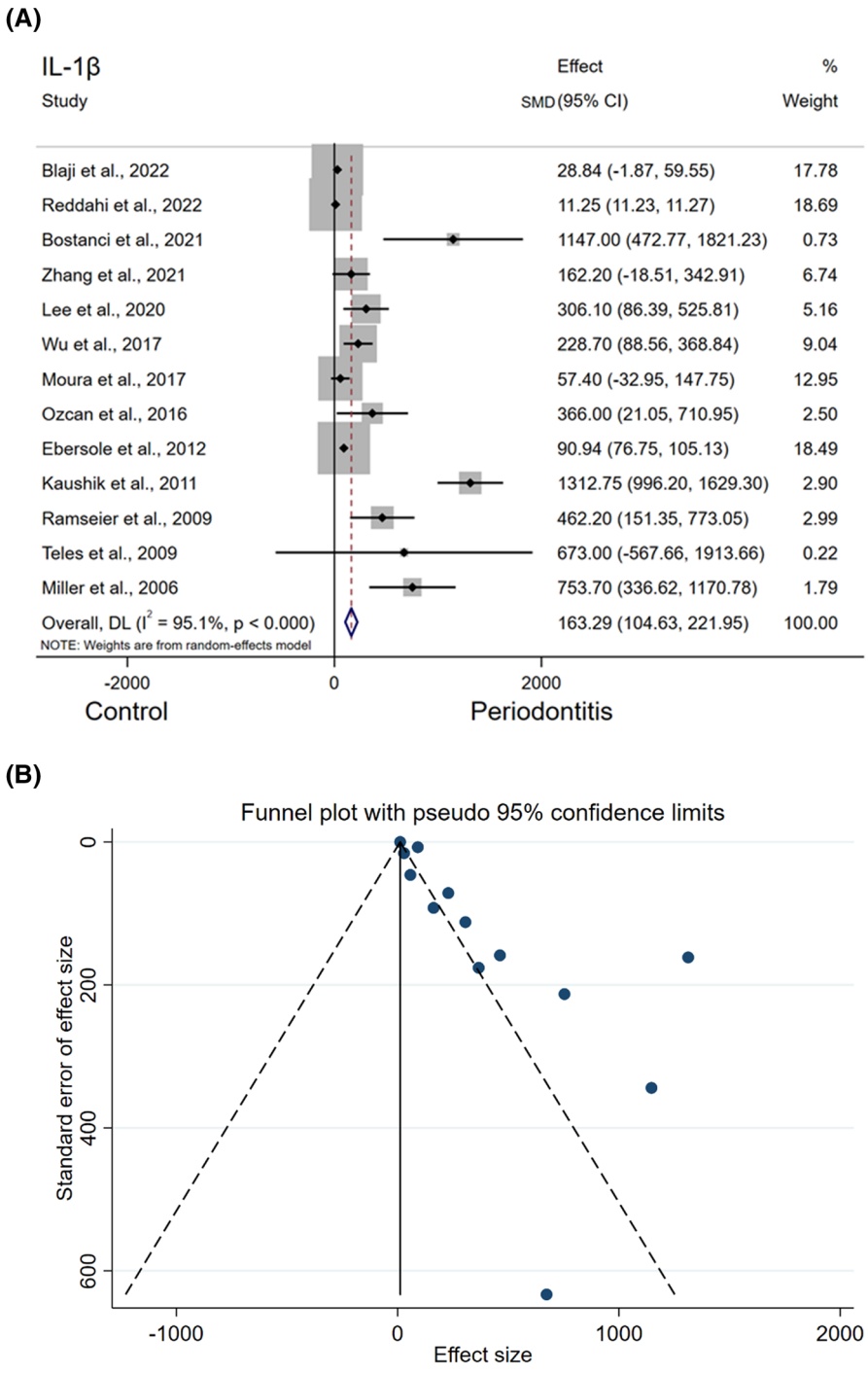

FIGURE 3 | Forest plot comparing the IL-1β levels in saliva of (A) control group versus periodontitis group. (B) Funnel plot to check the publication bias.

