SYSTEMATIC / NARRATIVE REVIEW ARTICLE

The clinical implications of *Porphyromonas gingivalis* and its detection methods – a systematic review

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ABSTRACT

Introduction: Mounting evidence has shown the significant correlation between periodontitis and the development of other comorbidities, such as cardiovascular disease due to periodontopathogenic bacterial migration and colonisation. As the main etiologic agent of periodontitis, the role of *Porphyromonas gingivalis (P. gingivalis)* has been widely explored as the main culprit and its early detection is crucial to control the exacerbation of diseases. This review aims to identify and summarise all clinical diseases that potentially developed due to the presence of *P. gingivalis* and discover all its detection methods that have been developed.

Materials and Methods: Full-text articles of case report, case control, cohort and cross-sectional studies that were published from 1st January 2012 until 30th June 2022, were searched using PubMed, CINAHL and Scopus. Periodontal related diseases were excluded in this review due to its well-known associated disease with *P. gingivalis*. A comparison studies of detection methods were also excluded in this review.

Results: Out of 612 articles that were screened, only 106 met the eligibility criteria to be selected for further review. Risk of bias was performed using FEAT principles and reviewers' discussion. A total of 21 final articles that were reviewed showed significant correlation with *P. gingivalis* and were classified into several clinical domains. Twelve out of 13 detection methods showed high sensitivity and specificity with short duration analysis.

Conclusion: Due to asymptomatic periodontal disease and the high prevalence of *P. gingivalis*-associated clinical diseases, this review suggests the need for oral public health awareness and early screening for the bacterium detection especially among elderly groups to maintain their quality of life.

KEYWORDS:
Clinical implication, Porphyromonas gingivalis, diseases, periodontal disease, detection method, systemic impact

INTRODUCTION

Porphyromonas gingivalis (P. gingivalis) is known to be the keystone and aetiologic agent in the progression of irreversible periodontitis, a chronic form of periodontal disease and had gained much interest globally due to its pathogenicity and virulence factors that cause destruction in the gingival and periodontal tissues.[1] In the early phase, periodontal disease is asymptomatic and painless, which could be the reason for most patients not seeking a dental treatment, subsequently becoming the site for bacterial colonisation, and leading to chronic periodontitis. The global prevalence of periodontitis demonstrated high occurrence in elderly group (82%) compared to adults (73%) and adolescents (59%), and thus, the disease is predicted to increase by years due to the increasing older population.[2] In the Indian population, *P. gingivalis* was highly detected in chronic periodontitis patients at 79.16%, and 29% in the healthy group.[3]

Over the last two decades, periodontal disease has been strongly associated with several systemic diseases such as cancer, atherosclerosis, rheumatoid arthritis, thus reducing individual performance and quality of life.[4] Numerous findings had revealed the role of *P. gingivalis* in the progression and exacerbations of existing disease by migrating from the bloodstream to the distant sites such as heart, liver, brain and placenta, then manipulating the immune system, causing immunosuppression and tissue damage.[5] Therefore, early detection of *P. gingivalis* is crucial to address the progression of other diseases and control their aggressiveness. Several advanced techniques have been developed such as polymerase chain reaction (PCR) and magnetic-nanobead based assay to detect the infection. Different sampling techniques were believed to affect the results.[6]

Most of the previous article reviews were only focused on the relationship between *P. gingivalis* and one specific disease or one class of disease, while the relationship between the bacterium and overall health, as well as the bacteria detection methods are still lacking. Therefore, in this review, our main objectives are: 1) to identify all the clinical diseases that are potentially due to the presence of *P. gingivalis* and 2)

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to identify all types of detection methods that have been developed for *P. gingivalis* detection.

MATERIALS AND METHODS

Criteria of selected studies in our review are as described as below:

1) Type of clinical disease: *P. gingivalis* is the main culprit and must be significant with a particular disease.
2) Type of detection: Multiple bacterial detection and comparison of detection method articles were excluded.

Search methods for identification of studies (including PRISMA 2009 flowchart)

Case report, case control, cohort and cross-sectional studies that was published from 1st Jan 2012 until 30th June 2022, were searched using PubMed, CINAHL and Scopus. A total of 612 full text articles were selected. Books, monograph, conference abstracts, editorials, letters, comments and reviews were excluded.

The search terms used were (*P. gingivalis* and clinical), (*P. gingivalis* and clinical), (*P. gingivalis* and health), (*P. gingivalis* and health), (*P. gingivalis* and disease), (*P. gingivalis* and disease), (*P. gingivalis* and importance), (*P. gingivalis* and importance), (*P. gingivalis* and significance), (*P. gingivalis* and significance), (*P. gingivalis* and implication), (*P. gingivalis* and implication), (*P. gingivalis* and association), (*P. gingivalis* and association), (*P. gingivalis* and detection), (*P. gingivalis* and detection), (*P. gingivalis* and culture), (*P. gingivalis* and culture), (*P. gingivalis* and isolation), (*P. gingivalis* and isolation), (*P. gingivalis* and cultivation), (*P. gingivalis* and cultivation), (*P. gingivalis* and cultivate), (*P. gingivalis* and cultivate), (*P. gingivalis* and identification), (*P. gingivalis* and identification), (*P. gingivalis* and methods), (P. gingivalis and methods), (*P. gingivalis* and ways), (*P. gingivalis* and ways), (*P. gingivalis* and technique), (*P. gingivalis* and technique), (*P. gingivalis* and techniques), (*P. gingivalis* and techniques), (*P. gingivalis* and assays), (*P. gingivalis* and assays), (*P. gingivalis* and assays), (*P. gingivalis* and assays). The results of each search terms were generated in Mendeley Reference Manager. From the main reference master page, subsequent subgroups references were generated based on the series of search terms mentioned. Any duplicated articles were deleted. Three independent reviewers checked and reviewed the articles independently.

The geographical area covered are all countries and the language of the publication was restricted to English in all databases. The PRISMA flow diagram for the search strategy is summarised in Figure 1 below.

