THE ASSOCIATION BETWEEN PERIODONTAL DISEASE AND PRETERM BIRTH

Nathaniel Weinstein

Department of Medicine
Division of Experimental Medicine
McGill University
Montreal, Quebec, Canada

December 2016

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Experimental Medicine

ABSTRACT

Objectives: While dental health and systemic health were once viewed as two distinct fields of research, recent empirical studies have begun evaluating the potential health-related associations connecting the two health realms. For instance, scientists have discovered biological pathways that may link poor oral health to certain diseases, including diabetes, cardiovascular disease, and adverse pregnancy outcomes. The principal aim of this thesis is to comprehensively examine the relationship existing between periodontal disease and preterm birth. This goal will be completed in two main steps. The first objective is to evaluate this putative relationship by conducting an observational study in a cohort of pregnant Canadian women. The second objective is to systematically assess and summarize the current literature examining whether or not there exists an association between periodontal disease and preterm birth.

Research design & methods: Two separate studies were conducted in order to fulfill the goal of this thesis. The first was a retrospective cohort study. We used a database consisting of 337 pregnant Canadian women recruited for a previously conducted case-control study that examined the association between periodontal disease and preeclampsia. These women were recruited at several hospitals in Quebec, Canada, and included 92 women with preeclampsia and 245 controls. We excluded the preeclamptic cases from our study as they were often induced preterm births, and evaluated the effect of periodontal disease on preterm birth in our new cohort of 245 pregnant women using multivariate logistic regression, adjusting models for maternal age, ethnicity, and income. The second consisted of a systematic review and meta-analysis, which included 37 observational studies assessing the effect of periodontal disease on the incidence of preterm birth. Studies were included if they consisted of an observational design (cohort or case-control), and if the exposure (periodontal disease) was clinically evaluated and diagnosed.

Results: In the cohort of Canadian women, the estimated risk of preterm birth and very preterm birth were, respectively, 1.51 (95% CI 0.61-3.71) and 0.90 (0.09-8.82) times higher in women with periodontal disease compared with periodontally healthy women, although results were not statistically significant. Separately, in the meta-analysis, we identified 842 total studies through our database search, from which we excluded 805 studies that did not meet our inclusion criteria. Out of 37 studies included in the meta-analysis, 26 were case-control studies and 11 were cohort studies. Twenty-four studies suggested that periodontal disease is a risk factor for preterm birth. The pooled OR was 2.20 (95% CI 1.80-2.69). We further stratified the studies by the type of observational study, the country it was conducted in, and the definition used to define the exposure (i.e. periodontal disease). All of the pooled ORs were greater than 1, and demonstrated a significant association.

Conclusion: The combined results of both of these studies suggest that although some individual studies may not be sufficiently powered to display a positive association, the overall literature does suggest such a relationship. The retrospective cohort study did not demonstrate a statistically significant association between periodontal health and preterm birth; however, the small sample size may have failed to provide us with the necessary power to detect an association that may have existed. On the other hand, the meta-analysis provided strong evidence that a relationship between the two variables does exist. Health professionals should use this information to counsel pregnant women about the importance of maintaining good oral health throughout pregnancy and the possible detrimental effects of not doing so. Further research involving treatment of periodontal disease should be undertaken to evaluate if the risk of preterm birth can be reduced in these high risk women.

Keywords: periodontitis; periodontal health; preterm birth; pregnancy

RÉSUMÉ

Objectifs : Bien que la santé dentaire et la santé systémique soient autrefois considérées comme deux domaines distincts de la recherche, des études récentes ont commencé à évaluer les associations potentielles reliant les deux ensemble. Les scientifiques ont découvert des voies biologiques pouvant lier une mauvaise santé buccodentaire à certaines maladies dont le diabète, des maladies cardiovasculaires, et des résultats défavorables de la grossesse. Le principal objet de cette thèse est de bien comprendre la relation existant entre la maladie parodontale et la naissance prématurée. Ce but sera atteint en deux étapes principales ; le premier objectif est d'évaluer cette relation en réalisant une étude observationnelle sur une cohorte de femmes enceintes au Canada. Le deuxième objectif est d'analyser la littérature actuelle étudiant si oui ou non il existe une association entre la maladie parodontale et l'accouchement prématuré.

Conception et méthodes de recherche : Deux études distinctes ont été menées afin d'atteindre le but de cette thèse. La première était une étude rétrospective de cohorte. Nous avons utilisé une base de données composée de 337 femmes canadiennes enceintes collectée pour une autre étude cas-contrôle sur l'association entre la maladie parodontale et la pré-éclampsie. Ces femmes ont été recrutées dans plusieurs hôpitaux au Québec, Canada et incluaient 92 femmes atteintes de pré-éclampsie et 245 contrôles. Nous avons exclu les cas pré-éclamptiques de notre étude, et avons évalué l'effet de la maladie parodontale sur les naissances prématurées dans notre nouvelle cohorte de 245 femmes enceintes par régression logistique multivariée. La deuxième consistait en une méta-analyse qui comprenait 37 études observationnelles évaluant l'effet de la maladie parodontale sur l'incidence des naissances prématurées. Les études ont été incluses si elles consistaient en une conception observationnelle (de cohorte ou cas-témoins) et si l'exposition (maladie parodontale) a été cliniquement évaluée et diagnostiquée.

Résultats : Dans la cohorte des femmes canadiennes, les chances de naissance prématurée et de naissance très prématurée étaient respectivement de 1,51 (IC à 95% = 0,61 à 3,71) et de 0,90 (0,09 à 8,82) fois plus grandes chez les femmes ayant une maladie parodontale comparativement aux femmes parodontalement saines. Ces résultats ont été ajustés pour l'âge de la mère, l'origine ethnique et le revenu. Dans la méta-analyse, nous avons identifié 842 résultats au total dans notre recherche de banques de données desquels nous avons exclu 805 études. Sur les 37 études incluses, 26 étaient des études cas-témoins et 11 étaient des études de cohorte. Vingt-quatre études ont suggéré que la maladie parodontale est un facteur de risque d'accouchement prématuré. Le rapport des cotes combiné était de 2,202 (IC à 95% = 1,801 à 2,692). Nous avons, de plus, stratifié les études par le type d'étude observationnelle, par le pays dans lequel elle a été menée et par la définition qui a été utilisée pour définir l'exposition. Tous les rapports de cotes combinés étaient supérieurs à 1, et ont démontré une association significative.

Conclusion : Les résultats combinés de ces deux études suggèrent que, même si certaines études individuelles ne puissent pas suffisamment afficher une association, la littérature suggère une telle relation. L'étude rétrospective de cohorte ne parvient pas à rapporter quelque association significative. Cependant, la petite taille de l'échantillon de notre étude pourrait avoir empêché de nous fournir le bagage nécessaire pour détecter une association. Entre-temps, la méta-analyse fournit des preuves solides qu'il existe une relation entre les deux variables. Les professionnels de la santé devraient utiliser cette information pour conseiller les femmes enceintes en expliquant les possibles effets néfastes du maintien d'une mauvaise santé buccodentaire. D'autres recherches impliquant le traitement de la maladie parodontale sont nécessaires pour évaluer si le risque de naissance prématurée peut être réduit chez les femmes à haut risque.

Mots clés : parodontite, santé parodontale, naissance prématurée, grossesse

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Haim A. Abenhaim for providing me with the opportunity to complete this master's program under his supervision. His guidance and support has allowed me to develop and explore my own interests, and conduct research in a domain relevant to my future career goals. Working with Dr. Abenhaim has given me the opportunity to become a more independent student and critical thinker, while expanding my areas of interest and expertise. I would also like to thank Dr. Abenhaim's research associate, Dr. Andrea Spence, for her helpful advice and guidance throughout the completion of my degree. I am very grateful for their combined direction in allowing me to recognize my potential.

I would like to thank Dr. Mervyn Gornitsky, the director of dental research at the Jewish General Hospital and a member of my thesis committee, for his mentorship and expertise in the field of dentistry. I would also like to thank Dr. William Fraser for providing me with the data used to conduct my retrospective cohort study. Thank you to Melisa Bohbot for participating in the retrieval of eligible studies to be included in the meta-analysis, to Nicholas Czuzoj-Shulman for his help in learning to use the software SAS, and to Gabriel Ouellette for his help in translating my thesis abstract from English to French.

I would especially like to thank my parents and my sister for their constant support and for always believing that I am capable of achieving my goals. Their love and encouragement has given me the courage to always work hard and be successful in following my dreams.

CONTRIBUTION OF AUTHORS

Nathaniel Weinstein is the primary author of this thesis under the supervision and guidance of Dr. Haim A. Abenhaim.

Nathaniel Weinstein and Dr. Haim A. Abenhaim devised the study concept and design.

Dr. William D. Fraser and his research team collected the data upon which the cohort study is based.

Nathaniel Weinstein conducted all analyses, interpreted the study results, and drafted all manuscripts included in this thesis

The thesis supervisor Dr. Haim A. Abenhaim reviewed and provided critical revision on all aspects of this thesis.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
(a)OR	(adjusted) odds ratio
(a)RR	(adjusted) risk ratio
BOP	bleeding on probing
CAL	clinical attachment level
CI	confidence interval
CPITN	Community Periodontal Index of Treatment Needs
GA	gestational age
GCF	gingival crevicular fluid
LBW	low birth weight
PD	probing depth
PDD	periodontal disease
PPROM	preterm premature rupture of membranes
PTB	preterm birth

CHAPTER 1: INTRODUCTION

The World Health Organization estimates that worldwide, nearly 20% of adults aged 35-44 years old have severe periodontal disease[1]. However, less severe cases of the disease can be found in up to 90% of the population, making periodontal disease one of the most common diseases of the oral cavity[2, 3]. In fact, gingivitis and periodontitis have been described as one of the most prevalent microbial diseases worldwide[4]. Periodontal disease consists of both gingivitis and periodontitis[5]. The former is the mildest form of the disease, consisting mainly of an accumulation of a bacterial biofilm on the teeth, which is referred to as dental plaque[3]. It physically manifests itself in the form of swollen, reddened, and bleeding gums[6], although it is reversible. However, without proper care and treatment, gingivitis may progress into a much more advanced and dangerous form of periodontal disease called periodontitis[6]. Inducing an inflammatory response, this form of the disease can lead to destruction of the attachment and alveolar support of the tooth, and eventually tooth loss[2]. Main treatments of periodontal disease include removal of the plaque via a deep-cleaning method called scaling and root planing, and certain medications may be prescribed in conjunction with the surgery[5]. Recent research in the field of dental medicine has revealed several bacterial species commonly involved in periodontal infection and inflammation, most notably *Treponema denticola*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*[7-10].

The broader health implications of periodontal disease have become apparent as studies have linked periodontal disease with a greater risk of various systemic diseases such as diabetes mellitus, respiratory disease and cardiovascular disease[10-12]. With these findings in mind, it has been surmised that periodontal disease in pregnancy may have an adverse impact on the health of both the mother and the neonate[13, 14].

Preterm birth, defined as any birth occurring prior to 37 weeks of gestation, remains the most common cause of death among children younger than five years of age[15]. There are significant racial, ethnic, and socioeconomic disparities regarding the incidence of preterm birth, with the highest rates occurring in non-Hispanic African Americans[16]. Whereas neonates born before 32 weeks of gestation constitute the majority of preterm-related deaths[17], surviving late preterm children tend to have more long-term cognitive, behavioral and psychiatric problems than children born at term[18].

It is currently theorized that the systemic consequences of periodontal disease are due to the release of inflammatory mediators in response to periodontal infection[10, 19]. Specifically with regards to the putative association between periodontal disease and the incidence of preterm birth, certain substances, such as cytokines, released during inflammation of the gums may diffuse into the amniotic fluid during pregnancy[16, 20]. In fact, analyses of amniotic fluid obtained from pregnant women at the time of preterm labor demonstrated elevated levels of these inflammatory substances[20].

There is a paucity of consistent, reliable information among published studies regarding the effect of periodontal disease on preterm birth due to the variability in study populations and heterogeneity of exposure definitions[21]. As a result, current research findings are inconclusive as to whether periodontal disease is truly a risk factor for preterm birth[22-26]. Certain studies reveal significant associations between the two variables[27, 28]; however, these observations are not consistent throughout the literature as other studies have found no association between periodontal disease and preterm births[29, 30]. This thesis seeks to evaluate this potential relationship, while providing more consistent observations regarding whether periodontal disease is a risk factor for preterm birth.

CHAPTER 2: LITERATURE REVIEW

The following chapter is divided into three seperate sections. The first part describes the clinical characteristics of periodontal disease, including the causes, symptoms and treatment options, while the second section outlines the pathophysiology of preterm birth. Finally, the third section presents studies evaluating the relationship between the two conditions, as well as other adverse pregnancy outcomes which have been shown to be associated with periodontal disease.

2.1 Overview of periodontal disease

2.1.1 Description and symptoms

Periodontal disease, also referred to as gum disease, is a chronic inflammatory disease occuring in the oral cavity, caused by an increase in bacteria along the gumline[2, 3]. Periodontal disease is separated into two distinct conditions based on the stage of progression.

2.1.1.1 Gingivitis

Gingivitis is the more milder form of periodontal disease, and is a lot more common. Gingivitis is characterized by swollen, reddened gums which are more prone to bleeding, usually upon stimulation (i.e. brushing)[6]. Due to a frequent absence of pain, gingivitis can often remain undetected for quite some time[6].

2.1.1.2 Periodontitis

If gingivitis remains untreated, it can progress into a more advanced stage of periodontal disease referred to as periodontitis. The major clinical sign of periodontitis is the pulling away of gums from the teeth, forming pockets. Bacteria often infiltrates these spaces, infecting the area and inciting a host immune response. The bacterial plaque

continues to spread while the immune system fights the toxins, and eventually the alveolar bone and connective tissue supporting the teeth starts to break down. Periodontitis is one of the leading causes of tooth loss in adults[3, 5].

2.1.2 Prevalence

Periodontal disease is one of the most common oral diseases, affecting up to 90% of the general population worldwide[3]. More severe forms of periodontal disease are found in 15-20% of adults between the ages of 35 and 44 years old[1]. In the United States alone, almost 50% of adults have mild, moderate or severe periodontitis. The prevalence rates continue to increase with age, affecting 70% of American adults older than 65 years of age[31]. Although there is little information available regarding the prevalence of periodontal disease in Canada, several studies have estimated that approximately 30% of adults have either severe gingivitis or periodontal disease[32]. Teenagers and young adults rarely develop periodontitis, although they are still at risk of developing gingivitis[5].