3.2 | Quality Assessment

The JBI criteria were applied to evaluate the quality in cross-sectional studies and clinical trials. According to the scores achieved, studies [30–36, 38, 40–43, 46–50, 52–56] demonstrated high quality with a low risk of bias, while studies [37, 39, 44, 51] exhibited moderate quality, as depicted in Figures S1 and S2.

3.3 | Meta-Analyses

3.3.1 | Measurement and Comparison of IL-1β Levels in Saliva of Subjects With Periodontitis Versus Control Subjects

As depicted in Figure 3, 13 articles [31–33, 37, 38, 42, 44, 47, 51, 53, 54, 56] compared IL-1β levels in the saliva of subjects

TABLE 2 | Summary of meta-analysis results.

Groups	No of studies	Test of comparison				Heterogeneity		
		SMD (95% CI)	*p*	Model	*Z*	Chi-square	*p*	I^2 (%)
IL-1β PG vs. CG	13	163.29 (104.64–221.95)	**0.001***	Random	5.456	243.51	**0.001***	95.1
MMP-8 PG vs. CG	20	282.22 (209.68–354.77)	**0.001***	Random	7.625	100.34	**0.001***	81.1
MMP-9 PG vs. CG	10	311.85 (179.64–444.05)	**0.001***	Random	4.623	61.15	**0.001***	85.3

Note: A *p*-value * ≤ 0.05 was considered statistically significant. Bold value represents significant differences.
Abbreviations: CG, control group; CI, confidence interval; IL-1β, interleukin 1 beta; MMP-8, matrix metalloprotease 8; MMP-9, matrix metalloprotease 9; PG, periodontitis group; SMD, standardized mean difference.

with periodontitis ($n = 451$) to those in periodontally healthy subjects ($n = 318$). The findings indicated that periodontitis is associated with higher salivary IL-1β levels compared to the control group (SMD = 163.29 (95% CI = 104.64–221.95); $p < 0.001$*). The chi-square test revealed significant heterogeneity among the studies ($I^2 = 95.1\%$, $p < 0.001$*), leading to the use of a random effects model to synthesize the primary results (Table 2). The funnel plot suggested asymmetry and the potential for publication bias, which was supported by Egger's test ($t = 3.90$, $p = 0.002$*), indicating evidence of bias (Figure 3A,B).

3.3.2 | Measurement and Comparison of MMP-8 Levels in Saliva of Subjects With Periodontitis Versus Control Subjects

As depicted in Figure 4, twenty studies [30, 31, 37–41, 43, 45, 48, 50, 52, 53, 55, 56] compared MMP-8 levels in the saliva of subjects with periodontitis ($n = 685$) to those of periodontally healthy individuals ($n = 585$). The findings indicated that periodontitis is associated with elevated salivary MMP-8 levels in comparison to the control group (SMD = 282.22, 95% CI = 209.68–354.77; $p < 0.001$*). The chi-square test revealed significant heterogeneity among the studies ($I^2 = 81.1\%$, $p < 0.001$*), leading to the use of a random effects model to synthesize the primary results (Table 2). The funnel plot suggested asymmetry and the potential for publication bias, which was supported by Egger's test ($t = 2.69$, $p < 0.001$*), indicating the presence of bias (Figure 4A,B).

3.3.3 | Measurement and Comparison of MMP-9 Levels in Saliva of Subjects With Periodontitis Versus Control Subjects

As depicted in Figure 5, 10 articles [33, 36–38, 40, 46, 48–50, 53] assessed the MMP-9 levels in saliva from subjects with periodontitis ($n = 499$) compared to periodontally healthy individuals ($n = 401$). The findings indicated a significant increase in salivary MMP-9 levels in those with periodontitis compared to the control group, with an SMD of 311.85 (95% CI = 179.64–444.05, $p < 0.001$*). The chi-square test revealed substantial heterogeneity across the studies ($I^2 = 85.3\%$, $p < 0.001$), leading to the adoption of a random effects model to integrate the primary outcomes (Table 2). The funnel plot was used to evaluate asymmetry and the potential for publication bias. Egger's test ($t = 2.00$, $p = 0.080$) found no significant evidence of bias (Figure 5A,B).