Data Collection and Analysis

Data collection was done by two reviewers (MSES & NANS) and checked by three reviewers (SA, EMA&, HAH) independently. After finalising the studies to be included for analysis, full texts of all the eligible studies were retrieved. Two reviewers (RM & MSES) independently screened titles and abstracts for eligible studies, followed by full-text reading for methodological validity. If multiple publications of the same study were retrieved, only the most recent relevant data was included from these publications.

Qualitative synthesis was done by descriptive comparison of the reviewed articles for the clinical implications of *P. gingivalis* in overall health, the detection methods of the bacterium and risk of bias comparison. The risk of bias is evaluated based on the author's judgement using FEAT principles and discussion with other reviewers.[7] Four core principles that risk of bias assessments must meet (FEAT: assessments must be Focused, Extensive, Applied and Transparent) to enable the risk of bias to be low. If one component is not fulfilled, the risk is considered moderate. Meanwhile high risk is equivalent to the study that is unable to meet two or more of the core principles. Meta-analysis was not performed due to difficulty in obtaining some of the estimates which were not reported in the articles.

RESULTS

P. gingivalis is associated with numerous clinical diseases and involves multiple systems in the human body. As summarised in Table I, the organism is responsible for gingivitis, carcinoma progression, cognitive deterioration, arthritis development, abnormal sugar control, cardiovascular diseases and fatty liver formation. The presence of *P. gingivalis* antibodies also increases the odds of having intracranial aneurysms and diabetic retinopathy. Antenatally, those with the presence of *P. gingivalis* are 6.7 times more likely to have a preterm birth and 2.8 times more likely to have a foetus with intrauterine growth restriction.

Detection Methods of *P. gingivalis*

Most of the detection methods of *P. gingivalis* used in all studies were just reused the established protocol, especially the primers used for the molecular detection technique, that sometimes were not reproducible by time due to the fact that primers are not 100% conserved. Numerous detection methods for *P. gingivalis* have been developed with the main objective to detect the species at a fast rate of detection, but with high specificity and sensitivity. A comparative table as shown in Table II representing the advantages and disadvantages of all developed detection techniques for *P. gingivalis*. Different sampling techniques were believed to affect the results.[6]

Risk of Bias in Included Studies

There were 26 studies with low risk of bias and seven articles with moderate risk category as highlighted in Table III. Methodologically, these studies were conducted cross sectionally, retrospectively or in a cohort study. Some are in the form of a case report in which the biases were looked at from the detailed description of the case and its objectives. Majority of the studies have low selection bias as the study population and eligibility criteria were clearly mentioned. Most of the articles highlighted a novel and standard way of performing the bacteria detection. However, one study by Brun et al.,[38] has high selection bias as within the study, different samples were taken from different specimen sites, without making the methodology to be homogenous to all sampling processes. Nevertheless, this study can be considered low to moderate bias as other categories of bias assessment were considered low.

Table I: List of reviewed articles on P. gingivalis, their characteristics and clinical importance

Authors	Study design	Sample size	Population	Period	Clinical importance of *P. gingivalis*	Clinical domain	Odds ratio (OR)/Relative risk (RR (confidence interval) [*p*-value]
Kong et al. (2021)[8]	Retrospective analysis	50 cases	Patients attending to the First Affiliated Hospital of Henan University of Science and Technology	Data collection between January 2012 and December 2018	*P. gingivalis* was highly detected in the late stage of oesophageal squamous cell carcinoma (ESCC)(64.7%) and showed positive correlation with lymph node metastasis, where *P. gingivalis* was also detected in lymphatic metastasis tissues of ESCC patients at 60%.	Oncology	[p<0.05]
Liu et al. (2021)[9]	Retrospective study	309 cases	Subjects were primarily obtained from Henan province, including the First Affiliated Hospital of Henan University of Science and Technology, Hospital of Zhengzhou University, and Anyang Tumor Hospital Patients were recruited from	Data collection between 2010 and 2013. Next follow up within 5 years.	The detection of *P. gingivalis* was significantly higher in lung squamous carcinoma tissues, compared to adjacent lung tissues.	Oncology	(CI:17.609-28.995) (χ2:6.365) [p<0.05]
Chang et al. (2019)[10]	Case control	61 cases, 30 controls	Affiliated Stomatological Hospital of China Medical University.	Data collection between 2013 and 2014.	*P. gingivalis* has been detected in the oral squamous cell carcinoma tissues at 60.7%, compared to healthy tissues, 13.3%.	Oncology	[p<0.05]
Ahn J et al. (2012)[11]	Prospective study	7852 participants	The participants were obtained from the National Health and Nutrition Examination Survey III (NHANES III) survey-based USA population.	Data collection between 1991 and 1994.	High levels of antibody *P. gingivalis* accompanied with an excess orodigestive cancer mortality.	Oncology	RR3.03 (CI: 0.99-9.31) [p=0.006]
Sansores-España et al. (2022)[12]	Case control	20 cases 10 controls	Control patients were recruited from the Faculty of Dentistry, Autonomous University of Yucatan.	Data collection from June and December 2019.	80% of Alzheimer's disease patients had a chronic periodontitis, with high abundance of *P. gingivalis* and its presence had negative correlation with Montreal cognitive assessment (MoCA) test values, suggesting the severe cognitive impairment patients tend to have a higher P. gingivalis load.	Neurology	-
Rasheed et al. (2013)[13]	Case report	1 case	34-years old-male patient admitted at Thammasat University Hospital, Thailand.	-	Patient was presented with subdural empyema and sinusitis. *P. gingivalis* was detected in the subdural empyema's patient in his yellowish purulent.	Neurology	-
Hallikainen et al. (2021)[14]	Case-control	227 cases, 1096 controls	Patients were recruited from Kuopio University Hospital (KUH). Controls were obtained from cross-sectional Finnish Health 2000 Health survey study	-	IgA antibodies against *P. gingivalis* were 1.5 times higher in intracranial aneurysms cases compared to control	Neurology	OR:1.4 (CI:1.1-1.8) [p≤0.003]
Wisutep et al. (2022)[15]	Case report	One case	80-years-old woman from Hospital of Mahidol University, Bangkok, Thailand.	-	Patient was presented with an acute stroke-like syndrome and *P. gingivalis* was detected in the pus aspirate sample. The patient was fully recovered after 8 weeks of taking antimicrobial treatment and dental therapy.	Neurology	-