2.1.3 Causes and risk factors

The primary cause of periodontal disease stems from a build up of bacterial plaque on the teeth adjacent to the gingiva[2, 3]. Recent studies do suggest that there are environmental, behavioral and genetic risk factors that may contribute to periodontal disease progression[2], such as tobacco use[3] and poor oral hygiene[6]. Certain diseases, such as diabetes and HIV/AIDS, and medications may also increase an individual's risk for developing infections, including periodontal disease[5]. In fact, most forms of periodontitis can be viewed as being an infectious disease[2]. Research in dental medicine has identified numerous anaerobic bacterial species

involved in periodontal disease, such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola*[8, 9].

2.1.4 Dental plaque development

More than 500 species of bacteria can be found in the oral cavity present as a biolfim. The most prevalent oral biofilm is dental plaque, which attaches to the surface of the teeth. As this biofilm continues to mature, it becomes predominated by anaerobic gram-negative bacterial species[2]. The bacteria present in this plaque release various substances, including certain toxins, enzymes and antigens. These compounds initiate an inflammatory response that aims to be protective, however it is also responsible for the loss of alveolar bone and connective tissue[8].

2.1.5 Types of periodontitis

There are two main categories of periodontal disease: chronic and aggressive periodontitis. These two types of disease share many clinical features. They are both caused by an increase in oral bacteria on tooth surfaces, initiating an inflammatory response against the supporting structures of the teeth[33, 34]. Moreover, both types of periodontitis may be diagnosed as localized or generalized. According to the 1999 Classification Workshop, the disease is localized if <30% of the sites/teeth are affected, and generalized if >30% of the sites/teeth are affected[33, 35]. However, there also exist many dissimilarities between the two types.

2.1.5.1 Chronic periodontitis

Chronic periodontitis was once referred to as adult periodontitis because it was mostly found in adults. However, epidemiologic data does suggest that adolescents are sometimes diagnosed with the same form of periodontitis, disproving the idea that only

adults are susceptible to this form. The name was changed to chronic periodontitis, however it remains true that this type of disease often has a later age of onset[33, 35]. Although it is now referred to as being "chronic", it does not mean that this disease is unresponsive to treatment[35]. Furthermore, chronic periodontitis has traditionally been viewed as a slowly progressing disease[34]. Some patients may experience short periods of rapid progression[35], however in general the disease progresses much slower than aggressive periodontitis[33].

2.1.5.2 Aggressive periodontitis

One of the main differences between chronic and aggressive periodontitis is the fact that in the latter, the localized and generalized versions can almost be considered different diseases[34]. In patients with localized aggressive periodontitis, there is only slight clinical inflammation, with a thin biofilm on the affected teeth[34]. In contrast, generalized aggressive periodontitis often causes extreme inflammation and a heavy build up of plaque on the tooth surfaces[34]. In addition, it is more common for younger patients to be diagnosed with aggressive periodontitis compared to chronic periodontitis[33]. This type is also associated with a faster rate of disease progression[33, 35].

2.1.6 Treatment

Earlier detection of periodontal disease allows for easier management and prevention of further damage and tooth loss[6]. Treatment aims to control the bacterial biofilm and arrest disease progression[3]. The principal form of periodontal therapy is removal of dental plaque rather than targeting and eliminating individual pathogens[2]. Dental professionals often use a deep-cleaning method called scaling and root planing. Scaling refers to the scraping off of tartar from above

and below the gum line, while planing involves eliminating the spots on the tooth root where bacteria and toxins gather[5]. Periodontal surgery is often performed in order to reduce the pocket depths by several millimeters. With the use of good toothbrushing techniques and frequent professional toothcleanings, patients should then be able to control and reduce the bacterial load[9].

2.2 Overview of preterm birth

2.2.1 Description

Preterm birth is defined as any birth occurring before 37 weeks of gestation[15]. The condition can be further separated into three distinct categories: moderate/late preterm (32-37 weeks), very preterm (28-32 weeks), and extremely preterm (before 28 weeks)[15]. Affecting an estimated 10% of every infant born in the United States[17], preterm birth is the number one contributor to infant death. Although the majority of preterm-related deaths occur in infants born extremely premature, those born late-preterm are at risk for severe long-term disabilities[17, 18, 36].

2.2.2 Incidence

Worldwide, approximately 15 million babies are born preterm every year, ranging between 5% and 18% of all babies born across 184 countries. Of these preterm infants, almost 1 million die each year due to complications of early birth[15]. This rate continues to increase steadily each year[16], mostly due to a rise in late preterm births, which account for 70% of all preterm births[18]. This increase in the incidence of preterm birth is thought to be due to increasing indicated preterm births and those caused by artifically conceived multiple pregnancies[37].

2.2.3 Causes of preterm birth

Preterm birth is a multifactorial disease, involving many different possible causes and risk factors. These may include behavioral and psychosocial factors, neighborhood and environmental exposures, medical conditions, and genetic and biological factors[16]. Seventy percent of preterm births are caused by spontaenous preterm labor[38]. Other conditions leading to preterm birth include preterm premature rupture of the membranes (PPROM), as well as labor induction or cesarean delivery for maternal or fetal indications. The latter can be caused by pre-eclampsia or eclampsia, and intrauterine growth restriction[37].

2.2.4 Consequences of preterm birth

As previously mentioned, preterm birth is the leading cause of perinatal mortality. However, there is an extreme inequality of survival rates in different countries and settings. In low-income areas, half of all infants born very preterm or extremely preterm do not survive, mostly due to a lack of available care[15]. The World Health Organization estimates that more than three-quarters of preterm infants can be saved with proper cost-effective care[15]. Although the rates of incidence of preterm birth have been steadily increasing throughout the last decade[16], actual global survival rates of these babies is increasing[36]. However, these infants born premature are at much higher risks of developing disorders, including long-term motor, cognitive, visual, hearing, behavioral, socio-emotional, health and growth problems[16].

2.3 Effect of periodontal disease on preterm birth

2.3.1 Possible biological pathways

Researchers have identified three main plausible biological pathways linking periodontal disease with certain systemic conditions. The first mechanism is spreading of infection from the oral cavity as a result of transient bacteremia, the second is injury from the effects of circulating oral microbial toxins, and the third is systemic inflammation caused by oral microorganisms[39-41].

2.3.1.1 Dissemination of infection

Infection can trigger both spontaneous preterm labor and PPROM[37, 42], which are considered two of the leading causes of preterm birth[40]. This theory of bacterial spreading is based on the idea that oral bacteria can infiltrate periodontal pockets, allowing easier diffusion into the bloodstream[20]. This bacteria then reaches the amniotic fluid, causing local infections that can trigger preterm birth[16]. Infection is thought to be the cause of up to 50% of extreme preterm births, which can potentially be prevented by stopping intrauterine infections[16].

2.3.1.2 Metastatic injury

Certain bacteria, especially gram-negative bacteria responsible for periodontal disease, are capable of producing diffusable proteins, namely endotoxins[39, 40]. These endotoxins are compositionally lipopolysaccharides that can have deleterious effects when introduced into the host[39]. They stimulate an increased production of cytokines and prostaglandins, further stimulating preterm labor[40].

2.3.1.3 Systemic inflammation

Throughout a normal pregnancy, hormones and cytokines have an important role in the regulation of labor, uterine contractions, and delivery[43]. Periodontitis is largely characterized by local inflammation, and certain compounds are released into the blood

flow[20]. This includes the secretion of various cytokines, namely prostaglandin E2, tumor necrosis factor-α, interleukin 6 or interleuin 1β. Research shows that in patients with preterm labor, there are elevated concentrations of these substances found in amniotic fluid samples[16, 20]. Once these pathogens reach the uterus, they are capable of inducing an inflammatory response, releasing substances that may cause preterm labor and preterm birth[16, 20].

2.3.2 Evidence for the relationship

Throughout the last decade, there has been an increase in the amount of research focusing on the detrimental systemic effects of periodontal disease. Several of the many outcomes of interest have been adverse pregnancy outcomes[44-46]. Focusing on the effect of periodontal disease on preterm birth, numerous observational studies have evaluated this potential association and concluded that it does, in fact, exist. A prospective cohort study conducted by Tellepragada et al. assessed different risk factors for both preterm birth and low birth weight among pregnant women in India[27]. After conducting both microbiological and clinical investigations for intrauterine infections, as well as dental check-ups for periodontitis, the authors concluded that periodontitis was significantly associated with preterm birth (aRR: 2., 95% CI 1.1-4.9).

Perunovic et al. evaluated the association between periodontal inflammation and labor triggers, particularly elevated cytokine levels, in preterm birth[23]. In their study, Walia et al. outline the mechanism of systemic inflammation due to the release of inflammatory substances from the oral cavity[20]. They concluded that women with preterm birth, compared to women with

term birth, demonstrated worse periodontal disease, as well as increased gingival crevicular fluid (GCF) levels of interleukin 6 and prostaglandin E2.

Other studies have also found a significant association between periodontitis and preterm birth, including Kumar et al.[26], Piscoya et al.[47], and Siqueira et al.[48], who have calculated significant odds ratios of 2.72 (95% CI 1.30-5.68), 6.05 (95% CI 3.01-12.16), and 1.77 (95% CI 1.12-2.59), respectively.

2.3.3 Evidence against the relationship

Although there does exist a fair amount of literature supporting the relationship between periodontal disease and preterm birth, the overall conclusions remain inconsistent as a large number of studies failed to report such an association. For example, in a prospective cohort study published just this year assessing the effect of periodontal disease on advserse pregnancy outcomes, Soucy-Giguère et al. concluded that there is no association between periodontal disease and preterm birth (RR: 2.27, 95% CI 0.74-6.96) or spontaneous preterm birth (RR: 0.90, 95% CI 0.20-4.11)[29].

A separate cohort study conducted by Santa Cruz et al. evaluated this relationship using both clinical and microbiological periodontal parameters[30]. The overall conclusion was that periodontal status had no effect on the adverse pregnancy outcomes evaluated, including preterm birth (OR: 1.325. 95% CI 0.455-3.855). However, the presence of a certain periodontal pathogen, *Eikenella corrodens*, was significantly related to preterm birth ($p = 0.008$).

A lack of significance in the association between periodontal disease and preterm birth has been concluded by a multitude of other studies, including ones conducted by Bulut et al. (OR: 1.48, 95% CI 0.54-4.06)[49] and Ryu et al. (aOR: 1.50, 95% CI 0.74-3.03)[50]. A case-control

study conducted by Macedo et al. attempted to evaluate this relationship using two separate definitions of periodontal disease[51]. The authors found that one of the definitions (PD ≥4 mm and CAL ≥4 mm in 1 or more sites) yielded a significant association, while the other one failed to produce the same results, providing evidence that the relationship between the two variables may vary depending on the chosen exposure definition.

2.3.4 Relationship between periodontal disease and other adverse pregnancy outcomes

Beyond preterm birth, numerous studies have been conducted on the potential effects of periodontal disease on other adverse pregnancy outcomes, including low birth weight and pre-eclampsia[44-46]. Babies born weighing less than 2.5kg/5.5lbs or 1.5kg/3.3lbs are referred to as having a low birth weight, or very low birth weight, respectively. In the U.S., low birth weight and very low birth weight increase a baby's risk of early death by 24 times and more than 100 times, respectively, as compared with normal weight infants[52]. Preeclampsia is a pregnancy-related condition characterized by a rise in blood pressure and potential damage to other organ systems, most often the kidneys. If left untreated, both the mother and the baby are at risk for very serious, even fatal, complications[53]. The literature remains inconclusive regarding these pregnancy outcomes as well, with certain studies providing significant results[44, 54-56], and others failing to report any association[57-59].

CHAPTER 3: RATIONALE, HYPOTHESES AND OBJECTIVES

Preterm birth is the leading cause of neonatal deaths, with a risk of mortality 75 times that of a full term newborn[13]. Each year, close to 15 million babies are born preterm, resulting in the demise of 1 million children due to related complications[15]. This number continues to increase, mostly due to a rise in late preterm births[18, 60]. The rising incidence of prematurity[36] and its detrimental impact on both the mother and the baby, long and short-term[16], underscore the importance of examining the potential relationship between maternal dental health and pregnancy outcomes.

Based on an interpretation of existing literature, we hypothesize that periodontal disease during pregnancy will be related to adverse outcomes for both the mother and the baby, specifically preterm birth. If this hypothesis proves to be correct, study results will allow physicians providing care to pregnant women to properly advise their patients on the importance of oral and dental health throughout the pregnancy. The potential goal of this research is to provide the empirical evidence to encourage pregnant women to maintain a healthy gingiva, ultimately leading to a decrease in the rates of preterm births.

Due to the ambiguity of current literature examining the association between periodontal disease and preterm birth[22, 26, 29, 51], the objective of this thesis is to further evaluate this relationship, and determine if periodontal disease truly is a risk factor for preterm birth. This goal will be completed in two steps. The first is to evaluate this relationship in a cohort of pregnant Canadian women by conducting an observational retrospective cohort study. The second step is to conduct a meta-analysis combining the results of numerous studies pertaining to this topic, calculating a pooled measure of effect and revealing a more accurate estimate of this association.

CHAPTER 4: THE ASSOCIATION BETWEEN PERIODONTAL DISEASE AND PRETERM BIRTH IN CANADIAN WOMEN: A RETROSPECTIVE COHORT STUDY

The following manuscript highlights the first objective of this thesis. An observational retrospective cohort study was conducted in order to assess the effect of periodontal disease on the incidence of preterm birth.

This chapter will be introduced with some background information, followed by a thorough explanation of the methods and materials, including a description of the study population and the statistical methods used. Next, the results will be described, with reference to the associated tables and figures. Finally, a detailed discussion provides important information on the topic of the study, as well as any limitations and future research directions.