4 | Discussion

This systematic review with meta-analyses systematically assessed salivary levels of IL-1β, MMP-8, and MMP-9 in individuals with and without periodontitis, drawing on 27 published studies from 15 different countries. The findings affirm a link between elevated salivary levels of IL-1β, MMP-8, and MMP-9 and the presence of periodontitis. Specifically, individuals diagnosed with periodontitis exhibited higher levels of these markers compared to those without the disease. These findings underscore the significant role that this cytokine and these enzymes play in the immunopathogenesis of periodontitis.

Periodontitis is an immunoinflammatory disease that leads to the progressive destruction of periodontal bone and connective tissue [59]. Cytokines play an important role in inflammatory cell chemotaxis, collagen degradation through regulation of MMPs secretion, and bone resorption through increased osteoclastogenesis mediated by activation of the RANK/RANKL/OPG axis [60].

One important factor in the inflammatory cascade associated with periodontitis is the pro-inflammatory character of IL-1β. It stimulates the recruitment of inflammatory cells to the infection site, increases the synthesis of other cytokines, and, by activating osteoclasts, contributes to bone resorption [61, 62].

Studies have shown that patients with periodontitis exhibit increased levels of IL-1β in their gingival crevicular fluid (GCF), which correlates with clinical indicators such as attachment loss and probing depth [63, 64]. IL-1β is overexpressed in subjects with gingivitis, periodontitis, mucositis, and peri-implantitis and correlates with disease progression [65, 66]. Furthermore, it has been shown that the levels of this cytokine decrease compared to baseline values 6 months after non-surgical periodontal therapy [44]; therefore, it can be hypothesized that this cytokine acts as a reliable biomarker to differentiate periodontal health from disease [48].

Furthermore, the inflammatory response is further complicated by the interaction of IL-1β with periodontal bacteria, including *Porphyromonas gingivalis*. To promote tissue damage and bone loss, pathogens can cause macrophages to secrete IL-1β [67].

Conversely, notable MMPs such as MMP-8 and MMP-13 (collagenases), along with MMP-2 and MMP-9 (gelatinases), are overexpressed in various biological samples, including serum, plasma, gingival tissue, gingival crevicular fluid (GCF), and

