Original Article

Prevalence of Anaerobic Bacteria *(P.gingivalis)* as Major Microbial Agent in the Incidence Periodontal Diseases by Meta-analysis

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KEY WORDS
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ABSTRACT

Statement of the Problem: Periodontal diseases are complex oral diseases characterized by bacterial-induced inflammatory destruction of tooth-supporting tissues. *Porphyromonas gingivalis (P. gingivalis)* is a common gram-negative anaerobic oral bacteria strongly associated with periodontal disease.

Purpose: The present study was conducted to estimate prevalence of *P. gingivalis* in patients with periodontal diseases by using meta-analysis method.

Martials and Method: Different databases including PubMed, EmBase, Scopus, the Institute for Scientific Information (ISI) Web of Science, and the Cochrane Library were searched to identify original English-language studies addressing prevalence of *P. gingivalis* in periodontal diseases up to December 2014. The random effects model was applied in the meta-analysis and the heterogeneity between studies was assessed using a Cochran test and the I^2 index. Funnel plots and Egger test were used to examine publication bias. Statistical analyses were performed using STATA version 12.

Results: Forty-two eligible studies published during 1993- 2016 were selected for meta-analysis. Considering all the included studies, the total sample size was 5,884 individuals containing 2,576 healthy people with a mean age of 37.21±7.45 years and 3,308 periodontal patients with a mean age of 44.16±8.35 years. Overall, the prevalence of *P. gingivalis* was 78% [95% CI: 74-81] in periodontal diseases group and 34% [95% CI: 26-41] in healthy individuals. There was a significantly higher prevalence of *P.gingivalis* in individuals with periodontal diseases compared to healthy subjects [78% versus 34%, respectively].

Conclusion: This study indicates that *P. gingivalis* is highly present in subjects with periodontal diseases and it also appears in periodontally healthy people, although to a lesser extent. Thus, the presence of P. *gingivalis* increases the chance of periodontal disease and it can be considered as a main potential risk factor.

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Introduction

Periodontal disease is an infectious clinical entity characterized by the destruction of supporting tissues of the teeth (periodontal ligament and alveolar bone)

and can lead to gum recession, soft tissue damage, bone loss and tooth loss. [1-2] It is also an important risk factor for multiple systemic diseases, such as rheumatoid arthritis and cardiovascular disease, high blood pressure, diabetes, pulmonary disease, pregnancy, cancer and MS in later years of life. The scientific rationale for these problems would probably be related to chronic and long-term aspects of inflammation in periodontal disease. [3-11]

The severity and progression of periodontal disease is influenced by multiple risk factors, including genetic, environmental and host factors. Moreover, polymicrobial biofilms present in subgingival crevices are the most important etiological factor in the pathogenesis of periodontal disease. [12-13] Studies show that the oral cavity is a source of different microorganisms and more than 700 species of bacteria have been detected in subgingival biofilms. [2, 12] Polymicrobial communities develop through interspecies interactions and adaptation within the surrounding microenvironments. [14] Some of the major periodontogenic pathogens are *aggregatibacter actinomycetemcomitans*, *porphyromonas gingivalis*, *prevotella nigrescens*, *fusobacterium nucleatum*, and *treponema denticola*. [15]

The most common bacteria associated with periodontal disease, *porphyromonas gingivalis* (*P. gingivalis*), is a gram-negative obligatory anaerobe, which resides in the mouth and is strongly associated with periodontal disease. [16-20] *P. gingivalis* is one of the species that constitute the red complex group and is the most important in the initiation or progression of periodontal disease. [21-22] The red complex group including *P. gingivalis*, *T. denticola* and *T. forsythia* from unattached subgingival plaque; occur in combination in periodontal pockets, appear in the developing biofilm and are considered the first pathogens involved in the clinical destruction of periodontal tissues in a cooperative manner. [16-22] Of the bacteria believed to be pathogenic in periodontal disease, *P. gingivalis* has been extensively studied due to its unique ability to evade the immune response; [23] as a result, it creates an environment that facilitates dysbiosis of subgingival microbiota, and the dysbiotic microbiota with increased pathogenicity overactivate inflammation in periodontal tissues. [24] *P. gingivalis* deregulates host immune systems by producing a number of virulence factors, such as lipopolysaccharide, fimbriae, and several proteases. [25] The various surface components of *P. gingivalis* enable the bacterium to formation of a biofilm that protects it against the host's defense. [26-27]