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Authors	Study design	Sample size	Population	Period	Clinical importance of *P. gingivalis*	Clinical domain	Odds ratio (OR)/ Relative risk (RR (confidence interval) [*p*-value]
Mougeot et al., (2017)[16]	Retrospective study	42 cases	-	Data collection between 2022 and 2003.	Among 245 species detected in the coronary and femoral arteries, *P. gingivalis* was the most abundance detected (46.8%), followed by another species, 19.3%.	Cardiology	[p=0.0005]
Totaro et al. (2013)[20]	Case-control	69 cases, 26 controls	Patients from the division of Rheumatology of the Catholic University of the Sacred Heart of Rome	From October 2010 to February 2012	High detection of *P. gingivalis i*n synovial tissue of rheumatoid arthritis patients (33.3%) suggested its role in the progression of disease.	Rheumatology	[p<0.01]
Ceccarelli et al, (2018)[21]	Case-control	143 cases, 94 controls	Patients were enrolled at the Rheumatology unit, La Sapienza University of Rome	-	P. gingivalis was significantly higher in rheumatoid arthritis patients compared to the control group.	Rheumatology	[p=0.01]
Arvikar et al. (2013)[22]	Cohort study	50 cases	Early rheumatoid arthritis patients were obtained from the Rheumatology clinic at Massachusetts General Hospital.	-	Rheumatoid arthritis patients tended to have a higher *P. gingivalis* antibody and the levels of anti-Pg antibodies were directly correlated with anti-cyclic citrullinated protein level. Moreover, patients with positive *P. gingivalis* had greater rheumatoid factor values and higher disease activity score (DAS) values.	Rheumatology	[p<0.01]
Kharlamova et al. (2016)[24]	Case control	1974 cases, 377 controls	Swedish population based	-	The autoantibodies *P. gingivalis* were more frequently detected in anti-citrullinated protein antibodies (ACPAs) positive rheumatoid arthritis patients.	Rheumatology	-
Radhakrishnan et al. (2019)[25]	Prospective study	37 cases	Patients attending to the outpatient clinic, in the tertiary care hospital of India.	-	Diabetes mellitus (Type 2) and periodontitis is closely associated and early detection of *P. gingivalis* is suggested to control glycaemic status.	Endocrine - diabetes	-
Al-Rawi & Al-Marzooq. (2017)[26]	Cross-sectional study	78 cases	Patients attending the University of Sharjah Dental Hospital, Sharjah, UAE.	From December 2015 to April 2016	The high levels of periodontopathogenic bacteria could trigger the release of salivary resistin in obese people, and the most prevalent bacterial species detected was T. denticola (100%), followed by *P. gingivalis* (97.4%).	Endocrine diabetes	-
Gogeneni et al. (2015)[27]	Case control	-	Patients attending the Endocrinology and Metabolism outpatient clinic, Aydın State Hospital, Aydın, Turkey.	Data collection between September 2012 to March 2013	Women with gestational diabetes mellitus (GDM) are more likely to have higher periodontopathogen, and *P. gingivalis* was detected at the most top three abundance bacteria at 52.6%	Endocrine - diabetes	-
Chiu et al. (2021)[28]	Cohort study	116 cases 116 controls	Patients were selected randomly from the US Third National Health and Nutrition Examination Survey.	The survey was conducted between 1988 and 1994.	Patients with high *P. gingivalis* IgG antibody levels had a high risk for developing early diabetic retinopathy over 60%.	Endocrine - diabetes	OR:1.64 (CI: 1.36-1.97) [p=0.0053]

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Table I: List of reviewed articles on P. gingivalis, their characteristics and clinical importance

Authors	Study design	Sample size	Population	Period	Clinical importance of *P gingivalis*	Clinical domain	Odds ratio (OR)/Relative risk (RR (confidence interval) [*p*-value]
Yoneda et al. (2012)[29]	Case control	150 cases, 60 controls	Nara City, Hospital, Japan.	-	The detection of *P. gingivalis* was significantly higher in non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) patients compared to control, suggesting its role in the progression of NAFLD and NASH.	Hepatology	OR:3.16
Omura et al. (2016)[30]	Case report	One case	45-years-old woman who died from sepsis	-	*P. gingivalis* was detected in NASH patient's hepatocytes, highlighting its significance in the progression of cirrhosis to NASH.	Hepatology	-
Andonova and Iliev. (2021)[31]	Case control	60 cases 50 controls	-	-	The presence of *P. gingivalis* in pregnant women showed a higher complication in pregnancy, where pregnant women with positive *P. gingivalis* were 6.7 times more likely to have a preterm birth and 2.8 times to have a foetus with intrauterine growth restriction.	Obstetric	RR: 6.65 (CI:1.38-32.11) [p<0.05]
Tellapragada et al. (2014)[32]	Cross-sectional study	390 pregnant women	Patients attending antenatal clinic at the Dr. TMA. Pai Hospital, Udupi, Karnataka, India.	Between July 2012 and June 2013.	Out of total pregnant women selected, 10% of patients were having periodontitis and 38% were diagnosed with gingivitis. The most bacterial species detected was *P. gingivalis* (36%).	Obstetrics	OR: 2.6 (CI: 1.35-5.15) [p< 0.05]

Table II: Detection methods for *P. gingivalis*, their advantages and limitations