The Association between Periodontal Disease and Preterm Birth in Canadian Women: A Retrospective Cohort Study

Nathaniel Weinstein
William D. Fraser, MD, MSc, FRCSC[1]
Mervyn Gornitsky, DDS, FRCDC[2]
Haim A. ABENHAIM, MD, MPH, FRCSC[3,4]

Montreal, Quebec, Canada

[1]Department of Obstetrics and Gynecology, University of Sherbrooke, Sherbrooke, Quebec, Canada
[2]Department of Dentistry, Jewish General Hospital, McGill University, Montreal, Quebec, Canada
[3]Department of Obstetrics and Gynecology, Jewish General Hospital, McGill University, Montreal, Quebec, Canada
[4]Centre for Clinical Epidemiology and Community Studies, Jewish General Hospital, Montreal, Quebec, Canada

The authors report no conflict of interest

Corresponding Author:
Haim Abenhaim, MD, MPH, FRCSC
Jewish General Hospital, Obstetrics & Gynecology, McGill University
Pav H, Room 325
5790 Cote-Des-Neiges Road,
Montreal, Quebec, H3S 1Y9
Tel: 514-340-8222 x 5488 Fax: 514-340-7941
Email: haim.abenhaim@gmail.com

Word count:
Abstract: 282
Main text: 3,062

4.1 Abstract

Background: Studies have linked periodontal disease with a greater risk of various systemic diseases and outcomes, including preterm birth. Current literature on the association between periodontal disease and preterm birth remains inconclusive. The aim of this retrospective cohort study is to further examine this relationship by comparing periodontal parameters among pregnant women who have delivered a full term baby with those who have delivered a preterm (<37 weeks gestation) or very preterm (<32 weeks gestation) baby.

Methods: A retrospective cohort study was conducted using data collected for a previous multi-center case-control study set in Quebec, Canada. This study included 49 pregnant women with periodontal disease and 196 periodontally healthy pregnant women. Periodontal disease was defined using the Community Periodontal Index of Treatment Needs (CPITN) as a pocket depth ≥ 6 mm, with gingival bleeding at the same site. Preterm birth and very preterm birth were defined as gestational age <37 weeks and <32 weeks, respectively. Multivariate logistic regression models, controlling for maternal age, ethnicity, and income, were used to determine the effect of periodontal health on the risk of preterm births through the estimation of odds ratios (OR) and 95% confidence intervals (CI).

Results: Of the 49 women characterized as having periodontal disease, 9 (18%) of them delivered preterm, compared with 28 (14%) of the 196 women without periodontal disease. No statistically significant associations were observed between periodontal disease and preterm birth (OR 1.51, 95% CI 0.61-3.71) or very preterm birth (OR 0.90, 95% CI 0.09-8.82).

Conclusion: Our findings do not support the hypothesis that clinical periodontal disease measured at delivery is an independent risk factor for preterm birth or very preterm birth.

Key words: periodontal disease; periodontitis; preterm birth

4.2 Introduction

The Centers for Disease Control and Prevention estimates that almost half of the American adult population suffers from either a mild, moderate, or advanced form of periodontal disease[31], while the World Health Organization suggests that a severe form of the disease can be found in 15-20% of middle aged adults (35-44 years)[1].

Periodontal disease is characterized by two distinct stages. Gingivitis is a mild inflammation of the gum tissue, manifesting symptoms such as reddened, bleeding gums with little to no discomfort[6, 61]. It is commonly caused by poor oral hygiene, which results in an increase of mainly gram-negative anaerobic bacteria along the gum line[6, 61]. If left untreated, gingivitis may continue to worsen, eventually advancing into periodontitis[61]. At this stage, the chronic inflammatory disease can cause damage to the bone and soft tissue anchoring the teeth, leading to possible tooth loss[6, 61].

Current literature continues to link periodontal disease to various systemic diseases including cardiovascular disease[62], rheumatoid arthritis[63], diabetes[10], and respiratory diseases[11]. Three plausible biological mechanisms have been proposed for the manifestation of this association, including bacterial spreading, inflammatory products dissemination, and the role of fetomaternal immune response against oral pathogens[20], which strengthen the causal nature of these relationships. These associations underscore the importance of advancing research on periodontal disease and its potential consequences on systemic health.

It was been hypothesized that periodontal disease in pregnant women is an independent risk factor for preterm birth, however the literature shows inconclusive results[29, 49, 64, 65]. Preterm birth affects an estimated 10% of births and it is responsible for the greatest contribution toward infant death[17]. Any birth occurring before 37 weeks of gestation is considered to be preterm,

although the majority of the associated deaths occur among infants born before 32 weeks[17]. There is an estimated 15 million preterm births worldwide per year, however this rate continues to rise[15]. Furthermore, while the actual survival of extremely premature infants has increased, these neonates continue to be at risk of developing serious health conditions later on in life[36], mainly neurodevelopmental impairments[16].

Based on the current literature available, we hypothesize that periodontal disease, through one or all of the previously proposed biological mechanisms, increases an expectant mother's risk of giving birth to a premature infant. Hence, the objective of this retrospective cohort study is to determine whether or not there is a significant relationship between periodontal disease and preterm birth, including very preterm birth.

4.3 Materials & Methods

4.3.1 Study Population

The data used for this study was from a case-control study conducted in four hospitals in Quebec, Canada, including Sainte-Justine Hospital and Jewish General Hospital in Montreal, and Saint-François d'Assise Hospital and Saint-Sacrement Hospital in Quebec City[57]. The previous study focused on the possible association existing between periodontal disease and preeclampsia, and included 92 preeclamptic women and 245 non-preeclamptic controls. Women who were eligible to participate in the initial study were at least 18 years of age, nulliparous, and spoke either French or English. Participants excluded from that study were multiparous, had 20 teeth or less, and had one or more of the following conditions: chronic hypertension or hypertension before 20 weeks of gestation, gestational hypertension (without proteinuria), pre-gestational diabetes, heart disorders, a history of fenfluramine-phentermine use, or human

immunodeficiency virus-positive serology[57]. All of the study subjects were recruited by obstetric nurses within 48 hours after delivery. Further, for our present study, we excluded all women who had preeclampsia and included only women who comprised the control group in the original case-control study so as not to introduce any bias given the strong relationship between preeclampsia and preterm birth[66, 67]. Hence, the final cohort for our study consisted of women who did not have preeclampsia while pregnant. From this new cohort, women were categorized into two groups: having periodontal disease and no periodontal disease. Further details regarding our definition of periodontal disease can be found below in subsection 4.3.3. Preterm birth, our outcome of interest, was defined as any pregnancy lasting less than 37 weeks[68]. This group was then further subdivided into women having had a very preterm infant, defined as any pregnancy shorter than 32 weeks[69].

All participants provided information on socio-demographics, health behavior, medical data history, and oral hygiene habits. A postpartum review of hospital medical records provided further information on pregnancy, labor and delivery outcomes. Of the 337 original participants used in the previous study, Saint-Françoise-D'Assise Hospital contributed 23, Sainte-Justine Hospital contributed 248, Saint- Sacrement Hospital contributed 9, and Jewish General Hospital contributed 57[57].

4.3.2 Oral Clinical Examination

Certified dental hygienists, calibrated for measurements of the clinical parameters of periodontal disease by two periodontists, performed oral examinations on all recruited patients within 48 hours after delivery. Using weighted κ scores for assessment, interexaminer and intraexaminer reliability was 85%. Intraclass correlation coefficients were ≥0.90. The dental hygienists and obstetricians were blind to the preeclamptic and periodontal status of the patients,

respectively. Measurements of the clinical parameters of periodontal disease were taken for each patient at six sites per tooth using a periodontal probe. This includes probing depth (PD), gingival recession, clinical attachment level (CAL), and bleeding on probing (BOP). PD is the distance in millimeters between the gingival margin and the tip of the periodontal probe. Gingival recession is the distance in millimeters between the cemento-enamel junction and the gingival margin. CAL is the distance in millimeters between the cemento-enamel junction and the top of the periodontal probe[57].

4.3.3 Classification of Periodontal Disease

In the current study, periodontal disease was categorized using the Community Periodontal Index of Treatment Needs (CPITN). This index was created by the World Health Organization in 1982, and was then adopted in 1985 by the International Dental Federation for use in epidemiological studies and clinical periodontal evaluation[70, 71]. The clinical parameters were used to score each tooth on a scale from 0 (no bleeding or pocketing) to 4 (severe disease, bleeding on probing, pocket depth ≥ 6 mm). The dentition was divided into six sections, each one representing the highest scored tooth as the sextant value. Any individual with at least one sextant score of 4 was considered to have periodontal disease[70, 72]. This is a different scale from that used in the previous study, where periodontal disease was defined as the presence of ≥4 sites with a probing depth ≥5 mm and a clinical attachment loss ≥3 mm at the same sites. Instead, we used the CPITN, a previously-defined diagnostic index, in an attempt to increase the validity of our results.

4.3.4 Statistical Analysis

Statistical analyses began with a comparison of the baseline characteristics of our two groups: subjects with periodontal disease and subjects without periodontal disease. Next, the

relationships between periodontal disease and preterm birth and very preterm birth were examined separately using Multivariate logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). All regression models were adjusted for maternal age, ethnicity, and income, which are potential confounders chosen for adjustment a priori.

As shown in Table 3, there are several different possible definitions for periodontal disease. We examined the varying effects of these distinct periodontal disease definitions on the outcome, preterm birth, using logistic regression models adjusted for maternal age, ethnicity, and income.

All statistical analyses were performed using commercially available SAS Enterprise Guide software (v6.1; SAS Enterprise Guide, Cary, North Carolina). P-values <0.05 were considered statistically significant. Ethics approval for the study was obtained from the Sainte-Justine Research Ethics Committee (See approval letter in Appendix 10.1)

4.4 Results

Our cohort consisted of 245 women, of which 49 had periodontal disease and 196 did not. Table 1 provides demographic, lifestyle, obstetric, and dental characteristics for the study participants. Although there were no significant differences demonstrated between the exposed and unexposed groups, there were certain observable differences between the two groups. On the whole, subjects with periodontal disease tended to be older, less likely to be white, and had a lower annual household income compared with subjects without periodontal disease. The two groups were similar in terms of smoking and alcohol consumption during pregnancy and obstetrical outcomes. Dental-related outcomes, including time since last dental visit, frequency of brushing, presence of bleeding gingiva, and possession of dental insurance during pregnancy, were also comparable between the groups.

Table 2 shows the effect of periodontal disease on the risk of preterm birth. Among women with periodontal disease, 18% had a preterm birth, compared with 14% of those without periodontal disease. The crude OR was calculated to be 1.35 (95% CI 0.59-3.09), revealing no statistically significant difference between the two groups. After controlling for potential confounding, the odds of giving birth preterm were 1.51 times higher in women with periodontal disease than in women without periodontal disease (aOR: 1.51 [95% CI 0.61-3.71]). Although the confidence interval spans 1 and is therefore concluded to be not significant, an adjusted point estimate of 1.51 may suggest a potential positive association. Logistic regression was also used to evaluate the effect of periodontal disease on very preterm birth. After controlling for the same variables as the previous analysis, no association was found (aOR: 0.90, 95% CI 0.09-8.82).

Table 3 displays the effect of periodontal disease on preterm birth using 6 different definitions for periodontal disease. The included definitions do not show that periodontal disease is a risk factor for preterm birth. However, the analysis using the AAP definition of periodontal disease (definition 5) did find a greater risk of preterm birth with poor dental health, although the estimate did not reach statistical significance. Even though the odds ratios from the models using the other definitions of periodontal disease were all below the null value of one, there is no reason to suggest that periodontal disease would be a protective factor for preterm birth.

4.5 Discussion

The main objective of our study was to assess the association between periodontal disease and preterm birth. We used a cohort of pregnant Canadian women previously defined for an earlier case-control study on the effect of periodontal disease on the incidence of preeclampsia[57]. For our study, we restricted our study population to the women who comprised the control group

of the earlier study; i.e. pregnant women who did not have preeclampsia. Our results, although not statistically significant, are suggestive of an association between periodontal disease and preterm birth.

The oral cavity contains many diverse species of microbes. Combined with mucus and other substances, they can adhere to the tooth surfaces and multiply, eventually causing gum disease, such as gingivitis, if left untreated[73]. Moreover, certain exposures, including smoking, genetic predispositions, and hormonal fluctuations, may further contribute to the development and progression of gum disease[61]. Once gingivitis has advanced into periodontitis, pockets form between the tooth and the gums. Bacteria is then able to infiltrate and infect these spaces and potentially cause tooth decay near the roots of the teeth[6].

Endocrine functions, specifically the action of sex hormones estrogen and progesterone, can have significant effects on an individual's periodontium through the hormones' influence on different cells of the gingiva, inducing cellular proliferation, differentiation and growth in target tissues[74]. Pregnancy causes an increase in the production of sex hormones, which can increase inflammation of the gingiva. As such, pregnant women are at a higher risk of progressive gingivitis[75].

An estimated 70% of preterm births are due to spontaneous preterm labor, caused by numerous pathological mechanisms, making it a challenge to determine proper methods of prevention and treatment[38]. Spontaneous preterm labor and preterm premature rupture of the membranes (PPROM), another cause of preterm birth, can both be caused by infection and inflammation[37, 42]. Inflammatory events are relatively common occurrences in normal pregnancies, however the level of inflammation appears to increase in preterm deliveries[13].

Periodontal disease in pregnant women has been linked to the onset of preterm birth and

other adverse maternal and fetal outcomes, including pre-eclampsia and low birth weight[44, 47, 64, 76]. Certain biological mechanisms have been proposed to explain the manifestation of such outcomes, including the translocation of periopathogenic bacteria to the fetal-placental unit[43, 55]. The affected periodontal tissues in pregnant women may harbor bacteria that can circulate to extra-oral sites, such as the fetal-placental unit, activating inflammatory signaling pathways that can cause premature labour[56]. A systematic review conducted by Stadelmann et al. concluded that there exists a positive association between inflammatory mediators present in gingival crevicular fluid (GCF) and adverse pregnancy outcomes. However, due to the heterogeneity among the studies included, further research that allows for appropriate analyses is required[45].

When examining the relationship between periodontal disease and preterm birth, Basha et al.[64], Alchalabi et al.[44], and Piscoya et al.[47] reported significant positive associations with odds ratios of 4.54 (95% CI: 1.98-5.46), 4.4 (95% CI: 1.7-11.7), and 6.05 (95% CI: 3.01-12.16) respectively. A systematic review conducted by Ide and Papapanou further synthesized current research on this association. Although they observed significant results, it was concluded that different exposure definitions based on periodontal disease being either a categorical or a continuous variable impacted the strength of the findings[21].