Due to the high prevalence and complications of periodontal disease, planning to prevention and treatment of this disease seemed necessary. A major aspect of periodontal disease prevention is the identification of potential periodontal pathogens; in the other hand, determining the risk factors that affect the incidences of periodontal disease is crucial for preventive and management strategies. [28] Since *P.gingivalis* has been known as a major etiological agent in periodontal disease and a risk factor for periodontal disease, it is of particular importance to investigate the prevalence of this periodontal pathogen, which can be an important approach for prevention, and treatment of periodontal disease. In addition, study of prevalence of oral microbes in periodontal patients is an important effort to provide the basic data for further control of the oral complications in these patients. [29] *P. gingivalis* has been extensively studied for well over a century and extensive studies have been conducted to control this pathogen causing dental diseases; in order to authenticate conducted studies, performing a meta-analysis seems to be necessary. Since combination of different studies via meta-analysis leads to a suitable sample size and better resolution, it can provide an overall precise and valid understanding of a desired subject compared to the separated reported studies. Therefore, it seems that assessment of prevalence of *P. gingivalis* in patients with periodontal disease via meta-analysis can be a useful tool for an overall and clear understanding of this disease. [30] The aim of this study was providing an overall summary measure of the prevalence of *P. gingivalis* in patients with periodontal disease by synthesizing available studies.

Materials and Method

Search strategies

This article was written according to the PRISMA guidelines. [31] We performed a literature search of the Scopus, ISI web of Science, PubMed, EmBase, and the Cochrane Library databases for original articles

that present the prevalence or incidence of *P. gingivalis* among patients with periodontal disease from 1993- 2016. The searches were applied by using the keywords *porphyromonas gingivalis*, chronic periodontitis, aggressive periodontitis, gingivitis, and related words; also, the study was limited to the English languages. We also used wildcard symbol '*' and combined the search words or phrases using Boolean operators ('AND', 'OR', 'NOT') and also scanned bibliographies of retrieved articles to expand the search. In addition, relevant original articles noted in the reference lists of each selected article were also evaluated as a further search tool. Furthermore, review articles were manually searched for additional references.

Inclusion and exclusion criteria

All papers with the selected keywords in their titles or abstracts were included in the initial list and other unrelated articles were eliminated. Accordingly, all original articles that reported the prevalence of *P. gingivalis* in periodontal disease were reviewed. The non-human studies were excluded. Studies that were conducted in patients with diseases other than periodontal diseases, non-epidemiologic studies, presented insufficient data, in languages other than English were excluded. In addition, review articles, congress abstracts, meta-analyses or systematic reviews and duplicate publication of the same study were omitted. In the