Authors	Methods	Method group	Sample	LOD	Duration analysis	Advantages	Limitations
Rajaram et al. (2016)[33]	Culturing on blood and kanamycin agar.	Culture-based	From root canal	-	Up to 3 days	Able to distinguish species in mixed microbial communities.	The method is time-consuming and has low rate of detection (44%). The sensitivity indicated as low, due to low rate of detection (44%) as stated in the table.
Mendes et al. (2016)[35]	PNA-FISH Technique	Direct detection methods	Subgingival plaque	-	Few hours	The method (peptic nucleic acid, FISH) is very high sensitive (100%), and very high specific (100%)	Need to pre-treat the sample and the assay is quite costing for microscopic visualisation.
Gu et el. (2020)[37]	Direct qPCR	Nucleic acid-based methods	Swabbing inside the cheek	1000 copies/ mL	1.5 hours	The method is highly sensitive (95.24%), and very high specificity (100%). Besides, the method is cost effective, and no DNA extraction is required.	Inconsistent results due to low level of DNA copies.
Brun et al. (2020)[38]	Nested PCR	Nucleic acid-based methods	-	-	-	Increased specificity by 22% from previous conventional PCR	Need a longer time for optimisation
Hamzan et al. (2018)[39]	Loop mediated isothermal amplification assay.	Nucleic acid-based methods	Subgingival plaque and saliva	1 ng of DNA	3 hours	The method was 10 times more sensitive than conventional PCR and needed fewer operations steps compared to PCR.	Complicated primer designs (six primers)
Kitano et al. (2016)[40]	LAMP combined with PCR	Nucleic acid-based detection method	Periodontitis tissue	21 copies/tube	20 minutes	Higher sensitivity and specificity than PCR technique.	Complicated design primers (need eight primers)
Ge et al. (2022)[41]	Isothermal amplification and Lateral Flow Strip Methods	Nucleic acid-based methods	-	9.27 CFU/rxn	30 minutes	The method is highly sensitive (95%), and highly specific (93.3%).	Good antibody preparation is obligatory
Imamura et al. (2015)[42]	Immunochro matographic assay	Immunologic al methods (IMs)	Subgingival plaque	10^4 copies/2 paper points	15 minutes	The method is highly sensitive (96.2%), and highly specific (91.8%). No expensive laboratory equipment is required and the portable device is suitable for point-of-care detection.	Inaccurate sample volume may reduce the accuracy.
Lee et al. (2021)[43]	Colorimetric membrane immunoassay	Immunologic al methods (IMs)	-	10^3 cells/mL	-	Cheap, easy to prepare, and can be observed by naked eyes.	Takes more times than other molecular techniques. It is easy to prepare as immunological methods, but the method takes more time compared to molecular techniques such as PCR assay.

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Table II: Detection methods for *P. gingivalis*, their advantages and limitations

Authors	Methods	Method group	Sample	LOD	Duration analysis	Advantages	Limitations
Witkowska et al. (2021)[44]	SERS-Based Magnetomicr ofluidic sensor	Biosensor methods	Saliva	10^3 CFU/mL	-	Able to detect multiple strains of *P. gingivalis*	Moderate accuracy of 82% and need further optimisations
Alhogail et al. (2018)[45]	Magnetic-nanobead based assay.	Biosensor methods	Saliva	49 CFU/mL	30 seconds	The method is highly sensitive and highly specific. Applicable for on-site detection	Requires minimal detection platform manipulation
Park et al. (2021)[46]	Electrochemic al sensor	Biosensor methods	Saliva	5×10^5 CFU/mL	-	The method is able to determine the exact concentration of *P. gingivalis* and applicable for point-of-care detection	Personnel trained is required to analyse the results from an instrument. and the detectable range was lower than qPCR
Yamanaka et al. (2018)[47]	Electrochemic al DNA sensor + PCR	Biosensor methods	Gingival crevicular fluid, saliva	10^4 CFU/mL	Few hours	Measurement principle is simple and applicable to other targets	Personnel trained is required to analyse the results from an instrument. and the detectable range was lower than qPCR

Table III: Risk of bias in reviewed articles

Author	Selection bias	Exposure assessment bias	Confounder	Other bias	Overall risk of bias
Kong et al. (2021)[8]	Low	Low	Moderate Other possible factors did not include such as family history, diet history, occupation which could lead to the development of malignancy.	Data were collected from medical records - information not verified with patient	Low
Liu et al. (2021)[9]	Low	Low	Low	Nil	Low
Chang et al. (2019)[10]	Low	Low	Low	Nil	Low
Ahn J et al. (2012)[11]	Moderate Subset of national health survey	Non-communicable diseases were not assessed directly but by patient self-reported	Moderate Family history/job exposure not included in co-variates	Nil	Moderate
Sansores-España et al. (2022)[12]	Moderate Small sample size	Nil	Other modifiable and non-modifiable risk factors were not included	Nil	Moderate
Rasheed et al. (2013)[13]	Moderate - one case report	Nil	Diabetes and immunocompromised status were not mentioned	Other relevant investigations were not included in the case report	Moderate
Hallikainen et al, (2021)[14]	Low	Low	Low	Nil	Low
Wisutep et al. (2022)[15]	Moderate - one case report	Low	Low	Nil	Low
Mougeot et al. (2017)[16]	Low	Low	Moderate - demographic information is too little. Other relevant modifiable and non-modifiable risk factors were not included.	Nil	Low
Totaro et al. (2013)[20]	Low	Low	Low	Nil	Low
Ceccarelli et al. (2018)[21]	Low	Low	Low	Nil	Low
Arvikar et al. (2013)[22]	Moderate - sample size questionable (relatively low for cohort study)	Low	Low	Nil	Low
Kharlamova et al. (2016)[24]	Low	Low	Low	Nil	Low
Radhakrishnan et al. (2019)[25]	Moderate - small sample size	Low	Low	Nil	Low
Al-Rawi & Al-Marzooq. (2017)[26]	Low	Low	Low	Nil	Low
Gogeneni et al. (2015)[27]	Low	Low	Other cofactors are not studied/ included	Nil	Low
Chiu et al. (2021)[28]	Moderate Subset of national health survey	Non-communicable diseases were not assessed directly but by patients self-reported.	Low	Low	Moderate
Yoneda et al. (2012)[29]	Low	Low	Other factors not included (alcohol, physical activity, diet, smokers)	Low	Low
Omura et al. (2016)[30]	Moderate - solely a case report	Low	Low	Dyslipidaemia status was not verified.	Moderate
Andonova & Iliev. (2021)[31]	Low	Low	Mothers with concomitant non-communicable diseases or co-morbidities were not excluded	Nil	Low
Tellapragada et al. (2014)[32]	Low	Low	Low	Nil	Low

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Table III: Risk of bias in reviewed articles