Furthermore, it has been suggested that providing periodontal treatment to women during their pregnancy may reduce the risk of such adverse events[77]. This hypothesis also continues to produce conflicting results[78-80], contributing to the ambiguity of the relationship between periodontal health and adverse pregnancy events. A meta- and trial sequential analysis conducted by Schwendicke et al. observed that, for populations with moderate occurrence of preterm birth and low birth weight, treatment of periodontal disease during pregnancy did not improve any of the outcomes. However, for populations with high occurrence (>20%), the results were

significant for preterm birth (OR 0.42, 95% CI 0.24-0.73) and low birth weight (OR 0.32, 95% CI 0.15-0.67). Overall, periodontal therapy had no significant effect on preterm birth (OR 0.79, 95% CI 0.57-1.10) or low birth weight (OR 0.69, 95% CI 0.43-1.13)[81]. These results were supported by another meta-analysis of 13 randomized controlled trials, which suggested that periodontal care during pregnancy had a non-significant effect on the reduction of preterm births[82].

Other studies have begun studying different oral pathogens commonly found in patients with periodontal disease[83]. Several articles suggest significant associations between the presence of certain periodontopathogens and the occurrence of preterm birth, low birth weight and pre-eclampsia[7, 27, 84]. However, a cross-sectional study conducted by Martinez-Martinez et al. found no association between periodontal disease, periodontopathogens, and preterm birth, suggesting that the exposures are not sufficient to produce the multifactorial condition that is preterm birth[22]. The main issue with this approach is that periodontal pathogens are used as a proxy for periodontal disease, and no clinical assessments are involved in the diagnosis. Since the presence of periodontopathogens may not necessarily imply the presence of clinically-diagnosed periodontal disease, associating the former with adverse pregnancy outcomes has little relevance to the possible effects of periodontal disease. Mitchell-Lewis et al. demonstrated this idea in a study examining the relationship between periodontal infection and preterm low-weight birth. When comparing the case and control groups, no differences were reported in clinical periodontal status. However, the preterm low-weight birth mothers had significantly higher levels of two periodontopathogens, *Bacteroides forsythus and Campylobacter rectus*[85].

One possible factor for the varying results across studies is the use of different definitions of periodontal disease. Since there is no universal clinical diagnosis criteria, researchers often

use their own definitions based on the different clinical parameters of periodontal disease, including probing depth, clinical attachment level, and bleeding on probing[55, 57, 86]. A study conducted by Macedo et al. assessed periodontal disease according to two different definitions: at least 4 teeth with ≥ 1 site showing a PD of ≥ 4 mm and CAL of ≥ 3 mm, and ≥ 1 site with a PD and CAL of ≥ 4 mm. The results varied according to the definition used, and a significant association was reported between periodontal disease and preterm birth for only the second definition (OR adjusted = 1.98; 95% CI = 1.14-3.43; p = 0.015)[51]. This reveals the importance of the chosen definition of an exposure variable when studying its effect on a specific outcome. This notion is demonstrated in Table 3 of this study. Using different definitions will produce varying odds ratios when evaluating the effect of periodontal disease on any outcome. Although the vast majority revealed similar conclusions regarding the significance of the association, the individual effect measures remained inconsistent.

In addition, the study population may alter the effect of poor dental health on pregnancy outcomes. According to our literature review, the vast majority of studies displaying significant positive associations between periodontal disease and adverse pregnancy outcomes were conducted in developing countries, and included mostly non-white women of lower socioeconomic status[44, 47, 51, 76]. On the contrary, our study population was predominantly white females, making up 74% of the exposed group and 87% of the unexposed group. The lack of a significant association in our study may represent a dissimilarity that exists between different populations when examining this relationship. This suggests that socioeconomic status and free/affordable access to dental health care may cause a difference in the effects of periodontal disease on pregnancy outcomes.

There are several limitations that exist in this study. First, the sample size was relatively

small, affecting the power of our study. Second, as mentioned in the above paragraph, the majority of the women included in this study were white. Due to this lack of ethnic diversity among the included patients and the strong effect of study population on the association between periodontal disease and preterm birth, our results can only be generalized to a Caucasian population. However, there are also certain strengths of our study, including the great number of baseline characteristics and clinical parameters measured for each participant. The researchers collecting the data gathered information regarding the patients' age, ethnicity, income, smoking and alcohol habits, parity, history of induced abortions, mode of delivery, birth weight, last dental visit, frequency of brushing, bleeding gingiva, and dental insurance during pregnancy, among others. In addition, detailed periodontal clinical parameters were provided for each of the study participants, allowing periodontal disease to be measured using several distinct definitions.

4.6 Conclusion

The findings of this study reveal that there is no significant association between maternal periodontal disease and preterm birth. However, given that our adjusted odds ratio was calculated to be 1.51, the results allude to the possibility that a positive association between periodontal disease and preterm birth may exist. Further examinations should be conducted using larger sample sizes in order to properly measure this association.

Table 1: Demographic, Lifestyle, Obstetric, and Dental Characteristics in Pregnant Women with and without Periodontal Disease

Characteristics	Periodontal disease (n = 49) (%)	No periodontal disease (n = 196) (%)
Maternal age (years)		
18 to 25	7 (14)	53 (27)
26 to 33	34 (70)	106 (54)
≥ 34	8 (16)	37 (19)
Race/Ethnicity***		
White	36 (74)	167 (87)
Black	4 (8)	7 (4)
Asian	4 (8)	4 (2)
Hispanic	4 (8)	6 (3)
Other	1 (2)	7 (4)
Annual Household Income		
< $20,000	6 (12)	12 (6)
$20,000 to $34,999	7 (14)	21 (11)
$35,000 to $49,999	3 (6)	26 (13)
$50,000 to $74,999	10 (21)	20 (10)
≥ $75,000	17 (35)	87 (45)
Refuse to answer or do not know	6 (12)	30 (15)
Smoking during pregnancy		
Yes	7 (14)	25 (13)
Alcohol consumption during pregnancy		
Yes	20 (41)	73 (37)
Multiple births		
Yes	1 (2)	5 (3)
History of induced abortions		

Yes	11 (22)	45 (23)
Mode of delivery		
Cesarean section	15 (31)	56 (29)
Birth weight (g)		
<2,500	5 (10)	25 (13)
2,500 to 3,000	13 (26)	36 (18)
>3,000	32 (64)	137 (69)
Last dental visit (months)**		
< 6	18 (38)	74 (38)
7 to 12	15 (31)	67 (35)
13 to 24	11 (23)	34 (17)
> 24	4 (8)	19 (10)
Frequency of brushing		
Two or more times a day	41 (84)	158 (81)
Once a day	8 (16)	35 (18)
Sometimes per week	0 (0)	3 (1)
Bleeding gingiva during pregnancy		
Always	12 (24)	25 (13)
Often	15 (31)	49 (25)
Occasionally	14 (29)	87 (44)
Never	8 (16)	35 (18)
Dental insurance during pregnancy*		
Yes	32 (71)	118 (62)

*There was information missing on ethnicity from 5 unexposed women
**There was information missing on last dental visit from 1 exposed and 2 unexposed women
***There was information missing on dental insurance from 4 exposed and 6 unexposed women

Table 2: Preterm Birth in Pregnant Women with and without Periodontal Disease, Stratified by Gestational Age				
Gestational Age	**Periodontal disease (n = 49) (%)**	**No periodontal disease (n = 196) (%)**	**Crude OR (95% CI)**	**Adjusted OR[+] (95% CI)**
< 37 weeks	9 (18)	28 (14)	1.35 (0.59-3.09)	1.51 (0.61-3.71)
< 32 weeks	1 (2)	6 (3)	0.66 (0.08-5.61)	0.90 (0.09-8.82)
[+]Adjusted for maternal age, ethnicity, and income Abbreviation: GA, gestational age				

Table 3: Preterm Birth in Pregnant Women with and without Periodontal Disease Using Multiple Exposure Definitions

Periodontal disease	Periodontal Disease n (%)	No Periodontal Disease n (%)	Crude OR (95% CI)	Adjusted OR[+] (95% CI)
Definition 1 ≥4 sites with PD≥5mm & CAL≥5mm	13 (14)	24 (16)	0.87 (0.42-1.80)	0.84 (0.39-1.81)
Definition 2 >30% bleeding sites	8 (14)	29 (16)	0.87 (0.38-2.03)	0.69 (0.28-1.72)
Definition 3 ≥90 sites with CAL≥3mm	14 (15)	23 (15)	1.02 (0.50-2.09)	0.96 (0.44-2.08)
Definition 4 Offenbacher's definition[87]	13 (14)	24 (16)	0.87 (0.42-1.80)	0.84 (0.39-1.81)
Definition 5 AAP definition[88]	7 (18)	30 (15)	1.28 (0.52-3.17)	1.78 (0.66-4.79)
Definition 6 ≥4 sites with PD≥5mm & CAL≥3mm	13 (14)	24 (16)	0.87 (0.42-1.80)	0.84 (0.39-1.81)

[+]Adjusted for maternal age, ethnicity, and income

Abbreviations: AAP, American Academy of Periodontology; CAL, clinical attachment level; PD, probing depth

CHAPTER 5: PERIODONTAL DISEASE AND PRETERM BIRTH: A META-ANALYSIS

The following manuscript addresses the second objective of this thesis. An evaluation of the current data available pertaining to the association between periodontal disease and preterm births was conducted using a meta-analysis.

This chapter will be introduced with some background information, followed by a thorough explanation of the methods and materials used, and the included observational studies. Next, the results will be described, including an overview of the statistical methods used and a description of the associated tables and figures. Finally, a detailed discussion provides important information on the topic of the study, as well as any limitations and future research directions.

Periodontal Disease and Preterm Birth: A Meta-Analysis

Nathaniel Weinstein
Melisa Bohbot
Mervyn Gornitsky, DDS, FRCDC[1]
Andrea R. Spence, PhD[2]
Haim A. ABENHAIM, MD, MPH, FRCSC[2,3]

Montreal, Quebec, Canada

[1]Department of Dentistry, Jewish General Hospital, McGill University, Montreal, Quebec, Canada
[2]Centre for Clinical Epidemiology and Community Studies, Jewish General Hospital, Montreal, Quebec, Canada
[3]Department of Obstetrics and Gynecology, Jewish General Hospital, McGill University, Montreal, Quebec, Canada

The authors report no conflict of interest

Corresponding Author:
Haim Abenhaim, MD, MPH, FRCSC
Jewish General Hospital, Obstetrics & Gynecology, McGill University
Pav H, Room 325
5790 Cote-Des-Neiges Road,
Montreal, Quebec, H3S 1Y9
Tel: 514-340-8222 x 5488 Fax: 514-340-7941
Email: haim.abenhaim@gmail.com

Word count
Abstract: 289
Main text: 3,203

Key words: preterm birth; periodontal disease

5.1 Abstract

Background: Periodontal disease continues to be linked to preterm birth, although the literature is conflicting. Oral infections may contribute to systemic infection, which may play a role in the rupture of membranes precipitating early labor. Our objective is to summarize the evidence regarding the effect of periodontal disease on preterm birth using a meta-analysis and review.

Methods: A total of 842 studies were identified by conducting searches through PubMed and Medline databases using the keywords "periodontal" and "preterm birth". Random-effects models were used to determine pooled odds ratios for the risk of preterm birth.

Results: We excluded 805 studies due to pre-established criteria. Twenty-six case-control studies and 11 cohort studies evaluating the effect of periodontal disease on preterm birth were included in this meta-analysis. Twenty-four of the studies suggested that periodontal disease is a risk factor for preterm birth, while 13 studies failed to report such a relationship. The calculated pooled OR was 2.61 (95% CI 2.06-3.31). We then stratified the studies by study design (case-control versus cohort study), study location (developed versus developing country), and by periodontal disease definition. All of the strata-specific pooled ORs demonstrated a statistically significant association between periodontal disease and preterm birth, with higher effect measures among cohort studies (OR: 2.89, 95% CI 1.69-4.97), studies conducted in developing countries (OR: 3.29, 95% CI 2.34-4.62), and studies defining periodontal disease using Definitions 1 (OR: 2.66, 95% CI 1.90-3.71) and 4 (OR: 6.14, 95% CI 3.35-11.28).

Conclusion: The findings from this meta-analysis suggest that periodontal disease in pregnant women is a risk factor for preterm birth. The pooled odds ratio remained significant irrespective of study type, study population and exposure definition. Studies evaluating preventative and treatment strategies in these high risk women are necessary.

5.2 Introduction

Oral health is an essential component of one's general health that can have a profound impact on overall quality of life. Periodontal disease is estimated to affect, to some degree, an estimated 47% of adults aged 30 years and older in the United States[89], and more than half of all adults in the United Kingdom[90]. Gingivitis is a mild form of periodontal disease characterized by gums that are inflamed, red, and bleed easily upon brushing[10]. The main cause of gingivitis is oral bacteria, mainly gram-negative anaerobic bacteria, which, along with mucus and other substances, form a plaque that builds up on teeth[3]. If left untreated, gingivitis can progress into a more serious form of periodontal disease called periodontitis. This disease is characterized by gums pulling away from the teeth, creating pockets in which debris collect and eventually gums can become infected. A combination of the toxins produced by the bacteria in plaque and the body's immune response can lead to the breakdown of bone and connective tissue supporting the teeth[5, 10]. Disease progression produces deepening of the pockets surrounding the teeth and further destruction of bone and gums, resulting in loosening of teeth and eventual tooth loss. Main treatments include removal of the plaque via a deep-cleaning method called scaling and root planing, and certain medications may be prescribed in conjunction with surgery[5].

Throughout the last decade, there has been an increase in studies evaluating the association between poor dental health and adverse pregnancy outcomes, both for the mother and the newborn. Preterm birth, defined as any birth occurring before 37 completed weeks of gestation[16], is the number one cause of newborn deaths due to developmental immaturity and the associated increased risk of illness and disability. In fact, in the U.S., it is estimated that the risk of early death in preterm infants is 75 times higher as compared with normal term infants[13]. Globally, approximately 15 million babies are born prematurely each year[15]. Although the survival rate of

premature babies has increased over the last 10 years, the rate of preterm births has also increased[36].

Although the specific etiology of preterm birth remains unclear, it is surmised that oral infections may contribute to maternal systemic infection, which may play a role in the rupture of membranes precipitating early labor[4]. Despite the current empirical evidence and a plausible biological mechanism, current data is not considered conclusive[56]. Moreover, while there have been several meta-analyses studying the effect of periodontal disease on adverse pregnancy outcome, there is a lack of studies specifically evaluating preterm birth as an outcome.