Table 1: Characteristics of the 48 trials included in the meta-analysis

First author, [Reference]	Country [year of publication]	Case	Control	Mean age		Prevalence of P.g [%]		Type of disease	Sample specimens	Methods of Assay
				Case	Control	Case	Control			
[27]	Jamaica [2000]	35	65	14-18	14-18	77	34	Periodontitis	subgingival plaque	PCR method
[28]	Japan [2003]	35	18	51.8 ±7.29	27.3±3.71	97.1	5.6	chronic periodontitis	subgingival plaque	PCR method
[29]	Brazil [2011]	33	32			35	0	apical periodontitis	purulent exudate aspirates	PCR method
[1]	Japan [2013]	85	20	57.4±13.1	45.9±17.0	65	40	Chronic periodontitis	plaque samples	PCR method
[30]	Korea [2005]	17	19	52±11.1	49±10.2	24.7	27.6	Gingivitis	subgingival plaque	PCR method
[31]	USA [2009	39	40	52±11.1	49±10.2	77	40	Periodontitis	subgingival plaque	stabilization *P. gingivalis* antibody seropositivity
[32]	Ohio State [1998]	130	181	51.4 ± 9.3	49.2± 9	79	25	Periodontitis	subgingival plaque	PCR method
[33]	Brazil [2002]	50	50	45.5 ±9.7	32.3±8.9	70	60	Periodontitis	subgingival plaque	PCR method
[34]	Netherlands [2002]	116	94	42.9 ± 9.8	40.4±11.9	59.5	10.6	Periodontitis	subgingival plaque	Anaerobic cultivation
[35]	Chile [2007]	20	6	27±5.2	22.7±4.9	50	50	Periodontitis/ gingivitis	subgingival plaque	Bacterial culture
[36]	Japan [2013]	139	380			87.1	36.8	Periodontitis	Dental plaque	PCR method
[37]	Japan [2001]	103	20			89.9	10	Periodontitis	Saliva and subgingival plaque	PCR method
[38]	Brazil [2004]	57	25			89.4	8	periodontal attachment loss	subgingivalplaque	PCR method
[38]	Brazil [2004]	20	25			30	8	Gingivitis	subgingival plaque	PCR method
[39]	Chile [2007]	115	136			81.7	22.1	Chronic periodontitis	subgingival samples	PCR method
[40]	Taiwan [2004]	407	91			85.7	23.1	periodontal disease	subgingival plaque	indirect immunofluorescent assay
[41]	Korea [2000]	29	20			96	18	Periodontitis	subgingival plaque	PCR method
[42]	Japan [2000]	29	15			61.8	0	Periodontitis	subgingival plaque	culture
[43]	Italy [2013]	66	46	48.9±18.2	31.6±18.6	52	83	Periodontitis	periodontal pocket	PCR method
[44]	USA [1993]	28	18	18-59	18-59	59	78	Periodontitis	subgingival plaque	ELISA
[45]	Lebanon [2010]	20	20	34.3±5.36	26.10 ±4.57	65	10	Periodontitis	Oral plaque	PCR method
[46]	Germany [2009]	46	21	55.2±11.2	66.6±1.5	76	62	Chronic periodontitis	subgingival plaque	PCR method
[46]	Germany [2009]	44	21	34.4 ± 6.5	66.6±1.5	65	62	Aggressive periodontitis	subgingival plaque	PCR method
[47]	Chine [2009]	48	25	38.9 ± 9.9	23.6±1.8	93.8	32	Periodontitis	subgingivalplaque	PCR method
[48]	Chine [2013]	27	20			96.3	30	Chronic periodontitis	subgingival	PCR and reverse hybridization assay
[49]	Chine [2013]	80	56			93.8	4.7	Aggressive periodontitis	gingival crevicular fluid	PCR method
[50]	Italy [1998]	33	21			56.5	4.7	severe periodontal disease	subgingival plaque	culture
[51]	Chine [2007]	61	30	42.4±8.7	37.35±7.3	62.3	10	chronic periodontitis	subgingival plaque	PCR method
[20]	Spain [2012]	33	37	43.39±7.4	40.68±7.1	66.7	27	chronic periodontitis	subgingival plaque	PCR method
[20]	Spain [2012]	16	37	38.81±6.9	40.68±7.1	37.5	27	Gingivitis	subgingival plaque	PCR method
[52]	China [2013]	25	20			92	35	Periodontitis	gingival crevicular fluid	PCR method
[53]	Colombia [2007]	143	40	39.5±9.85	32.6±10.6	64.3	7.5	Periodontitis	subgingival plaque	culture
[54]	Italy [2012]	127	66	48.9±18.2	31.6 ±18.6	71.7	33.4	Periodontitis	periodontal pocket microbiota	PCR method
[55]	China[2014]	25	29			84	24.1	Periodontitis	subgingival plaque	PCR method
[56]	Thailand[2009]	20	20			95	45	chronic periodontitis	subgingival plaque	PCR method
[57]	Iran [2007]	61	40	43±11	41.35±9.8	83.6	4	chronic periodontitis	subgingival plaque	PCR method
[58]	China [2006]	55	17			81.8	17.6	Aggressive periodontitis	subgingival plaque	PCR method
[59]	China [2005]	152	30			91.5	3.3	chronic periodontitis	Periodontal pocket and gingival sulcus	PCR method
[60]	Spain [2004]	30	30			30	13.3	Gingivitis	subgingival plaque	PCR method
[60]	Spain [2004]	32	30			81.3	13.3	Periodontitis	subgingival plaque	PCR method
[2]	China [2015]	42	32			75	63	chronic gingivitis	Gingival crevicular fluid	PCR method
[2]	China [2015]	95	32			91	63	chronic periodontitis	Gingival crevicular fluid	PCR method
[6]	Japan [2013]	20	10	43.6±11.1	28.7 ± 3.2	75	0	chronic periodontitis	subgingival plaque	PCR method
[10]	Korea [2013]	284	128	48.3±9.5	42.3±13.5	97.5	57.5	chronic periodontitis	subgingival plaque	PCR method
[61]	Germany [2012]	33	20	33.39 ±10.47	37.65±10.88	51.6	10	chronic periodontitis	subgingival plaque	PCR method
[62]	Colombia [2007]	80	30	33.91 ±9.32	26.90± 7.17	79.8	10	Periodontitis	subgingival plaque	PCR method
[63]	Switzerland[2004]	17	33	53.1 ±8.53	26.8±5.3	71	90	chronic periodontitis	subgingival plaque	PCR method