Author	Selection bias	Exposure assessment bias	Confounder	Other bias	Overall risk of bias
Rajaram et al. (2016)[33]	Low detailed recruitment and methodological process has been spelled out	Low	Low	Nil	Low
Mendes et al. (2016)[35]	High name of strain providers were mentioned in the methodology - which may lead to conflict of interest and bias.	Low	Low	Low	Moderate
Gu et al. (2020)[37]	Low	Low	Low	Nil	Low
Brun et al. (2020)[38]	High - different sample sites for different studies	Low	Low	Nil	Moderate
Hamzan et al. (2018)[39]	Low	Low	Low	Nil	Low
Kitano et al. (2016)[40]	Low	Low	Low	Nil	Low
Ge et al. (2022)[41]	Low - detailed methodology steps	Low	Low	Nil	Low
Imamura et al. (2015)[42]	Moderate - detailed of the person responsible to differential and classify to groups are not clear	Low	Low	Nil	Low
Lee et al. (2021)[43]	Supplier of bacterial strains were highlighted	Low	Low	Nil	Low
Witkowska et al. (2021)[44]	Low	Low	Low	Nil	Low
Alhogail et al. (2018)[45]	Low	Low	Low	Nil	Low
Park et al. (2021)[46]	Low	Low	Low	Nil	Low
Yamanaka et al. (2018)[47]	Low	Low	Low	Nil	Low

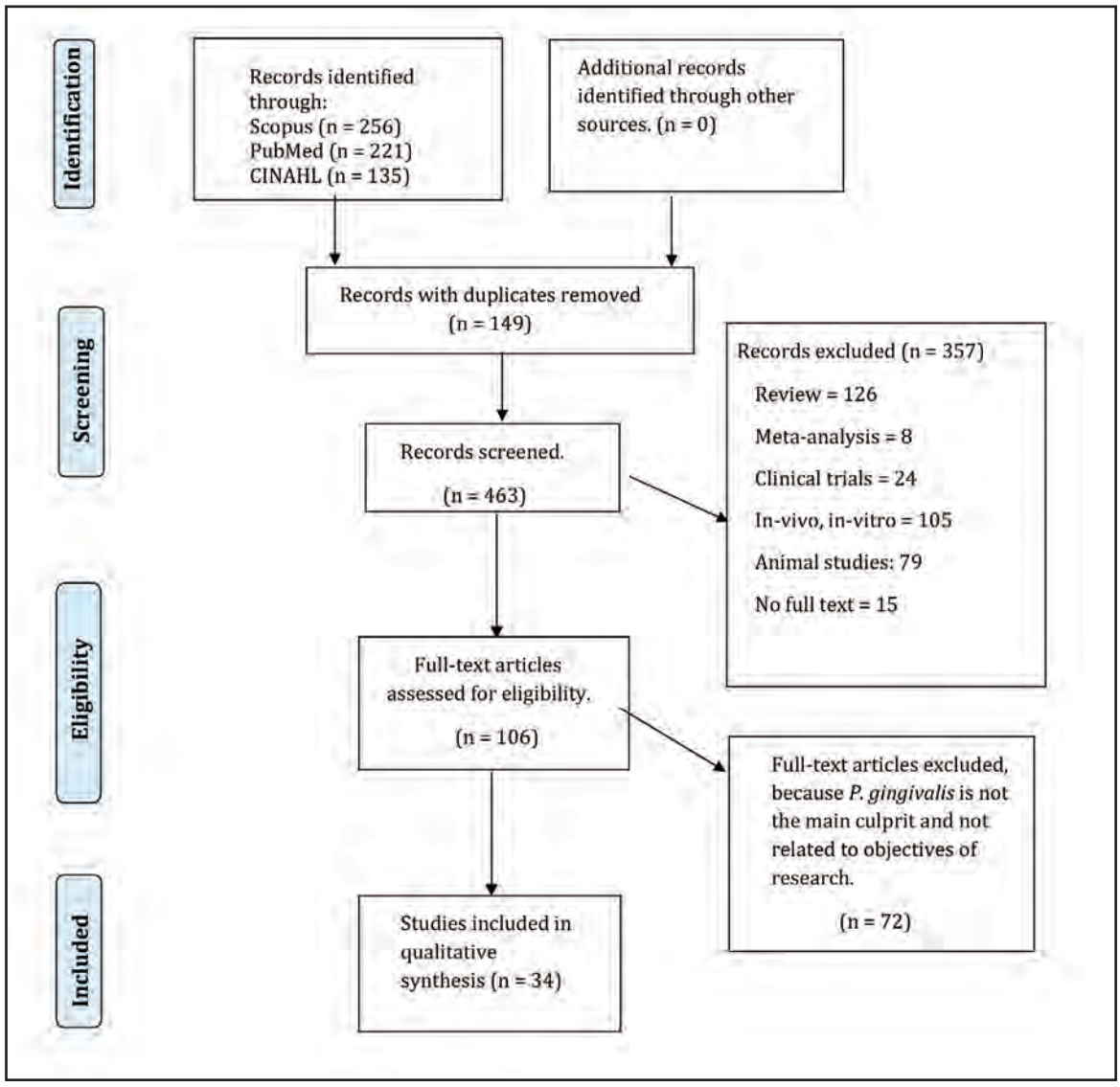

Fig. 1: PRISMA 2009 Flow Diagram.

In terms of confounding factors, they are present in the form of background of the patients recruited. For example, the study conducted by Kong et al.,[8] Ahn et al.,[11] Sansores-España et al.,[12] and Rasheed et al.,[13] the sociodemographic factors and patient related profiles were not captured completely. These include family history, diet history, comorbidities, and some important results.

In the form of data collection, the study by Kong et al.,[8] has high risk of bias as the information was gathered from self-report which can lead to recall bias and cannot be verified. Overall, 16 studies have low risk in all categories of bias risks.

DISCUSSION

A. Clinical Importance of *P. gingivalis* and Disease Implications

Due to growing evidence, *P. gingivalis* has been an important risk factor in the exacerbations of a particular disease as shown in Table I either via its virulence factors or *P. gingivalis* alone.