5.3 Materials & Methods

We used two computerized bibliographic databases, PubMed and Medline, to conduct an extensive search for the studies included in this meta-analysis. Two researchers (NW and MB) independently searched the databases to June 7, 2016 using the keywords "periodontal" and "preterm birth". Publications were restricted to epidemiologic studies involving human subjects and written in English or French. There were no restrictions in terms of year of publication. This produced a total of 476 studies in PubMed and 366 studies in Medline. Figure 1. outlines the results of our search scheme using PubMed. Starting with the PubMed search, we reviewed the titles and abstracts, selecting relevant studies for inclusion in our meta-analysis. All studies selected by both researchers were kept for further analysis. In the first round of selection, 106 studies were kept. The other 370 studies were excluded for one or more of the following reasons: if they were review articles or meta-analyses (132), if the article was not in English or French (2), if the study was not conducted on humans (6), if the entire article was not available and there was insufficient information in the abstract (11), and if the topic was not relevant to our meta-

analysis (219). The latter includes studies not evaluating the association between periodontal disease and preterm birth, studies without clinically-defined measures of periodontal disease (such as using periodontal pathogens or self-reported measures of gum disease), and studies including treatment of periodontal disease, among others. In the next round of selection, we read through full articles and further excluded another 59 studies. They were not included in this analysis for some of the same reasons already stated, as well as the inclusion of other pregnancy outcomes in the case group, a lack of proper information to extract an odds or risk ratio, and no comparison group. Finally, a third round of selection was conducted on the remaining 47 studies. Ten more studies were deemed ineligible and were thus excluded due to missing study sample information required by the chosen statistical software.

Next, we screened the records identified through the Medline search for any new, relevant studies to include. Out of 366 results, 109 were reviews or meta-analyses, 17 were neither written in English or French, 26 were not relevant, 6 were not conducted using humans. The remaining 207 studies were duplicates found in PubMed and had been part of the selection process described above. No further articles were included in the meta-analysis.

Any discrepancies that arose between the primary reviewers were brought to a third independent researcher (HAA) who made the final decision. Odds ratios and risk ratios were extracted from each study. If neither were present, an odds ratio was calculated using data from the study. Other information, including study type, sample size, periodontal disease definitions used, and study conclusions, was also independently extracted from each selected study.

Several of the included studies provide multiple analyses using different definitions previously defined in published literature. In order to avoid including the same study sample multiple times in the meta-analysis, only the definition most widely used in the literature was

kept for analysis. The studies containing multiple definitions were those conducted by Macedo et al., Martinez-Martinez et al., Guimaraes et al, and Martinez de Tejada et al. The chosen definitions are highlighted in Table 1.

5.3.1 Statistical Analysis

The software OpenMeta[Analyst] was used to determine the pooled effect estimates. The results were graphically presented by means of forest plots. The I^2 statistic test was used to test the heterogeneity between the individual studies, evaluating the percentage of variation existing across the studies due to heterogeneity rather than chance[91, 92]. A higher I^2 percentage is indicative of increasing heterogeneity, and a random-effects model was deemed appropriate to use in calculating a pooled OR and 95% confidence interval[91, 92]. This model was selected due to the variability between the selected studies caused by different study populations and diagnostic criteria[93]. An overall pooled odds ratio was obtained for all 37 included studies and then sensitivity analyses were performed by stratifying data by study type (case-control, cohort) and study location (developed country, developing country). Further, due to the wide array of clinical characteristics used to define periodontal disease created by the researchers of each study, we stratified the studies into 4 broad subgroups. The first, referred to as Definition 1, consists of any combination of probing depth (PD) and clinical attachment level (CAL), where PD ≥4 mm and CAL ≥3 mm. Definition 2 includes any studies defining periodontal disease using only the probing depth, while Definition 3 is used for the studies defining periodontal disease using only the clinical attachment level. Finally, Definition 4 is used for the remaining studies, where periodontal disease is defined as either gingival recession, PD and CAL less than 4 mm and 3 mm respectively, or where no detailed definition is available.

5.4 Results

Table 1 outlines the 37 studies used for the total meta-analysis, including 26 case-control studies and 11 cohort studies. These studies were conducted between 2001 and 2016, and the sample sizes range from 59 to 3576 pregnant women.

All of the included studies examined the association between periodontal disease and preterm birth. The majority of the studies (24) concluded that there does exist a positive relationship between these two variables, while the remaining 13 studies failed to report such an association. It should be noted though that all included studies, except for two, had point estimates greater than 1.0. The effect measures reported in the individual studies varied widely from 0.55 to 137.50, resulting in an inability to come to a definitive conclusion regarding this relationship. Figure 2 shows a forest plot of the pooled results of all of the included studies. It was found that the odds of preterm birth in pregnant women with periodontal disease are 2.61 (95% CI 2.06-3.31) times the odds of preterm birth in healthy women. This result supports the idea that periodontal disease is in fact associated with preterm birth.

For further specification, we stratified analyses by type of study (case-control studies and cohort studies). These results are presented in Figure 3 and Figure 4, respectively. For the pooled odds ratio of all of the case-control studies, the odds of giving birth preterm are 2.53 (95% CI 1.93-3.31) times higher in women with periodontal disease than in women with healthy gingiva. Moreover, in cohort studies, the pooled odds are 2.89 (95% CI 1.69-4.97) times higher in women with periodontal disease. We also decided to stratify the results by study population and country of origin. Previous literature has suggested that the results may vary based on whether the study population is from Canada or a European country, or from the United States or a developing country[94]. These results can be seen in Figure 5 (developed countries), Figure 6 (developing

countries), and Figure 7 (United States). The pooled odds of preterm birth in developed countries are 1.64 (95% CI 1.27-2.12) times higher in women with periodontal disease. In developing countries, where the women were mostly non-white and of lower socioeconomic status, the pooled odds of preterm birth are 3.29 (95% CI 2.34-4.62) times higher in women with periodontal disease. Although both analyses reveal significant associations, the observed odds ratios are evidence of a stronger relationship between periodontal disease and preterm birth in study populations of developing countries when compared to developed countries. When studies were limited to those only conducted in the U.S., the pooled odds ratio was 1.83 (95% CI 1.03-3.23).

Lastly, we stratified the studies by clinical characteristics used to define periodontal disease. The forest plots for these subgroup meta-analyses can be seen in Figures 8-11. The pooled odds of preterm birth are 2.66 (95% CI 1.90-3.71) times higher in women with periodontal disease defined using Definition 1 when compared to healthy women. The pooled odds ratios using Definitions 2, 3 and 4 are 2.33 (95% CI 1.29-4.23), 1.81 (95% CI 1.16-2.81), and 6.14 (95% CI 3.35-11.28), respectively. All of these results reveal a significant relationship between the exposure and outcome variables, providing evidence that the association exists regardless of the definition used.

Each forest plot also displays an I^2 value, demonstrating whether there is a high degree of heterogeneity present among the included studies. In figure 2, the I^2 value is equal to 84.66% with a p-value < 0.001, which is suggestive of heterogeneity. We observed the same results in all of the following sub-group meta-analyses, with the exception of the meta-analysis including studies conducted in developed countries, and the meta-analysis including studies using Definition 4 to define periodontal disease.

5.5 Discussion

The main objective of our meta-analysis was to assess the association between periodontal disease and preterm birth by gathering all of the published literature pertaining to this topic and combining the effect measures into pooled odds ratios. We conducted searches through two separate databases, PubMed and Medline, and extracted 37 out of a total of 842 studies. The rest of the articles did not comply with our inclusion criteria and were therefore excluded. Using the software OpenMeta[Analyst], we determined that the pooled odds ratio for the effect of periodontal disease on preterm birth was 2.61 (95% CI 2.06-3.31). We also stratified the studies by study design (case-control versus cohort study), study location (developed versus developing country), and by periodontal disease definition. All of the strata-specific pooled odds ratios are suggestive of a significant positive association between periodontal disease and preterm birth.

There is an increasingly large amount of evidence in current literature pointing to the detrimental effects of periodontal disease on an individual's systemic health[10]. Studies have linked poor oral health to the onset of multiple diseases, including abnormalities with cardiovascular, respiratory, musculoskeletal, and endocrine systems[12, 19]. Furthermore, this association is supported by biologically plausible mechanisms, most notably either systemic inflammation or direct infection by periodontal pathogens[39]. While dental medicine was once viewed as separate from allopathic medicine, evidence of a strong link between the two suggests that perhaps this gap is not as wide as once thought[10, 19].

It has been hypothesized that a change in periodontal health is a risk factor for preterm birth, specifically preterm premature rupture of membranes (PPROM), via contamination of the fetoplacental unit by periodontal pathogens[20, 41]. Cellular changes occurring at the site of rupture result from the release of certain substances, including phospholipases, cytokines, and

proteases[95]. Maternal bacterial infection and inflammation further promote changes in cytokine and hormone-regulated gestation[41], leading to early release of these substances and premature labor. A study conducted by Al Riyami et al. revealed that the most important risk factor for extreme PPROM is a history of infection[96]. Gingival pockets formed in patients with periodontal disease allow infiltration of bacterial substances, causing local inflammatory responses[10, 20]. Oral bacteria and lipopolysaccharide endotoxins from sub gingival plaque in diseased gums can produce systemic host responses by entering the bloodstream and circulating to the amniotic fluid, triggering preterm birth[16, 41].

A major factor contributing to the conflicting results between studies is the lack of universal clinical criteria for diagnosing periodontal disease[97]. For this reason, each researcher decides on their own exposure definition based on the measured clinical parameters of periodontal disease, namely probing depth, gingival recession, bleeding on probing, and clinical attachment level. Probing depth is defined as the distance between the tip of the periodontal probe to the gingival margin. Gingival recession is the distance between the cemento-enamel junction and the gingival margin. Clinical attachment level is the distance between the cements-enamel junction and the tip of the periodontal probe[57]. The majority of the studies included in this meta-analysis use some sort of combination of these parameters to create a diagnostic definition. However, the disparity between each separate researcher's criteria may alter the observed association with any outcome. There have been several indices developed in order to establish a concrete definition for diagnosing periodontal disease, including the Community Periodontal Index for Treatment Needs (CPITN) and Russell Periodontal Index[98]. Unfortunately, many of these have limited validity and sensitivity for disease detection[99]. For this reason, we have stratified the included studies by the general criteria used to define periodontal disease. As

stated in the results, all of the subgroup meta-analyses still demonstrated a significant association with preterm birth.

A common observation in studies examining the association between dental health and pregnancy outcomes is the effect of the study population on the results. The majority of studies in current literature reporting a significant association between periodontal disease and preterm birth take place either in the United States or in developing countries[94]. These studies included a higher percentage of non-white women, or women of lower socioeconomic status[26, 27, 76, 100, 101]. The majority of studies conducted in Canada or European countries included mostly white women or women of higher socioeconomic status, and tended to reveal insignificant associations for the effect of periodontal disease on preterm birth[29, 30, 102, 103]. This observation suggests that the relationship may be influenced not only by one's socioeconomic status, but also on a country's access to free or affordable dental care. Due to the strict inclusion and exclusion criteria for this meta-analysis, several studies displaying this observation were unable to be included. Although the pooled OR for developed countries reveals a significant association, it remains less than the pooled OR for developing countries. Furthermore, our pooled OR for the studies conducted in the United States contradicts the hypothesis that study populations in the U.S. are more similar to those of developing countries than developed countries[94]. However, while this subgroup meta-analysis reveals an odds ratio closer in value to that of the developed countries, the literature on the effect of periodontal disease on preterm birth conducted in the United States is still suggestive of a significantly positive relationship[100, 101, 104, 105].

There are several limitations to our meta-analysis. As noted in Table 1, a large number of the studies controlled for potential confounding variables and reported adjusted odds or risk ratios. However, the software used for the analysis was only able to calculate crude odds ratios

using the sample sizes for each group. The resulting pooled measures of association are completely uncontrolled and likely affected by multiple confounders. This includes, but is not limited to, maternal age, race/ethnicity, previous preterm birth, smoking, and income or socioeconomic status. The latter has been shown, in a systematic review conducted by Xiong et al.[94], to affect the association between periodontal disease and preterm birth. For this reason, we conducted subgroup meta-analyses for pooled effect estimates of developed countries and of developing countries (and the United States). A second limitation of this meta-analysis, and in general, the other studies that have examined the association between periodontal disease and preterm birth, is the use of different diagnostic criteria for defining periodontal disease. Because of the lack of a universal definition, there is a great amount of heterogeneity between the individual studies in terms of how the exposure is defined. A third limitation, and one that may be caused by the previous two limitations, is the presence of a high degree of heterogeneity between the included studies. This is demonstrated by the significant p-values of the I^2 values listed in Figures 2-4 and Figured 6-10. Because of this heterogeneity, a random-effects model was used to conduct the individual meta-analyses.

While the amount of research being conducted on this subject has been steadily increasing, it is important for any further studies to apply more rigorous methodology. The majority of the studies included in this meta-analysis did provide clear definitions for periodontal disease, however they varied greatly from one another. Each definition was created or chosen by the investigators conducting the study, causing a great variety among those used. There is a strong need for a proper universal research definition of periodontal disease measured in an objective manner. Moreover, large sample sizes are required in order to truly estimate the exposure-outcome relationship. Most of the published studies used in this meta-analysis had sample sizes

below 500 patients. More research needs to be conducted in samples sufficiently large to assess the effect of periodontal disease on preterm birth.

5.6 Conclusion

The findings from this meta-analysis suggest that periodontal disease in pregnant women is a risk factor for preterm birth. The pooled odds ratio was 2.61 (95% CI 2.06-3.31), and remained significant despite further stratifications based on study population and exposure definition. These results provide further evidence upon which health professionals should counsel women, both before and during conception, regarding the potential ramifications of their dental health. However, due to possible heterogeneity between studies and a lack of adjustment of effect estimates and the inclusion of studies of small sample size, further research is required.