necessary cases, authors were contacted for additional information. The STROBE (strengthening the reporting of observational studies in epidemiology) statement was used for quality control of the studies. [32] Non-qualified studies were excluded.

Data Extraction

Abstracts and full articles were reviewed independently by two of the authors, and if results were discordant, papers were reviewed jointly until the differences were resolved.

The following items were extracted from the studies: first author, year of publication, study location, sample size, sample age, *P. gingivalis* screening method, sample specimens and percentage of *P.gingivalis* in patient and healthy individual (Table 1). Two of the authors independently reviewed the abstracts and full articles and extracted data according to a standard protocol. In which cases the results were discordant, papers were reviewed jointly until the differences were resolved. The data were entered into a standardized data extraction form and entered into Microsoft Excel.

Data Synthesis and Analysis

The main objective of the study was to evaluate the prevalence of *P.gingivalis*; therefore, the binomial distribution was used to calculate the variance of each study, since the prevalence of *P.gingivalis* and the sample number have been extracted in each study. To combine the prevalence of various studies, the average weight was used and each study was weighted in proportion to its variance. The heterogeneity between studies was assessed using a Cochran test and the I2 index. Considering the significant heterogeneity of the studies, the random effects model was applied in the meta-analysis and the findings are described in forest plots (the point estimations and their 95% CI). Sensitivity analyses were also performed. To examine publication bias, Funnel plots and Egger test were used. *p* values <0.05 were considered significant in heterogeneity tests. Statistical analyses were performed using STATA version 12.

Results

In the search process, 172 articles were identified through the literature search. The screening process of studies was completed based on titles, abstracts, and full texts evaluation in the first step and after the initial screening of abstracts and titles, 85 papers were excluded (of these, 24 were on non-periodontal diseases, 61 were unrelated) and 87 papers remained for full-text evaluation. In a secondary screening and after full text review, we excluded another 45 articles (Eighteen studies were non-human (animal) studies, six studies collected insufficient data, five studies was not published in English, seven were duplicated articles, nine studies were retrospective, review and meta-analyses studies. Finally, 42 case-control studies that were published between 1993 and 2015 selected for the final analysis. [1, 2, 12, 16, 26, 33-69] (Figure 1) The characteristics and extracted data from these studies are summarized in Table 1, including quality scores.

Considering all the included studies, the total sample size was 5,884 individuals containing, 576 healthy people, ranging from 14 to 67 years of age