(i) Oncology

Upper Gastrointestinal Malignancy

As both oesophagus and oral cavity are structurally closed to each other, the oral microbiome is more likely to infect oesophagus compared to other parts in the digestive system and *P. gingivalis* has been confirmed to have a strong correlation with oesophageal cancer. Previous study demonstrated that *P. gingivalis* was highly detected in the late stage of oesophageal squamous cell carcinoma (64.7%) compared to those in the early stage (30.3%). Furthermore, it must be noted that there was no significant correlation between positive *P. gingivalis* detection and other factors including smoking history, alcohol status, age and gender in the oesophageal squamous cell carcinoma (ESCC) patients. In addition, the detection of *P. gingivalis* in the ESCC showed positive correlation with lymph node metastasis, where *P.*

gingivalis was also highly detected in lymphatic metastasis tissues in ESCC at 60%. These findings suggested the specific role of *P. gingivalis* as an etiologic agent and could be a potential prognostic indicator in the progression of esophageal cancer.[7]

Lung Cancer

Due to the clinical importance and close association between *P. gingivalis* and oesophageal cancer that has been discussed in previous discussion, it has been suggested that *P. gingivalis* also could migrated and colonised into the lung cells as the oesophagus and trachea are anatomically closed to each other. To support this speculation, Liu Y et al. 2021,[9] found a significantly higher detection of *P. gingivalis* in the carcinoma tissues of patients with lung adenocarcinoma (26.89%), lung squamous cell carcinoma (39%), and small cell lung cancer (35%), compared to the adjacent lung tissues (3%, 3% and 4%, respectively). Importantly, the 5-years survival rate of these three types of lung cancer patients with positive *P. gingivalis* were significantly lower than those survival rates of patients with negative *P. gingivalis*. Hence, the authors suggested that *P. gingivalis* infection is closely associated with survival rate of lung cancer patients. Besides, the highest detection rate of *P. gingivalis* in lung squamous cell carcinoma was frequently observed in many patients with smoking history, suggesting that smoking habit may increase the risk of *P. gingivalis* infection, subsequently promoting the progression of lung cancer. In fact, long-term smoking could damage the body's immune function, and thus, allowing a better colonisation of *P. gingivalis* and the bacterium may induce invasion, proliferation, and metastasis of lung cancer.[8]

Oropharyngeal Cancer

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy that occurs due to the mutation of squamous cell that lining up the lips, mouth, tongue and gums. Besides alcohol consumption, poor dietary, and other environmental factors, several studies have indicated the significance of oral microbes in the carcinogenesis of OSCC, where high abundance of P. gingivalis has been detected in the OSCC tissues at 60.7%.[9] Next, *P. gingivalis* also has been suggested to be a biomarker for bacterial-associated risk of death in orodigestive cancer. Previous study had found that greater levels of serum *P. gingivalis*-IgG was associated with an increased orodigestive cancer mortality. *P. gingivalis* that is associated with orodigestive mortality was also detected in patients without periodontal disease, suggesting a strong association of *P. gingivalis* in the orodigestive cancer mortality regardless of periodontal health.[10]

(ii) Neurology

Neurodegenerative Disorder

Research interests in exploring the association of periodontal microbes and neurodegenerative disorder such as Alzheimer's disease (AD) has increases in recent decade, suggesting that the presence of microbes could lead to the overproduction of amyloid-β peptides in the brain which may clumps into plaque and cause neuroinflammation. A statistical data showed that 80% of AD patients had a chronic periodontitis, with higher abundance of *P. gingivalis* and its pro-inflammatory molecules, compared to non-AD patients, where only 20% had a chronic periodontitis. Moreover, it has been identified that the more *P. gingivalis* is present in AD patients, the lower their Montreal Cognitive Assessment (MoCA) test values are, suggesting that the severe cognitive impairment patients tend to have a higher *P. gingivalis* load.[11]

Subdural Empyema

Although *S. pneumoniae* is the most common species to cause subdural empyema, the first case study in 2013 by Rasheed et al.,[13] had discovered that *P. gingivalis* could be the main culprit in the disease progression where an adult male patient was presented with precedent dental and sinus infection. After microbiological examinations, the subdural empyema's patient was positive *P. gingivalis* in his yellowish purulent. Meanwhile, no organisms were observed in an aerobic environment. Hence, the author and his colleagues suggested *P. gingivalis* should be considered in differential diagnostic measure of subdural empyema or CNS abscesses.[12]

Intracranial Aneurysms

Previous study had found that patients with intracranial aneurysms (IAs) were more likely to have gingivitis and severe periodontitis (2 times and 1.5 times, respectively) as compared to the control. Interestingly, the *P. gingivalis* epitope was found to be present in the IA wall, suggesting its role in the IAs formation and rupture. Moreover, further examinations have confirmed that the IgA antibodies level against *P. gingivalis* in both ruptured and unruptured IAs patients was 1.5 times higher than control patients. Meanwhile, the IgG antibodies against *P. gingivalis* were 1.8 times lower than control patients. Thus, exposure to *P. gingivalis* and dysfunctional acquired immune response against the bacterium could exacerbate the risk of IAs formation and rupture.[13]

Stroke

Odontogenic infection, including periodontitis is one of the commonest sources of brain abscess formation. A recent case study has reported that a patient who had a brain abscess and presented as an acute stroke-like syndrome was having multiple periodontal infection sites during oral examination. Further microbiological diagnostic was confirmed that the pus aspirate sample isolated from that patient was positive with oral anaerobes, *P. gingivalis* and Filifactor alocis. Interestingly, that patient was fully recovered after 8 weeks of taking antimicrobial treatment and dental therapy. Therefore, the authors speculated that both *P. gingivalis* and *F. alocis* were suspected to be the main culprits for the brain abscess formation.[14]

(iii) Cardiology

Atherosclerosis (AS) is a progressive disease that develops due to lipid accumulation in the arterial walls that may harden and narrows the arteries and could lead to occlusion. Although periodontopathogens may not be the main factor in the inflammatory diseases associated with AS, but it may be considered as a potential risk factor. The association between the *P. gingivalis* and AS may be supported by the evidence of its DNA detection in the healthy arterial tissues, where P. gingivalis was the most abundant species detected at 79.2% of all bacterial species counts. These findings suggested the possible role of P. gingivalis in the initiation or exacerbation of early atherosclerosis where the bacterium

may invade the arterial walls from the subgingival tissues and survive intracellularly.[15]