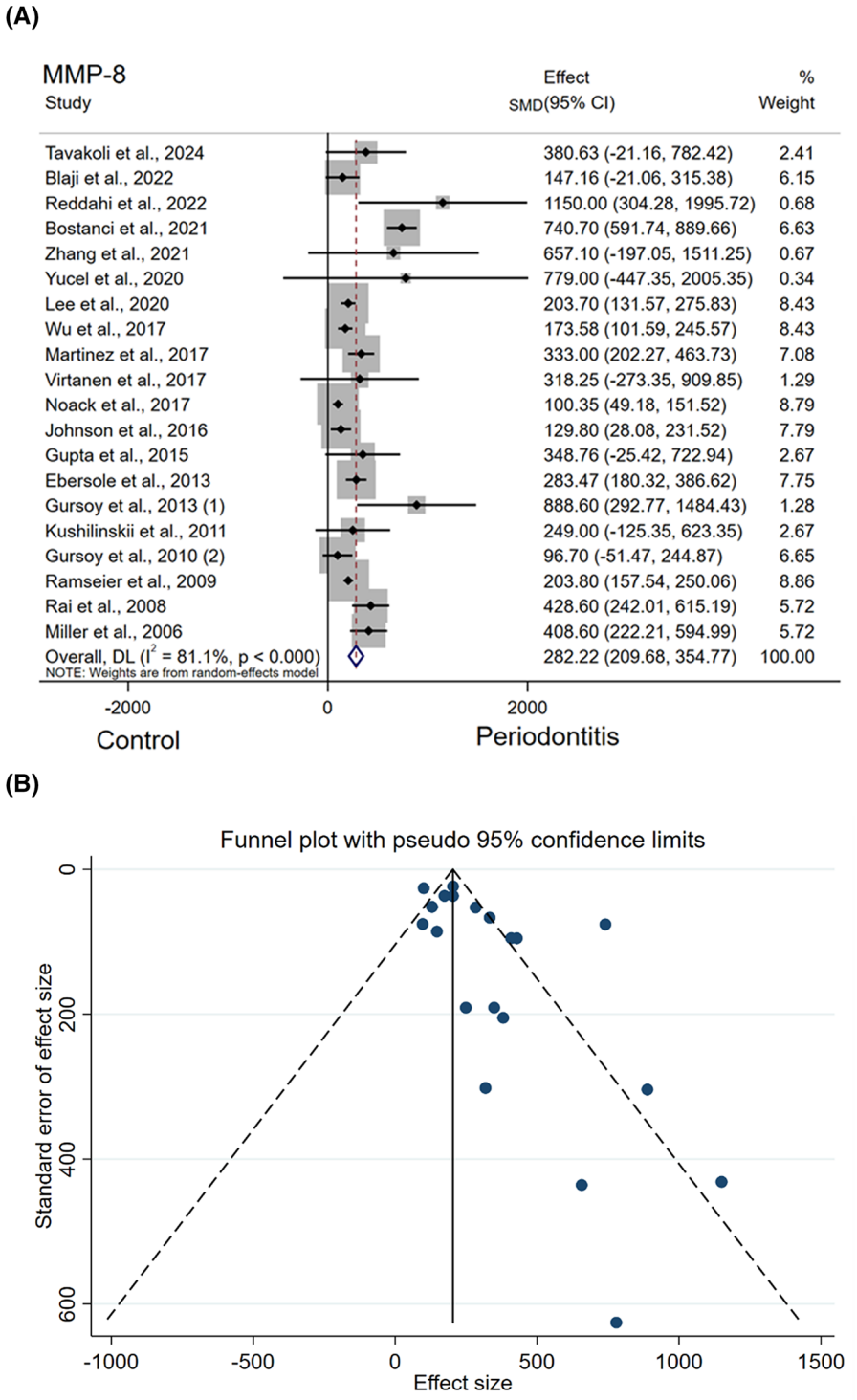

FIGURE 4 | Forest plot comparing the MMP-8 levels in saliva of (A) control group versus periodontitis group. (B) Funnel plot to check the publication bias.

saliva in individuals with gingivitis, periodontitis, mucositis, and peri-implantitis. This overexpression correlates with the progression of these diseases [68–70], primarily because their main target is type I collagen, which is abundant in the periodontal extracellular matrix [69].

A crucial player in the pathophysiology of periodontitis, matrix metalloproteinase-8 (MMP-8) is mainly responsible for the breakdown of collagen and other extracellular matrix constituents. The presence of active periodontal disease has been associated with elevated MMP-8 levels in GCF, and the concentration of MMP-8 tends to drop after periodontal therapy, indicating that it serves as a marker for disease activity [71]. MMP-8 activity has the potential to degrade the extracellular matrix, which is essential for preserving the structural integrity of the periodontal ligament (PDL) in the context of PDL health [72]. It has been shown that inflammatory stimuli, like nicotine exposure, cause MMP-8 to become overexpressed, suggesting