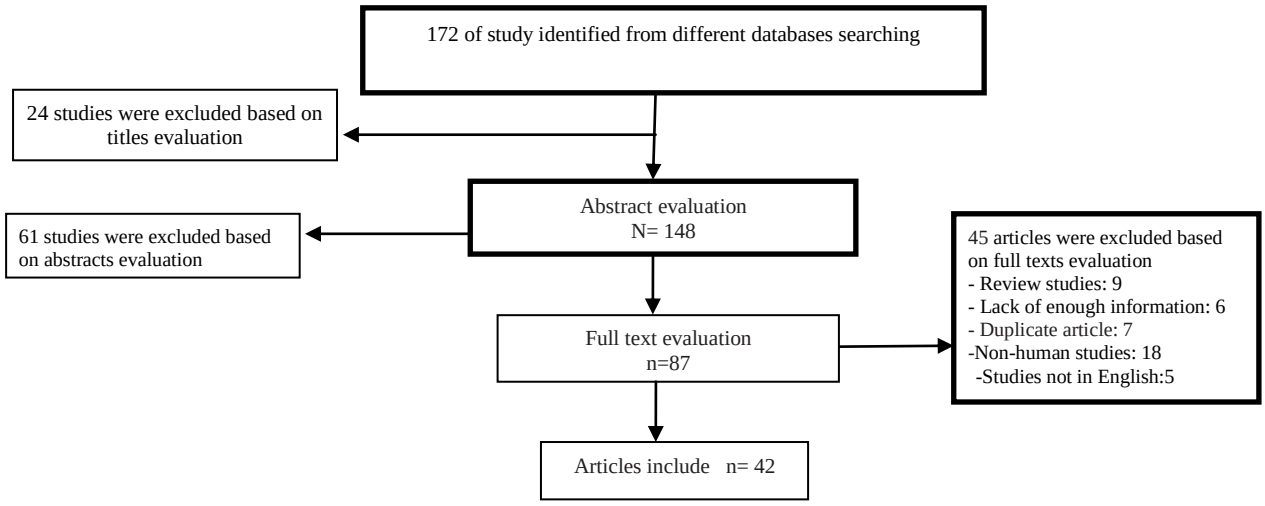

Figure 1: Flow diagram of the studies identified in the systematic review and meta-analysis