(iv) Rheumatology

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease that causes destruction, pain and swelling in the joints. Periodontitis is known to be one of the risk factors of RA, where both shared the same histopathological characteristics, inflammatory pathways and risk factors for susceptibility, such as cigarette smoking and genetic factors by HLA-DRB1 shared epitope (SE) alleles.[16] Moreover, several studies have highlighted that periodontitis is more frequent in RA patients compared to healthy subjects.[16-18] A previous study had demonstrated that *P. gingivalis* could migrate to the joints and its persistent exposure may exacerbate the chronicity of inflammation in arthritis progression. The authors found a higher detection of *P. gingivalis* in the synovial tissue of RA patients at 33.3% compared to healthy subjects at 5.9%.[19] Additionally, Ceccarelli and his colleagues reported that there was a significant association between *P. gingivalis* composition and RA disease activity score in 28 joints (DAS28), where the higher DAS values was observed frequently in *P. gingivalis*-positive patients (8.2%) compared to *P. gingivalis*-negative patients (1.7%). The authors also found that the RA patients in remission state had a lower prevalence of *P. gingivalis* compared to non-remission RA patients, indicating the presence of *P. gingivalis* may trigger an autoimmune system regardless of whether periodontitis is present or not.[20]

The autoantibodies against citrullinated proteins, also known as anti-citrullinated protein antibodies (ACPAs) or its subset namely anti-cyclic citrullinated protein (anti-CCP) is highly specific for RA and became one of an important diagnostic measure for the disease. The citrullinated proteins could be catalysed by peptidylarginine deiminase (PAD) enzyme, where *P. gingivalis* is the only known bacteria that generates PAD.21 Moreover, it has been reported that people at high risk of RA with positive anti-CCP were having a dysbiotic microbiome, and the *P. gingivalis* was found to be higher in the risk group compared to other groups. Therefore, it is suggested that the *P. gingivalis* infection could contribute to the progression of RA by generating citrullinated proteins via PAD enzymes.22 Others, the glutaminyl cyclases (QC) expressed by *P. gingivalis* also has been proposed to play important role in maintaining inflammatory conditions and destructions of RA, where the QC mRNA was detected more frequently in the gingival crevicular fluid of RA patients.[23]

Although both periodontitis and RA shared the same risk factor which is a cigarette smoking, it has been revealed that the anti-P gingivalis arginine gingipain type B (anti-RgpB) antibody level and RA had even stronger association as compared to the association between smoking and RA. Moreover, the increased anti-RgpB antibody levels, as well as smoking and HLA-DRB1 SE alleles were only observed in ACPAs positive patients only. These findings supported that P. gingivalis is an etiologic agent in RA progression, along with smoking and HLA-DRB1 SE alleles as a well-established risk factor.[24]

(v) Diabetology

Diabetes is commonly associated with periodontal disease and recently *P. gingivalis* was detected at 30% in diabetic patients with periodontal disease.[25] In addition, previous study had suggested that the release of salivary resistin (resist insulin) could be upregulated by high abundance of periodontopathogenic bacteria in obese patients, where *P. gingivalis* was detected at 97.4% associated with high amount of salivary resistin. It is speculated that *P. gingivalis* could trigger the release of salivary resistin due to its lipopolysaccharide (LPS) virulence factor based on previous in-vitro studies.[26]

Previously, *P. gingivalis* was detected at the most top three among periodontopathogenic bacteria at 52.6% in gestational diabetes mellitus (GDM) patients or a pregnant woman who had been diagnosed with diabetes for the first-time during pregnancy with the presence of gingivitis.[27] Besides, recent findings also demonstrated that a high amount of *P. gingivalis*-IgG serum was measured in early diabetic retinopathy patients over 60%.[28] Based on this evidence, *P. gingivalis* may be a risk factor in the development of diabetes, and further studies on the mechanisms on how the bacteria involved in the disease progression are needed.

(vi) Hepatology

Non-alcoholic fatty liver disease (NAFLD) also known as metabolic (dysfunction) associated fatty liver disease (MAFLD). The higher prevalence of *P. gingivalis* infection in the NAFLD patients (46.7%) compared to healthy subjects (21.7%) suggested that the *P. gingivalis* may be involved in the progression of onset of NAFLD. The study findings showed that there is no significant difference in the persistence of diabetes mellitus (DM) was noted between both positive and negative *P. gingivalis* in NAFLD subjects. Thus, it is suggested that the high detection of *P. gingivalis* in NAFLD patients was not due to the presence of DM, as reported by some previous studies due to the correlation between NAFLD and DM. However, there is a significant difference in the persistence of DM between positive and negative *P. gingivalis* among non-alcoholic steatohepatitis (NASH) subjects. These findings suggested that the presence of both DM and *P. gingivalis* may cooperatively contributed to the risk of the progression of NAFLD to NASH. In addition, the prevalence of *P. gingivalis* in NASH patients (52.0%) was higher than NAFLD patients.[29] Interestingly, previous case study has reported that the *P. gingivalis* was detected in the hepatocytes of NASH patients who died from sepsis. Further autopsy found that the NASH patients had progressed to cirrhosis. Therefore, this case suggested that the *P. gingivalis* does contribute to the progression of NASH to cirrhosis.[30]

(vii) Obstetrics

Most findings have been focused on bacterial vaginitis as a primary infection in pregnant women with an adverse pregnancy outcome. However, since pregnant women were more susceptible to periodontal disease, oral anaerobic bacteria had gained much interest among researchers as a distant site of infection that could reach the fetoplacental unit, leading to pregnancy complications. Among oral bacterial species detected, *P. gingivalis* was the most abundant species detected in pregnant women at 56%.[31,32] Recent study

also discovered that a group of pregnant women with positive *P. gingivalis* in their oral swabs were 6.7 times more likely to have a preterm birth compared to those negative *P. gingivalis*, and 2.8 times to have a foetus with intrauterine growth restriction.[31]