Table 1: Study and Participant Characteristics for the Included Studies			
Author, Year, ID, Country	**Sample Size**	**PDD Definition**	**Conclusions**
Tellapragada C., et al.[27] (2016) 27255075 India	N: 710 Case: 54 Control: 656	CPITN: PD ≥4 mm (CPI score ≥3) among any of the 6 index teeth	Periodontal disease is a risk factor for preterm birth
Soucy-Giguere L., et al.[29] (2016) 27208603 Canada	N: 258 Exposed: 117 Unexposed: 141	PD ≥4 mm and ≥10% BOP in 1 or more sites	Periodontal disease is not a risk factor for preterm birth
Martínez-Martinez R.E., et al.[22] (2015) 26576767 Mexico	N: 70 Case: 25 Control: 45	Multiple definitions using a combination of the measured clinical parameters (Definitions used: PD ≥4 mm and CAL ≥3 mm in 1 or more sites in ≥4 teeth)	Periodontal disease is not a risk factor for preterm birth
Perunovic N.D., et al.[23] (2016) 26447753 Serbia	N: 120 Case: 60 Control: 60	Combination of PD, CAL, BOP, and plaque index	Periodontal disease is a risk factor for preterm birth
Bulut G., et al.[49] (2014) 24850505 Turkey	N: 100 Case: 50 Control: 50	PD ≥4 mm and CAL ≥3 mm at the same site in ≥4 teeth	Periodontal disease is not a risk factor for preterm birth
Muwazi L., et al.[24] (2014) 24773772 Uganda	N: 400 Case: 194 Control: 206	1: PD 4-5 mm 2: PD ≥ 6 mm 3: gingival recession	Only gingival recession was found to be significantly associated with preterm birth
Macedo J.F., et al.[51] (2014) 23947938 Brazil	N: 296 Case: 74 Control: 222	1: PD ≥4 mm and CAL ≥3 mm in 1 or more sites in ≥4 teeth 2: PD ≥4 mm and CAL ≥4 mm in 1 or more sites	Periodontal disease is a risk factor for preterm birth according to Definition 2
Wang Y.L., et al.[25] (2013) 23548222 Taipei	N: 211 Exposed: 62 Unexposed: 149	>5% gingival bleeding, CAL >6 mm in ≥2 sites, PD ≥5 mm in 1 or more sites	Periodontal disease is not a risk factor for preterm birth

Santa Cruz I., et al.[30] (2013) 23083427 Spain	N: 170 Exposed: 54 Unexposed: 116	CAL ≥3 mm in ≥15 sites	Periodontal disease is not a risk factor for preterm birth
Kumar A., et al.[26] (2013) 22845916 India	N: 340 Exposed: 61 Unexposed: 279	PD ≥4 mm and CAL ≥4 mm in 1 or more sites	Periodontal disease is a risk factor for preterm birth
Martinez de Tejada B, et al.[106] (2012) 22548257 Switzerland	N: 429 Case: 84 Control: 345	European: Severe: CAL ≥5 mm in ≥30% of teeth USA: Moderate: ≥2 interproximal sites with CAL ≥4 mm, not on the same tooth, or ≥2 interproximal sites with PD ≥5 mm, not on the same tooth Severe: ≥2 interproximal sites with CAL ≥6 mm, not on the same tooth, and ≥1 interproximal sites with PD ≥5 mm	Periodontal disease is a risk factor for preterm birth according to the USA definition
Lauren M., et al.[28] (2012) 23922515 Bosnia and Herzegovina	N: 230 Exposed: 57 Unexposed: 173	PD, gingival recession, CAL	Periodontal disease is a risk factor for preterm birth
Piscoya M.D., et al.[47] (2012) 22044450 Brazil	N: 718 Case: 360 Control: 358	PD ≥4 mm and CAL ≥3 mm at the same site in ≥4 teeth	Periodontal disease is a risk factor for preterm birth
Mannem S., Chava V.K.[76] (2011) 21957382 India	N: 104 Case: 52 Control: 52	PD ≥4 mm and CAL ≥3 mm at the same site in ≥4 teeth	Periodontal disease is a risk factor for preterm birth
Baskaradoss J.K., et al.[107] (2011) 21507002 India	N: 300 Case: 100 Control: 200	PD ≥4 mm and CAL ≥3 mm at the same site in ≥4 teeth	Periodontal disease is a risk factor for preterm birth

Vogt M., et al.[108] (2010) 21047427 Brazil	N: 327 Exposed: 156 Unexposed: 171	PD ≥4 mm and CAL ≥4 mm in at least 1 site in ≥4 teeth, with BOP	Periodontal disease is a risk factor for preterm birth
Guimaraes A.N., et al.[109] (2010) 20192860 Spain	N: 1207 Case: 161 Control: 1046	1: PD ≥4 mm and CAL ≥3 mm in 1 or more sites in ≥4 teeth 2: PD ≥4 mm and CAL ≥4 mm in 1 or more sites	Periodontal disease is a risk factor for preterm birth
Ryu J.I., et al.[50] (2010) 20192615 Korea	N: 172 Case: 59 Control: 113	CAL >3.5 mm in ≥2 teeth	Periodontal disease is not a risk factor for preterm birth
Rakoto-Alson S., et al.[110] (2010) 20151798 Madagascar	N: 204 Exposed: 23 Unexposed: 181	CAL ≥4 mm in ≥3 sites from different teeth	Periodontal disease is a risk factor for preterm birth
Nabet C., et al.[111] (2010) 20096065 France	N: 2202 Case: 1108 Control: 1094	Localized: PD ≥4 mm and CAL ≥3 mm at the same site in 2 or 3 teeth Generalized: PD ≥4 mm and CAL ≥3 mm at the same site in ≥4 teeth	Periodontal disease is not a risk factor for preterm birth
Srinivas S.K., et al.[112] (2009) 19375568 United States	N: 786 Exposed: 311 Unexposed: 475	CAL ≥3 mm in ≥3 teeth	Periodontal disease is not a risk factor for preterm birth
Lohsoonthorn V., et al.[113] (2009) 19131565 Thailand	N: 934 Case: 467 Control: 467	PD ≥4 mm and CAL ≥6 mm (severe) or ≥5 mm (moderate) in ≥2 nonadjacent teeth with interproximal sites	Periodontal disease is not a risk factor for preterm birth
Kushtagi P., et al.[114] (2008) 18316087 India	N: 239 Case: 89 Control: 150	Poor oral hygiene index (simplified), gingival index >1, or PD ≥4 mm	Periodontal disease is a risk factor for preterm birth
Siqueira F.M., et al.[48] (2007) 18052698 Brazil	N: 1280 Case: 238 Control: 1042	PD ≥4 mm and CAL ≥3 mm in one or more sites in ≥4 teeth	Periodontal disease is a risk factor for preterm birth

Toygar H.U., et al.[115] (2007) 17970674 Turkey	N: 3576 Case: 447 Control: 3129	1: PD is 4 or 5 mm, supra- or sub-gingival calculus/overhanging restoration margins 2: PD ≥6 mm	Periodontal disease is a risk factor for preterm birth
Le H.T., et al.[116] (2007) 17877238 Vietnam	N: 390 Case: 130 Control: 260	PD ≥4 mm and CAL ≥3 mm in one or more sites in ≥4 teeth, BOP after 10 sec at the same site	Periodontal disease is a risk factor for preterm birth
Mumghamba E.G., Manji K.P.[117] (2007) 17594498 Tanzania	N: 373 Case: 150 Control: 223	PD ≥4 mm and ≥30% BOP in 4 or more sites	Periodontal disease is not a risk factor for preterm birth
Santos-Pereira S.A., et al.[118] (2007) 17309595 Brazil	N: 124 Case: 48 Control: 76	CAL ≥1 mm in at least 1 of 6 sites, and BOP at the same site	Periodontal disease is a risk factor for preterm birth
Alves R.T., Ribeiro, R.A.[119] (2006) 17242792 Brazil	N: 59 Case: 19 Control: 40	Periodontal Screening and Recording (PSR)	Periodontal disease is a risk factor for preterm birth
Bassani D.G., et al.[99] (2007) 17116160 Brazil	N: 803 Case: 196 Control: 607	CAL ≥5 mm in ≥3 sites	Periodontal disease is not a risk factor for preterm birth
Bosnjak A., et al.[120] (2006) 16889630 Croatia	N: 81 Case: 17 Control: 64	E2: % of sites with CAL ≥2 mm E3: % of sites with CAL ≥3 mm E4: % of sites with CAL ≥4 mm	Periodontal disease is a risk factor for preterm birth
Offenbacher S., et al.[105] (2006) 16394036 United States	N: 1020 Exposed: 147 Unexposed: 873	Most disease: PD ≥4 mm in ≥15 sites Mild disease: AAP classifications of gingivitis and mild periodontal disease	Periodontal disease is a risk factor for preterm birth
Lunardelli A.N., Peres M.A.[121] (2005) 16104956 Brazil	N: 449 Case: 32 Control: 417	1: at least 1 site with PD ≥3.5 mm 2: pockets at ≥4 sites	Periodontal disease is not a risk factor for preterm birth (significance lost when adjusting for

			infections/pathologies during pregnancy)
Rajapakse P.S., et al.[122] (2005) 15723870 Sri Lanka	N: 227 Exposed: 66 Unexposed: 161	3 individual plaque scores, bleeding scores and pocket depth values higher than the median values in the total cohort	Periodontal disease is not a risk factor for preterm birth
Jarjoura K., et al.[101] (2005) 15695995 United States	N: 203 Case: 83 Control: 120	CAL ≥3 mm in ≥5 sites	Periodontal disease is a risk factor for preterm birth
Goepfert A.R., et al.[100] (2004) 15458901 United States	N: 139 Case 1 (early spontaneous PTB): 59 Case 2 (early indicated PTB): 36 Control 2: 44	1: inflammation, no CAL 2: CAL 3-5 mm 3: CAL >5 mm in any sextant	Periodontal disease is a risk factor for early spontaneous preterm birth, but not for early indicated preterm birth
Offenbacher S., et al.[104] (2001) 11887460 United States	N: 812 Exposed: 45 Unexposed: 767	Moderate-severe PDD: PD ≥5 mm and CAL ≥2 mm at ≥4 sites	Periodontal disease is a risk factor for preterm birth

List of Abbreviations (Table 1)

PDD periodontal disease

CAL clinical attachment level

PD probing depth

CPITN community periodontal index of treatment needs

OR odds ratio

RR risk ratio

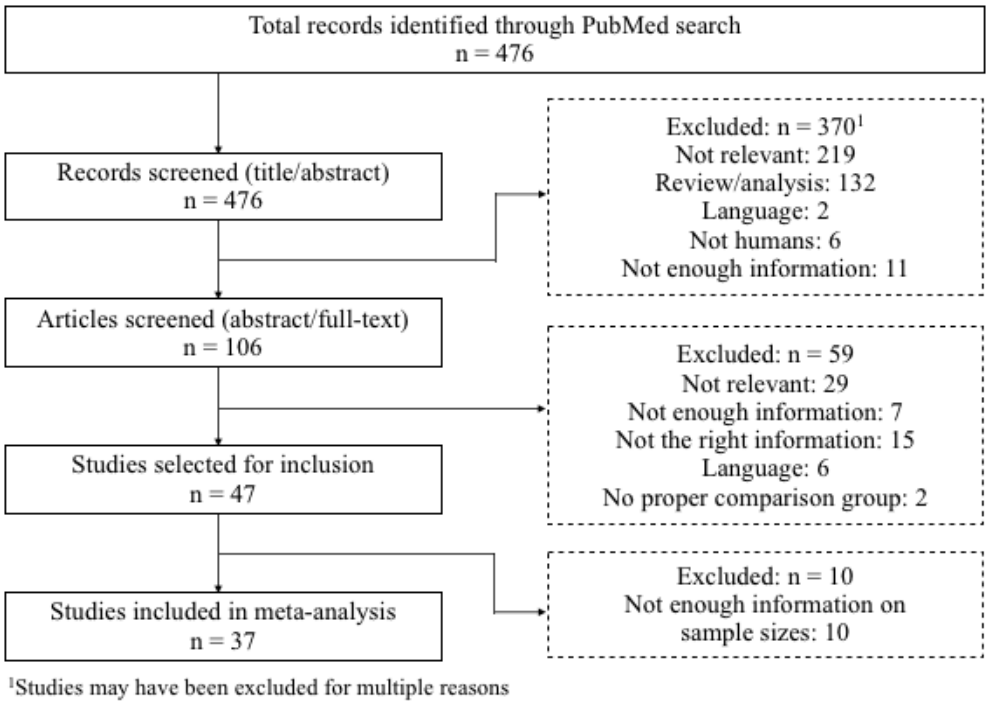

Figure 1: Flow diagram showing study selection process for meta-analysis

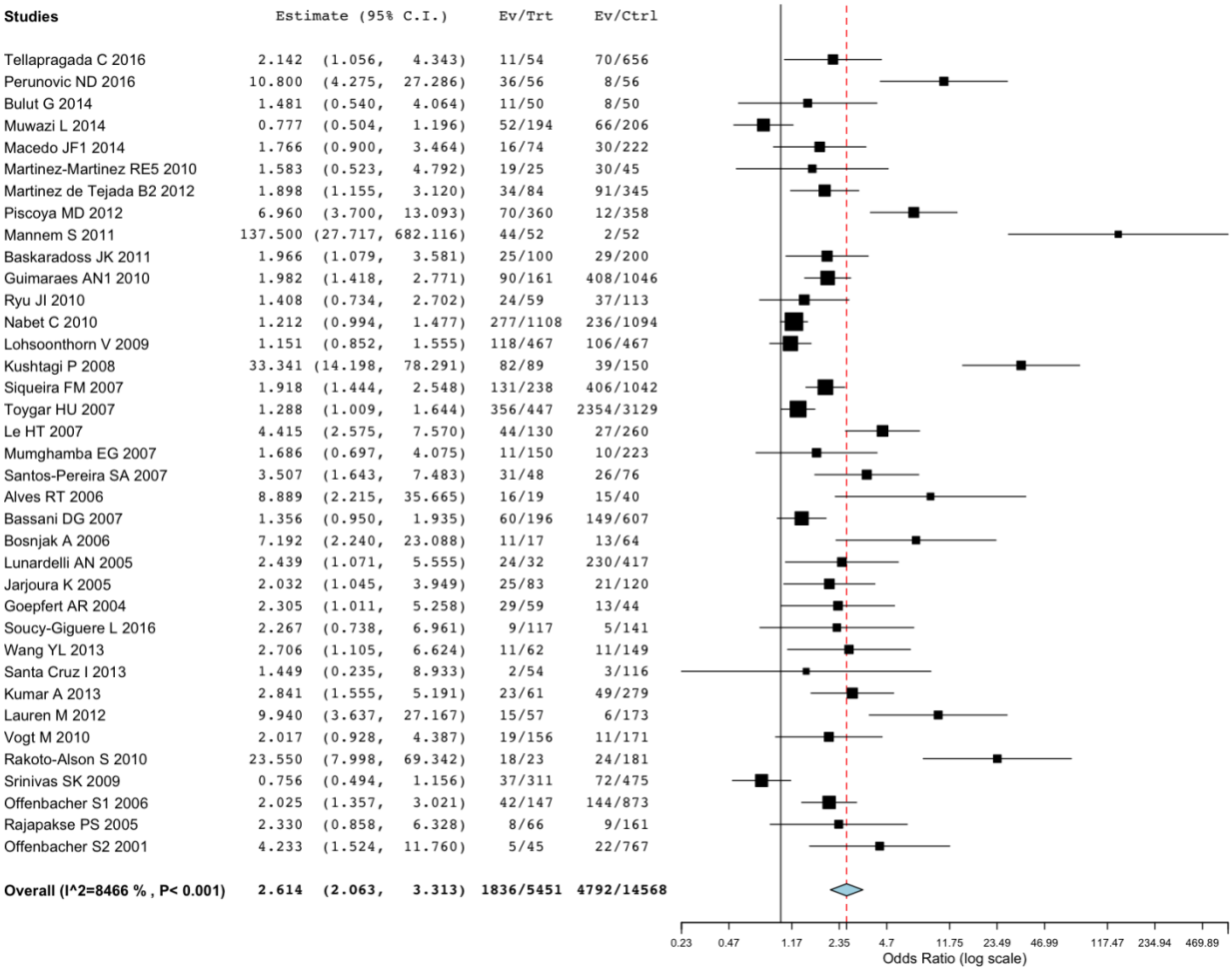

Figure 2: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies included in meta-analysis