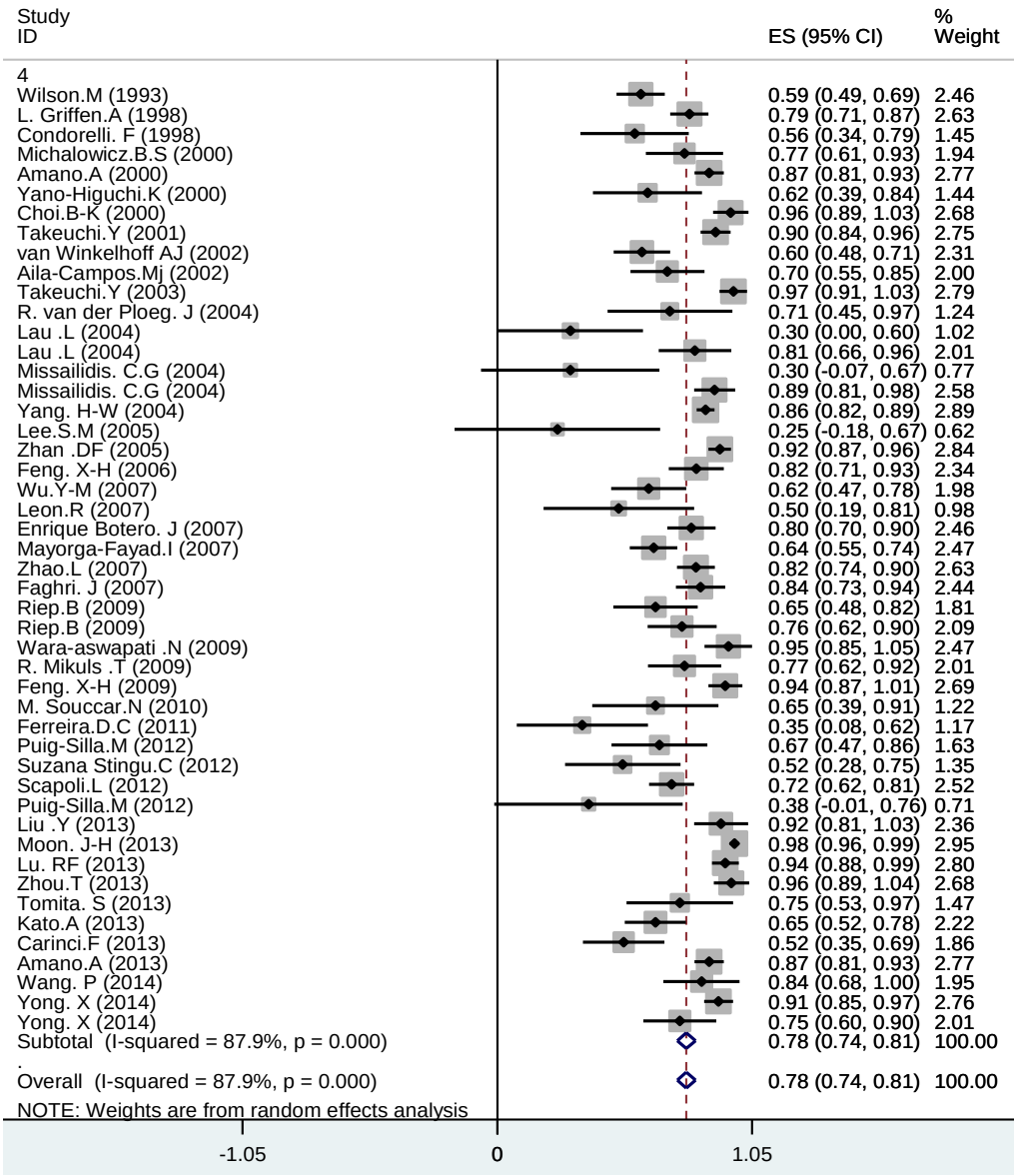

Figure 2: Meta-analysis of the prevalence of *P. gingivalis* in periodontal diseases: studies are sorted in order to their research author's names and publication years

with a mean age of 37.21±7.452 years and 3,308 periodontal patients, ranging from 14 to 59 years of age with a mean age of 44.16±8.35 years.

Among the 42 studies included in this meta-analysis, the prevalence of *P. gingivalis* in periodontal diseases group was 78% (95% CI: 74-81; Figure 2). Considering 45 included studies (three studies were excluded due to major difference in reported prevalence with the other studies), the prevalence of *P. gingivalis* in healthy individuals was 34% (95% CI: 26-41, Figure 3). The significant differences observed between the prevalence of *P. gingivalis* in periodontal diseases and healthy individuals (Figures 2 and 3). As seen in Figures 2 and 3, the prevalence of *P.gingivalis* in periodontal disease was significantly higher compared to healthy subjects (78% versus 34%, respectively).

Figure 4 presents the Begg's funnel plot of the included trials related to the prevalence of *P. gingivalis* in periodontal diseases. No sign of publication bias was observed, when the funnel plot was examined. In fact, most studies were located inside the Funnel Plot, and thus the results of most relevant studies were included into the analysis (p= 0.005). (Figure 4)

Discussion

The present study systematically reviewed the published studies on *P. gingivalis* in periodontal diseases. Periodontal disease comprises a group of conditions that affect gingiva, periodontal ligament, cementum, a-