B. Detection Methods

(i) Culture-Based Technique

Bacterial culture is considered as a gold standard detection method due to its ability to identify a wide range of unexpected species in the mixed microbial communities in a clinical sample. However, the cultivation of *P. gingivalis* may take several days at minimum of three to four days and need further biochemical tests for identification. Furthermore, *P. gingivalis* is an anaerobic and fastidious organism that needs specific conditions to grow and sometimes are uncultivable. Based on our literature, although the cultivation process was done with proper and adequate precautions, the highest detection rate of *P. gingivalis* through culture was only 44%.[33]

(ii) Direct Detection Methods

This method can visualise the desired organism directly by using a microscope or an optical instrument where the amplification efficiency is not considerable. However, these direct detection methods need a longer time to pre-treat the sample and require an expensive instrument.[34] The highest specificity and sensitivity for direct detection method was 100% for both that was done by a fluorescence in situ hybridisation (FISH) technique. The developed technique is also able to localise the organism and observe the spatial distribution of polymicrobial communities in the clinical sample.[35] However, the FISH technique usually takes around two to three days and takes some time to set up the reaction.[36]

(iii) Nucleic Acid-Based Detection Methods

These identification methods are based on the amplification of single-stranded DNA that binds to its complementary strand.[34] The current nucleic acid-based detection methods for *P. gingivalis* are PCR and a loop-mediated isothermal amplification (LAMP) method. Based on our literature, the detection of *P. gingivalis* by PCR assay has been greatly evolved over time. The study by Gu et al. 2020,[37] had performed a direct qPCR assay without DNA extraction and only took 1.5 hours when compared to DNA extraction-based qPCR (kit-qPCR). Although the specificity was 100%, the positivity of P. gingivalis by direct qPCR was inconsistent and the retest results also showed weak positive to negative results compared to kit-qPCR. These might be due to low levels of target DNA and the presence of inhibitors.[37] Next, a nested PCR for *P. gingivalis* detection involving two primer sets has been developed, where the large fragments outside the targeted DNA were amplified first by one set of primer (first PCR), allowing a specific amplification of the 16s rRNA gene by the second primer set. The protocol had successfully improved the specificity of the amplification by 22.2%.[38]

LAMP is an alternative PCR method that provides a better sensitivity and specificity by using six to eight primers compared to only two primers by PCR. The detection of *P. gingivalis* by LAMP method was proven to be ten times more sensitive than a conventional PCR.[39] Nucleic acid-based methods can be combined each other, such as done by Kitano et al. 2016[40] that demonstrated a great combination between LAMP and PCR assay (LAMP-PCR), and the combination was much more sensitivity where only two or more copies of *P. gingivalis* DNA were needed for the detection, compared to 21 copies by LAMP assay alone.[40] Another successful combination was done by Ge et al.,[41] where a recombinase polymerase amplification (RPA) is combined with a lateral flow strips assay (RPA-LFS), where the amplification time was two times more rapid than qPCR assay, and the accuracy showed 100%.[41]

(iv) Immunology-Based Methods

An immunology-based detection method is a highly portable and rapid method by visualising the antigen-antibody interactions in the clinical specimens.[34] Some developed immunology-based methods for *P. gingivalis* detection include an immunochromatographic device and colorimetric membrane enzyme immunoassay (EIA) technique.[42] The highest sensitivity of immunology-based methods was established by EIA technique where the sensitivity was 100 times more sensitive than other lateral flow immunoassay. In addition, no device is needed for the visualisation as the results can be observed by naked eyes.[43] However, this immunology-based method is at high risk of giving false negative results if the antigens are partially denatured and need pre-enrichment to expose the surface of antigens, thus, extending the detection time. Moreover, this method was also less employed in detecting the desired organism due to its lower sensitivity compared to other molecular techniques.[34]

(v) Biosensor-Based Methods

Biosensor-based detection method is an analytical platform that comprises a bio-receptor to recognise the desired targets. Once recognised, the transducer will convert the bioreaction into a measurable electrical signal such as electrochemical, magnetic, or optical. There are many biosensor devices that have been developed for *P. gingivalis* detection that can process many specimens at once and is suitable for point-of-care detection.[34] The first study of surface-enhanced Raman spectroscopy (SERS) technique for *P. gingivalis* detection has been successfully developed, where the microfluidic and magnetic separation were employed that allows multiple strains of *P. gingivalis* detection. However, the accuracy of the developed protocol was only 89% and needed further optimisations.[44] Based on our overall literature, the magnetic nanobead-based assay is one of a notable assay where the method had the fastest detection rate which is only within 30 seconds with high specificity.[45] Next, an electrochemical biosensor done by Park et al.,[46] had demonstrated that the developed device is highly specific and sensitive, although washing and separating steps were not included in processing their saliva samples, thus reducing the detection time to only 30 minutes.[46] A newly quantitative electrochemical analysis for *P. gingivalis* detection was developed where a portable electrochemical DNA sensor was linked with PCR. This method was primarily developed to quantify the *P. gingivalis* load in an easier way compared to conventional real-time PCR (RT-PCR) by using disposable electrodes, which may reduce cross-contamination. However, this method had a lower dynamic range compared to RT-PCR and the detection limit was just the same as RT-PCR.[47]

LIMITATION AND RECOMMENDATION

This review has some limitations. First, there was no review of the association between the presence of systemic implications and periodontal disease. Second, the mechanism or the way on how the bacterium contributed to systemic implications was not explained clearly. Therefore, we suggested reviewing the association between particular systemic diseases, such as rheumatoid arthritis and periodontal disease, in the future. Lastly, we also recommended a review of the mechanisms involved in the development of the particular systemic diseases due to the presence of *P. gingivalis*.

CONCLUSION

In this review, the modulation effect of *P. gingivalis* on the major clinical diseases, as well as the established detection methods for the bacterium have been summarised. The high prevalence of *P. gingivalis*-associated diseases suggests the need for oral public health awareness and encouragement for oral screening regularly, although there are no apparent symptoms developed. Almost all detection methods were able to detect the desired organism at a fast detection rate, while maintaining the high sensitivity and specificity for more accurate results. Therefore, it is recommended for health practitioners to take oral samples from patients who attend for medical help with a chronic inflammatory disease and employ the suitable detection methods based on availability, convenience, and patients' concern, as each method has its own benefits and drawbacks.

DISCLOSURES

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