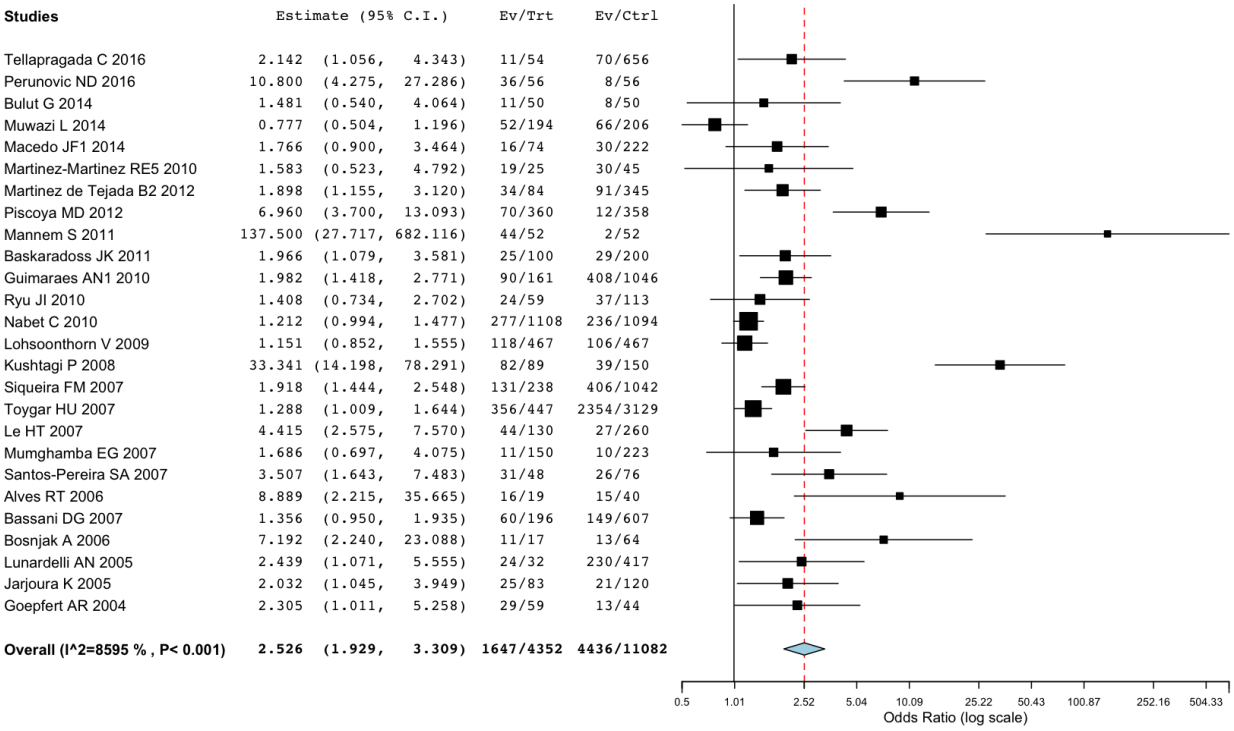

Figure 3: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all case-control studies

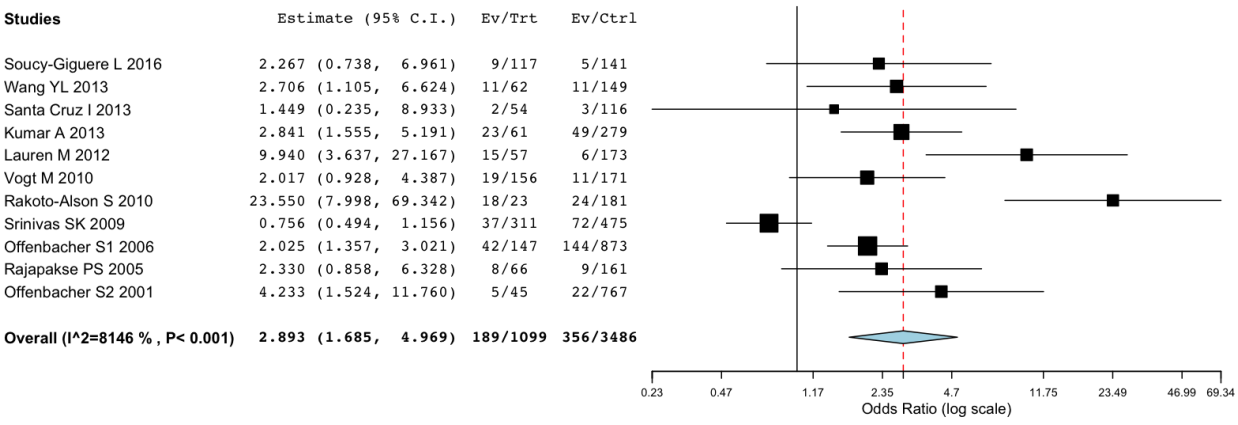

Figure 4: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all cohort studies

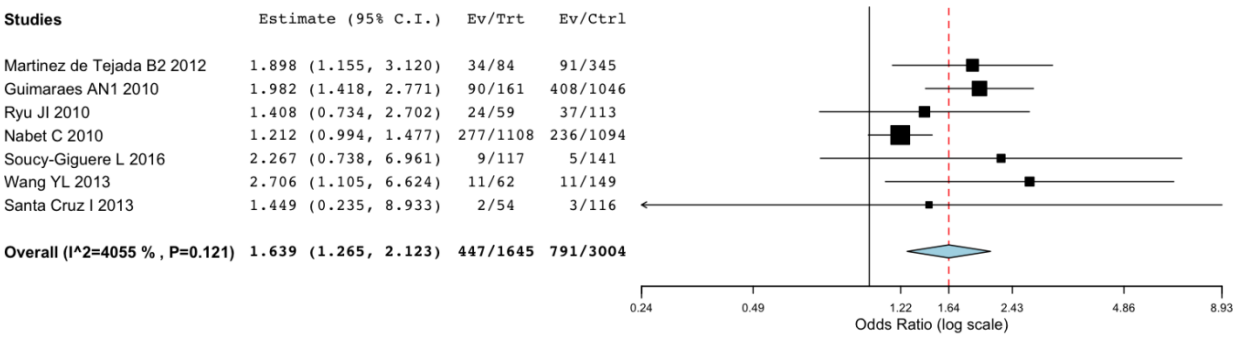

Figure 5: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies conducted in developed countries

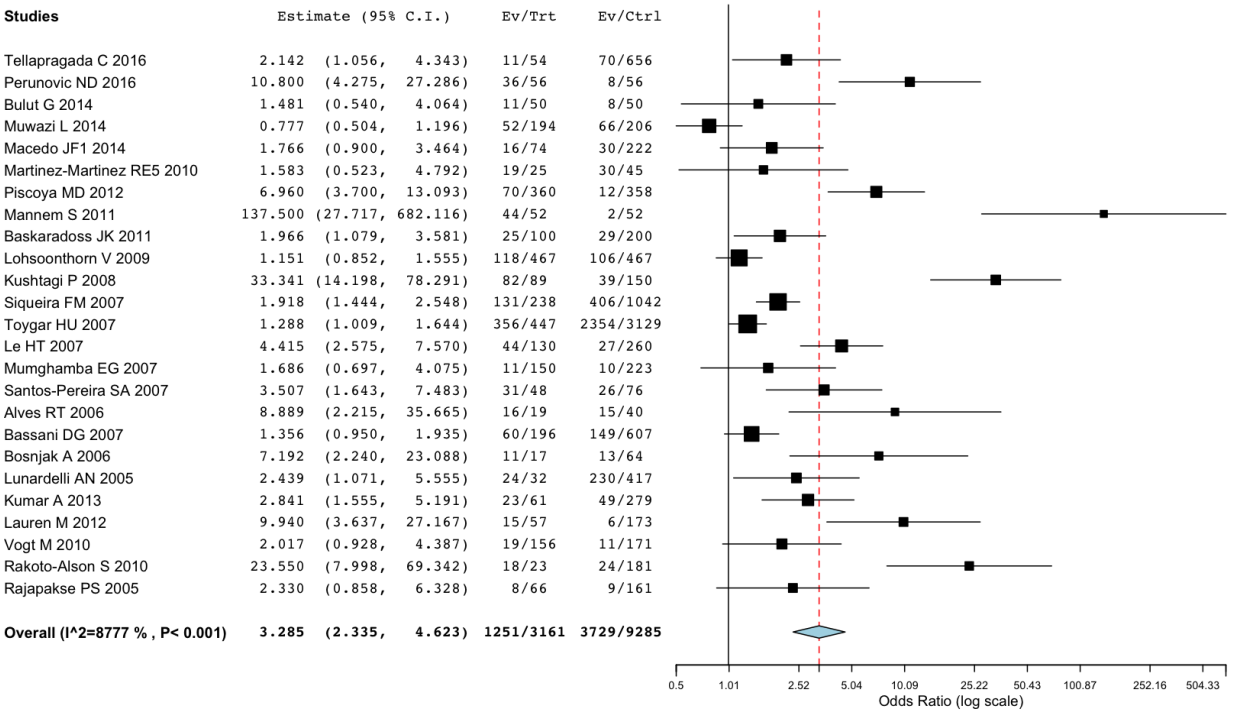

Figure 6: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies conducting in developing countries

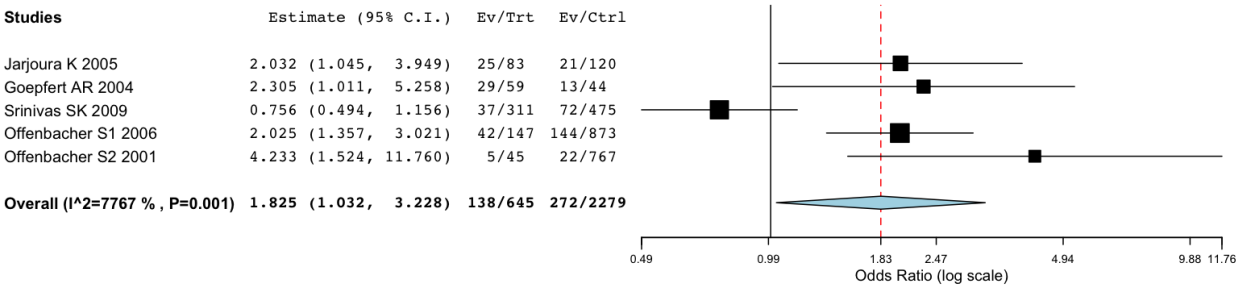

Figure 7: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies conducted in the United States

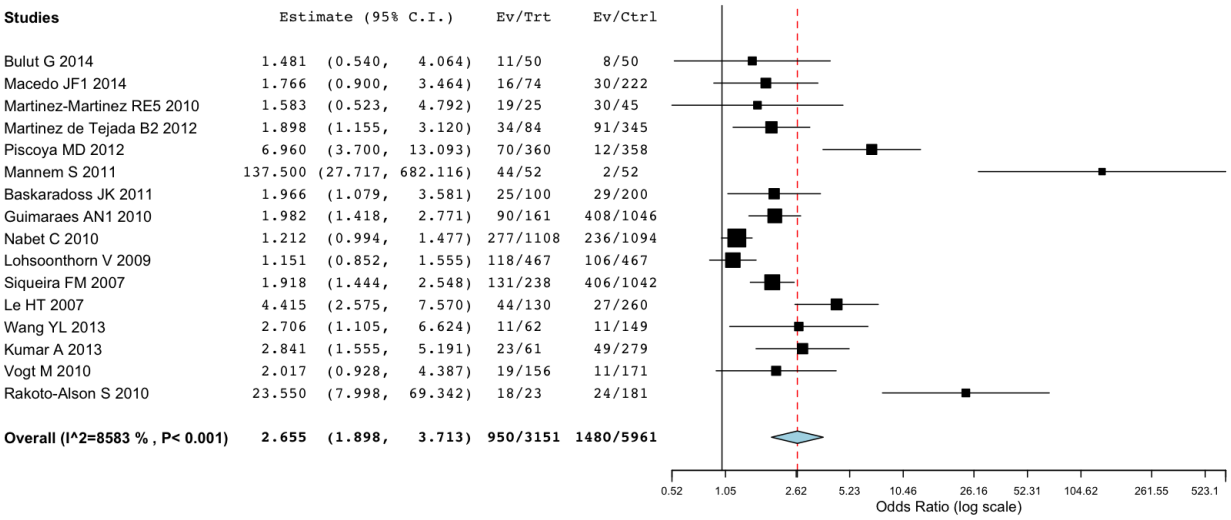

Figure 8: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies using Definition 1 for periodontal disease