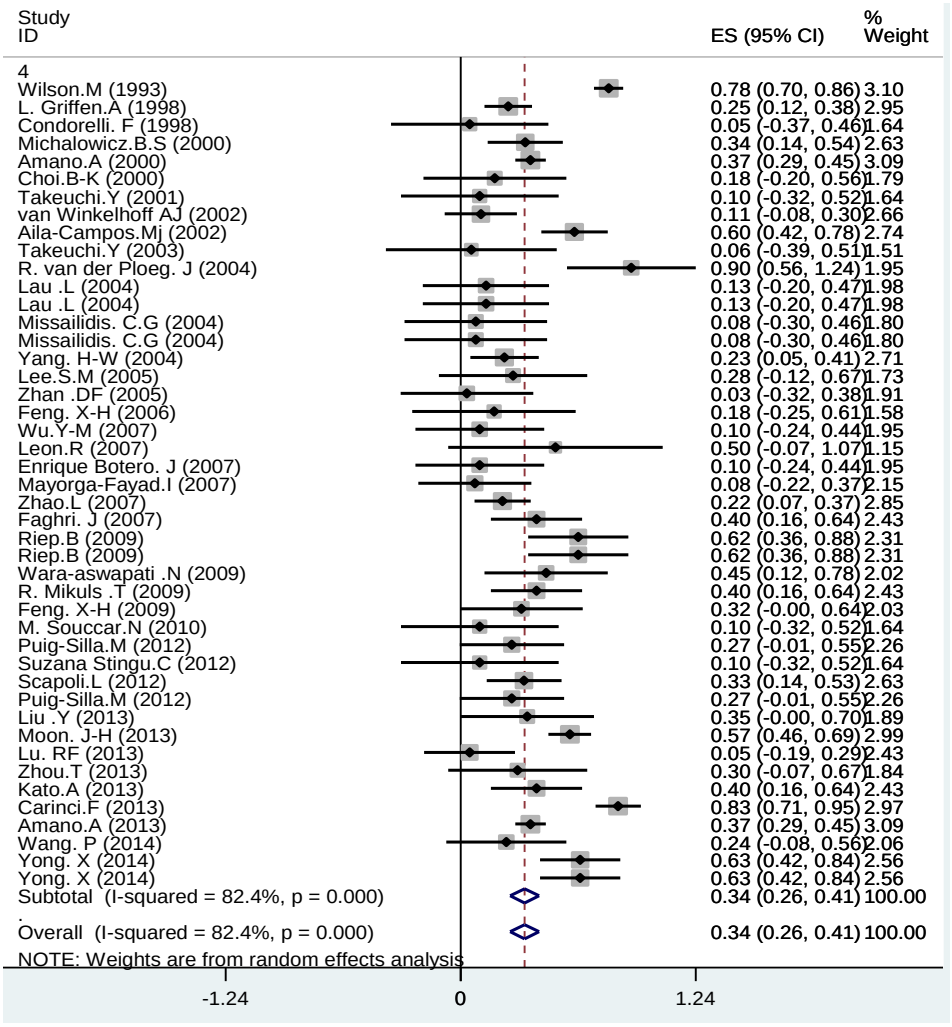

Figure 3: Meta-analysis of the prevalence of *P. gingivalis* in healthy individuals. Studies are sorted in order to their research author's names and publication years

lveolar bone, and tissue structures that support the teeth. [28] Initiation and progression of this disease are influenced by the interaction of a lot of genetic, environmental, host, and microbial factors. [2-3, 10] Studies have revealed unexpectedly high diversity of microorganisms involved in periodontal disease; it has proven that the primary microbial factor contributing to this disease has been a shift in the content of the oral microflora. [49] The content of the microflora, associated with periodontal health and disease, has been intensely studied for well over a century and the available literature in this regard is rapidly growing in scope.

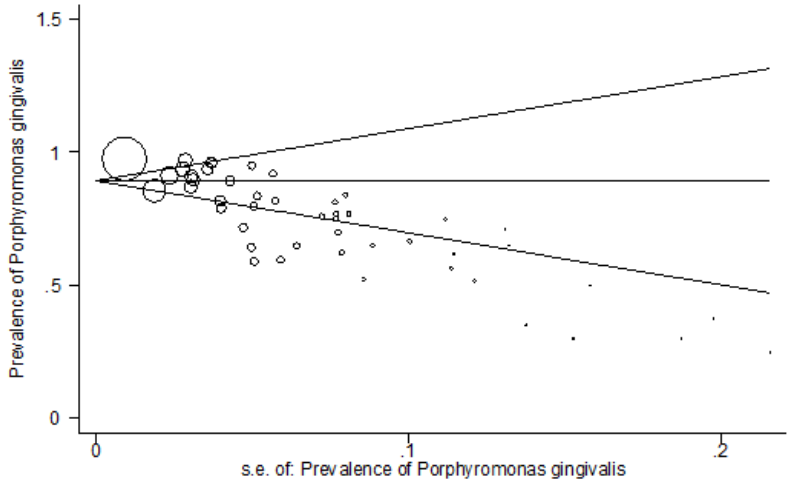

Figure 4: Begg's funnel plot with pseudo 95% confidence limits

[70-71] Since *P. gingivalis* has been known as a major etiological agent in periodontal diseases; we conducted a meta-analysis to comprehensively review previous studies and then quantitatively evaluate the prevalence of *P. gingivalis* in periodontal disease. This systematic review and meta-analysis included 5,884 individuals from 42 case-control studies (all studies received high quality ratings) demonstrated high prevalence of *P. gingivalis* in periodontal disease.