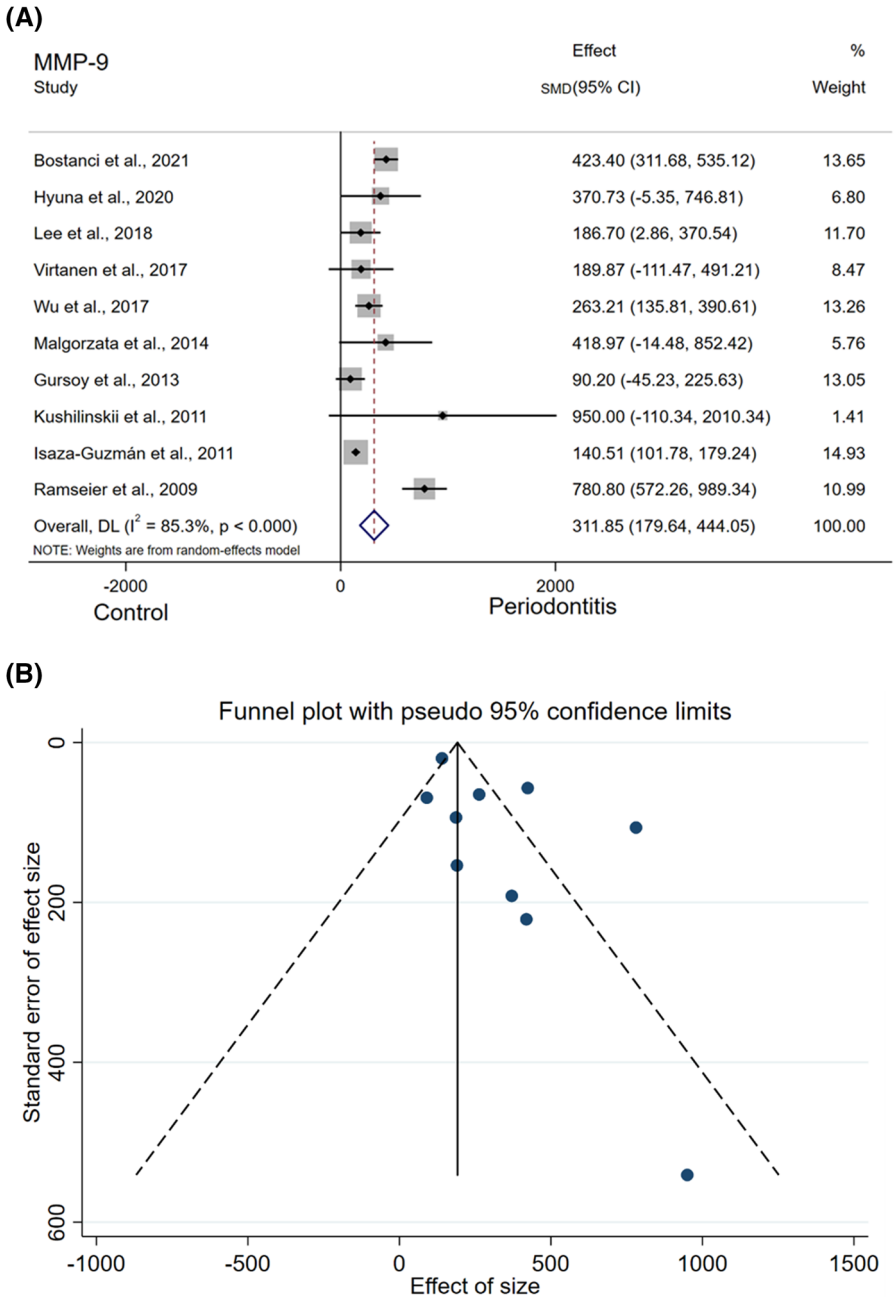

FIGURE 5 | Forest plot comparing the MMP-9 levels in saliva of (A) control group versus periodontitis group. (B) Funnel plot to check the publication bias.

that environmental factors may worsen the deterioration of periodontal tissue.

The MMP-9 and MMP-8 perform essentially the same tasks. Its involvement in the degradation of the extracellular matrix in the gingival tissues and the periodontal ligament (PDL) is especially noticeable when periodontal disease is present [73, 74]. Periodontal tissue degradation may result from an imbalance between MMPs and their tissue inhibitors (TIMPs). The correlation between elevated MMP-9 and reduced TIMP-1 levels has been linked to periodontal degradation, underscoring the significance of this equilibrium for maintaining periodontal health [36, 75]. Even Kaise et al. discovered a clear correlation between MMP activity and periodontal tissue destruction in canine gingival tissues affected by periodontitis, as evidenced by the increased expression of MMP-9 and the decreased levels of TIMP-1 [74]. Research has indicated that MMP-9 levels in gingival crevicular fluid (GCF) are markedly higher in patients with chronic periodontitis than in healthy individuals, suggesting that MMP-9 functions as a marker for the severity of periodontal disease [76–78]. For instance, it was discovered that MMP-9 levels are correlated with periodontitis clinical parameters, like probing depth and attachment loss, confirming the possibility of using the biomarker for diagnostic purposes [73].

Reducing MMP-9 activity might lessen tissue damage and enhance periodontal health. For instance, natural substances like neem and *aloe vera* extracts have demonstrated promise in vitro

in decreasing MMP-9 activity, indicating a possible path for adjuvant therapies for periodontal disease treatment [79].

The systematic reviews by Morais et al. [80] and Zhang et al. [81] supported the results of the present meta-analysis. The authors found higher concentrations of MMP-8 in saliva and GCF of individuals with periodontal disease compared to healthy controls, as well as in subjects with more advanced stages of the disease. However, to the authors' knowledge, a meta-analysis involving IL-1β and MMP-9 had not been performed.

It is now known that the increased expression, release, and uncontrolled activation of MMP-8 and MMP-9 contribute to inflammation and tissue destruction associated with periodontitis [82].

The present systematic review and meta-analyses had some limitations. Most of the included studies were assessed as having a low risk of bias; the presence of even a small proportion (15%) with moderate risk could influence the overall results The heterogeneity of the included studies, although addressed by a random-effects model, could still contribute to variability in the observed effect sizes. Factors such as different patient populations, disease severity, sample collection methods, and testing techniques could contribute to this heterogeneity. Furthermore, although the meta-analysis demonstrates a statistically significant association, it does not establish causality. Elevated biomarker levels may be a consequence of periodontitis rather than a predictor of its development.

5 | Conclusion

Within the limitations of this study, it could be concluded that increased salivary levels of IL-1β, MMP-8, and MMP-9 are associated with periodontitis. Further high-quality, follow-up studies with randomized designs and a larger sample size are required to verify our results in the future.

Author Contributions

Conceptualization: Mario Alberto Alarcón-Sánchez and Ruth Rodríguez-Montaño. Methodology: Mario Alberto Alarcón-Sánchez. Software: Mario Alberto Alarcón-Sánchez. Validation: Mario Alberto Alarcón-Sánchez, Ruth Rodríguez-Montaño, and Artak Heboyan. Formal analysis: Mario Alberto Alarcón-Sánchez, Ruth Rodríguez-Montaño, and Artak Heboyan. Investigation: Mario Alberto Alarcón-Sánchez and Ruth Rodríguez-Montaño. Resources: Mario Alberto Alarcón-Sánchez and Artak Heboyan. Data curation: Mario Alberto Alarcón-Sánchez and Ruth Rodríguez-Montaño. Writing – original draft preparation: Mario Alberto Alarcón-Sánchez and Ruth Rodríguez-Montaño. Writing – review and editing: Mario Alberto Alarcón-Sánchez, Ruth Rodríguez-Montaño, Artak Heboyan, and Seyed Ali Mosaddad. Visualization: Mario Alberto Alarcón-Sánchez, Ruth Rodríguez-Montaño, and Artak Heboyan. Supervision: Mario Alberto Alarcón-Sánchez, Ruth Rodríguez-Montaño, and Artak Heboyan. Project administration: Mario Alberto Alarcón-Sánchez and Artak Heboyan. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors have nothing to report.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.