To the best of our knowledge, there are no other meta-analyses similar to ours in terms of comparing the results; however, our findings were consistent with most previous studies that indicated high prevalence of *P. gingivalis* in patients with periodontal disease. The results of our quantitative meta-analysis showed the prevalence of *P. gingivalis* in periodontal disease was 78.7%. The high prevalence of *P. gingivalis* found here is similar to those in other studies examining periodontal disease; they report that *P. gingivalis* is very frequently present in periodontal patients, ranging from 50.3% to 89.4% of cases. [1, 12, 42, 49, 51, 61, 67-68] Our findings and these results suggest that *P. gingivalis* is highly associated with incidence of periodontal disease; this bacterium increases the chance of periodontal disease and may be considered as a main potential risk factor. The authors of various studies on *P. gingivalis* reported that *P. gingivalis* was the marker of a destructive lesion; this pathogen is able to infect soft tissues along with virulence factors, such as lipopolysaccharide, fimbriae, and several proteases, and then flee the surgical debridement of periodontal lesions; this could account for some cases of resistant periodontitis or lesions. [72-73]

Our study also showed that the prevalence of *P. gingivalis* was 34% in periodontally healthy subjects. These results were in accordance with previous studies, which reported *P. gingivalis* appeared in periodontally healthy subjects, ranging from 22.1% to 36.8%. [18, 48] The authors demonstrate that this bacterium does not appear exclusively in periodontal patients but is also present in periodontally healthy individual, although to a lesser extent. [58, 60]

The results of our quantitative meta-analysis provided an overall estimate of the prevalence of *P. gingivalis* in periodontal patients and healthy individuals, and found that both these percentages were in the upper ranges. Whereas, the prevalence of *P.gingivalis* in periodontal disease was significantly higher than healthy subjects (78.7% versus 34%), in accordance with results in other studies examining the prevalence of *P. gingivalis* in both group and concluded that *P. gingivalis* was more prevalent among patients with periodontal disease than healthy people. [35, 58, 74-75] Contrary to our results, some studies showed no differences in prevalence of *P. gingivalis* between the two groups. [52, 76] This discrepancy may be associated with different conditions such as patients' health status and types of periodontal disease. Furthermore, it should be noted the presence of periodontal pathogens in healthy people and patients might indicate that the presence of periodontal pathogens does not necessarily lead to periodontal disease. [2]

We observed that the prevalence of *P. gingivalis* was different in various included studies. Type of strain [ATCC 53978 and ATCC 33277], can be one of the causes of this difference. These two strains are quite distinct as *P. gingivalis* ATCC 53978 has a capsule known as a major antigen associated with pathogenicity of the strain [77] while *P.gingivalis* ATCC 33277 lacks this antigen and is minimally inflammatory. [78]

This meta-analysis had several limitations: First, in a meta-analysis of published studies, publication bias is an inevitable problem. Secondly, we were unable to evaluate the impact of some important factors such as age, gender, smoking and alcohol consumption because of insufficient data; these factors influence the prevalence of *P. gingivalis* and the incidence of periodontal disease because they may affect the ability of the bacteria to invade the gingival tissue and potentially impact the malignant process. Thirdly, the studies varied in types of periodontal diseases and demographic features of population (age, severity, complications) that could have influenced the results. Finally, some studies associated with the prevalence of *P. gingivalis* in periodontal disease were not accessible.

In summary, the results of the present study indicate that *P. gingivalis* is highly present in subjects with periodontal disease and it also appears in periodontally healthy individuals, although to a lesser extent. Thus, this bacterium increases the chance of periodontal disease and it can be considered as a main potential risk

factor. This result suggests that further research is needed to investigate its pathogenicity.

Conclusion

This study indicates that *P. gingivalis* is highly present in subjects with periodontal diseases and it also appears in periodontally healthy people, although to a lesser extent. Thus, the presence of P. *gingivalis* increases the chance of periodontal disease and it can be considered as a main potential risk factor.

Acknowledgments

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Conflict of Interest

There is no benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this article.

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