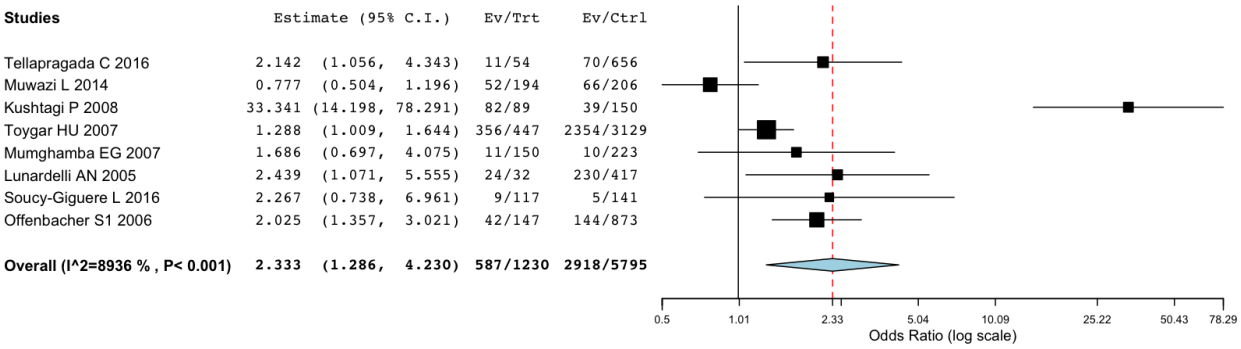

Figure 9: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies using Definition 2 for periodontal disease

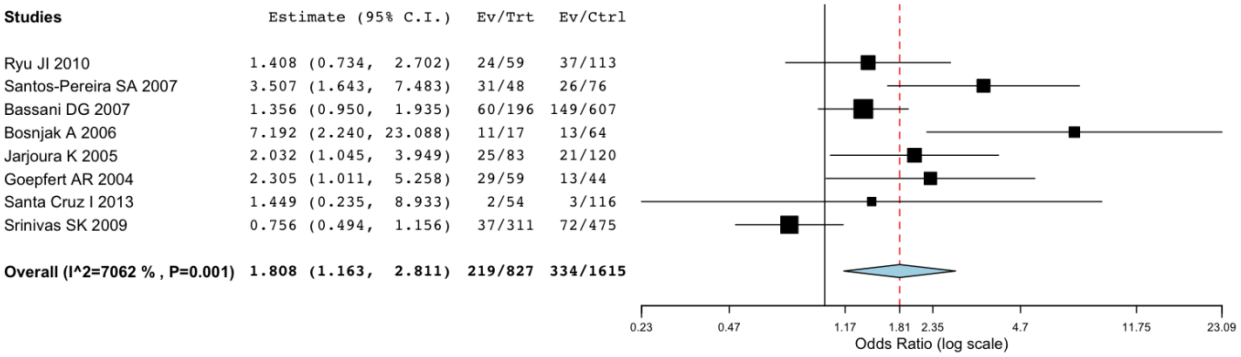

Figure 10: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies using Definition 3 for periodontal disease

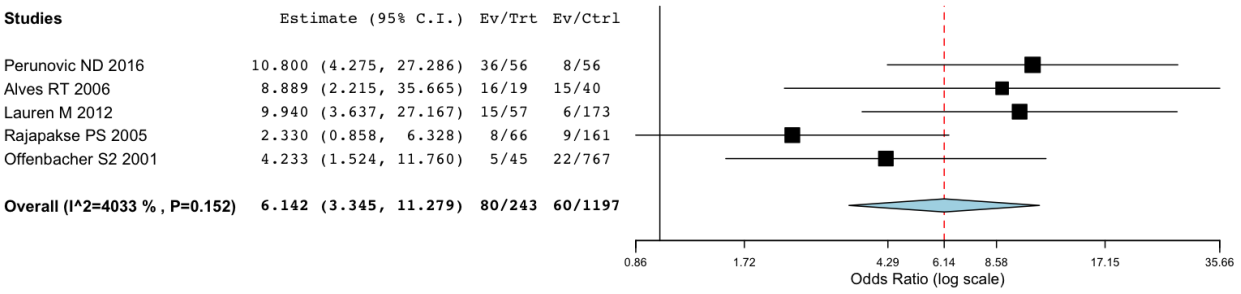

Figure 11: Forest plot showing pooled OR for the effect of periodontal disease on preterm birth for all studies using Definition 4 for periodontal disease

CHAPTER 6: GENERAL DISCUSSION

The results provided by the manuscripts included in this thesis reveal conflicting conclusions. The meta-analysis suggests that periodontal disease is a risk factor for preterm birth, while the retrospective cohort study fails to report such an association.

6.1 Findings and summary for the effect of periodontal disease on the incidence of preterm birth

Tbe main objective of this thesis was to further evaluate the effect of periodontal disease on the incidence of preterm birth. This objective was split into two separate steps. The first step was conducting a retrospective cohort study. Using a database created in Quebec, Canada, for a study assessing the effect of periodontal disease on preeclampsia[57], a cohort of 245 pregnant women was used for this study. Multivariate logistic regression was used to analyze the data, and the observed results consisted of an increased odds of preterm birth of 1.51 (95% CI 0.61-3.71) in women with periodontal disease compared to those without. It is evident that these results are not indicative of any significant relationship between the two variables, contradicting the results obtained in the second manuscript. However, the overall results of this thesis seem to reflect what exists in the literature, where the overall conclusions regarding this topic are ambiguous.

Numerous observational studies on this subject have been conducted throughout the last 15 years as research relating to periodontal disease has gained a lot of interest[22, 23, 27, 29]. More literature is being published on the systemic consequences of poor oral health, suggesting that it is associated with several diseases such as diabetes, cardiovascular disease, rheumatoid arthritis, and respiratory diseases[11, 19]. A review conducted by Kim and Amar underlines the importance of

linking allopathic medicine with dental medicine due to the strong associations found between the two[10]. With this idea in mind, there is hope of potentially ameliorating systemic illness by aiming to treat periodontal disease. This notion has been applied to adverse pregnancy outcomes as well, establishing the rationale for assessing the potential effect periodontal disease can have on outcomes such as preterm birth. A cross-sectional study conducted by Perunovic et al. compared a case group of women with preterm birth to a control group of women who delivered a baby at term[23]. The results indicated that the women in the case group had both worse clinical periodontal parameters and increased levels of gingival crevicular fluid inflammatory cytokines. However, a separate case-control study conducted by Bulut et al. failed to report any statistically significant differences between the two groups regarding periodontal disease and preterm birth[49]. These inconsistencies are demonstrated throughout the literature, and are especially noticeable in studies displaying both significant and insignificant results. For example, a case-control study conducted by Macedo et al. used two separate clinical definitions for the diagnosis of periodontal disease[51]. The first definition required four or more teeth to display at least one site with a probind depth of at least 4 mm and a clinical attachment level of at least 3 mm. The second one required only one site with both a probing depth and a clinical attachment level of at least 4 mm. After performing multiple logistic regression analyses, only periodontal disease defined according to the second definition was found to be significantly associated with preterm birth.

The second part of the objective toward understanding the potential relationship between periodontal disease and preterm birth involved collecting eligible observational studies evaluating the association between periodontal disease and preterm birth for inclusion in a meta-analysis. The results of this analysis revealed a significant pooled OR of 2.61 (95% CI 2.06-3.31), suggesting that periodontal disease is, in fact, a risk factor for preterm birth. The studies

were further stratified into distinct groups in an attempt to better understand the exposure-outcome relationship. This stratification involved separating the studies based on the type of observational study conducted, the population country of origin, and the included exposure definition.

The majority of meta-analyses and systematic reviews currently published are more general, including studies with various adverse pregancy outcomes, such as preeclampsia and low birth weight as well. This subject has been vastly studied throughout the last decade, however the findings remain inconclusive[46, 94, 123, 124]. In 2013, Stadelmann et al. conducted a systematic review on the potential association between GCF inflammatory mediators, a sign of periodontal disease, and adverse pregnancy outcomes[45]. The outcomes consisted of preterm birth, low birth weight, and preeclampsia. Eight studies were included, the majority of which confirmed a positive association. However, due to heterogeneity and variability between the studies, especially concerning the outcomes investigated, they were unable to conduct a meta-analysis. Furthermore, several of the studies did not adjust for confounding variables. Therefore, the observed association in these studies between periodontal disease and adverse pregnancy outcomes may be due to inadequate adjustment.

Another study, conducted by Khader et al., also examined the relationship between periodontal disease and preterm birth and low birth weight[125]. Two case-control and three cohort studies were included in the study, and a meta-analysis was performed. The calculated pooled adjusted risk of preterm birth in women with periodontal disease was 4.28 (95% CI 2.62-6.99) times the risk for periodontally-healthy women. For preterm low birth weight, the overall adjusted OR was 5.28 (95% CI 2.21-12.62), while the overall adjusted OR for either preterm birth or low birth weight was 2.30 (95% CI 1.21-4.38). These observations are consistent with

the results of our meta-analysis, demonstrating that the assocation does exist. However, there are certain limitations to this study, including the fact that most of the included studies are of poor to fair quality, as demonstrated by a quality score.

A common observation in research evaluating the effect of periodontal disease on the occurrence of adverse pregnancy outcomes is the strong association between the disease and the country of origin of the participants. More specific, patients from developing countries, as well as the United States, are more likely to display a significant association between periodontal disease and preterm birth[94]. In such countries, the majority of the subjects tend to be non-white women of lower socio-economic, and access to health care is more difficult[26, 27, 94, 100, 126]. On the contrary, studies conducted in more developed countries, specifically in Europe or Canada where health care is more accessible and more affordable, include higher percentages of white women and often fail to report any association between periodontal disease and preterm birth[29, 57, 94, 102, 103].

One of the most prominent limitations surrounding research on periodontal disease is the lack of a universal clinical diagnostic definition. Certain indices have been established in an attempt to regulate what researchers use to define periodontal disease, such as the Community Periodontal Index of Treatment Needs (CPITN). This procedure requires clinical assessment for the presence or absence of markers of periodontal disease, such as periodontal pockets and gingival bleeding[72]. Depending on the age of the patient, a certain amount of teeth are examined using a special CPITN periodontal probe, or its equivalent. Six scores are reported, representing the highest score for each sextant of the mouth. Although this procedure is commonly used to identify and diagnose periodontal disease, a study conducted by Bassani et al. observed a significant discrepancy when comparing CPITN results to gold standard results, failing to

demonstrate the index's validity[99]. Another index, called the Russell's Periodontal Index, is also a recording system scoring the presence and severity of periodontal clinical parameters[98]. All teeth are examined and scored using a five-level system, with higher values representing more severe disease. However, Sheiham and Striffler discovered that when compared with radiographic methods, the index reported lower disease levels[127].

6.2 Strengths and limitations

There are several strengths and limitations of both studies included in this thesis. A major strength of the retrospective cohort study is the number of demographic characteristics and clinical parameters, both dental and obstetrical, obtained by the researchers collecting the data. Information was collected regarding maternal age, ethnicity, income, smoking and alcohol habits, parity, history of induced abortions, mode of delivery, birth weight, last dental visit, frequency of brushing, bleeding gingiva, and dental insurance during pregnancy, among others. The dental hygienists examining the included patients provided all of the clinical parameters, making it easy to evaluate periodontal disease using various available definitions. However, there do exist several limitations of this study. First of all, the sample size was relatively small due to the fact that we were restricted to using only the control arm of the previous study for which the data was collected. Second, over 80% of the study population consisted of white women, which may affect the association of interest.

Continuing with the meta-analysis, we were able to include 37 observational studies conducted in the last 15 years evaluating the effect of periodontal disease on preterm birth. All of these studies eligible for inclusion provided sufficient information regarding the sample sizes for each group, as well as the country of origin, clear periodontal disease definitions, odds or risk

ratios (or the appropriate information to allow us to extract such data), and overall conclusions regarding the association of interest. However, not all of these studies controlled for confounding variables, thus several of the effect measures provided in Table 1 of the meta-analyses were crude ratios. This means that the observed results may have been due to external variables, not necessarily the exposure of interest. Furthermore, the software used to conduct the analyses calculated the odds ratios for each study using sample size information, and therefore no adjustment was included in the pooled results. As mentioned, failure to adjust for the effects of these external variables, may have confounded the association. Moreover, relating to the previously mentioned, multiple studies included in the meta-analysis had differing diagnostic criteria for identifying periodontal disease in patients.

Due to the conflicting results both in the literature and in this thesis, additional studies investigating the effect of periodontal disease on preterm birth are required.

CHAPTER 7: CONCLUSION

Periodontal disease has been increasingly linked to various systemic diseases[10, 12, 19], and is hypothesized to be associated with adverse pregnancy outcomes[44, 64, 76], including preterm birth. Identifying and evaluating this association is crucial toward properly treating and advising pregnant women on the possible detrimental effects poor dental health can have on both the mother and the neonate. Using the current literature available on this topic, the retrospective cohort study conducted on a group of pregnant Canadian women failed to report any significant relationship between the two variables. However, our meta-analysis has determined that there is indeed a significant positive association between periodontal disease in pregnant women and a higher risk for preterm birth. These results suggest that while single observational studies may not have sufficient power to identify a relationship, the overall literature does provide evidence for an association. There is evidently a need for further validation and research in this field in order to truly identify whether or not periodontal disease is a risk factor for preterm birth. In the meantime, given the large amount of evidence available, clinicians should still take into account a woman's dental health throughout her pregnancy.

CHAPTER 8: FUTURE RESEARCH DIRECTIONS

Due to the inconsistent results and conclusions observed in the literature currently published regarding the effect of periodontal disease on the incidence of preterm birth[27, 48, 106, 111, 112], there is a clear need for additional research in this field. This conclusion is further supported by the conflicting results of the individual manuscripts presented in this thesis. While the retrospective cohort study conducted on a cohort of pregnant women with and without periodontal disease fails to display a significant relationship between the exposure and preterm birth, the meta-analysis consisting of an amalgamation of already-published observational studies does demonstrate a significant positive association.

The most prominent issue surrounding research focused on periodontal disease remains the lack of a universal clinical diagnostic definition for the disease[97]. This creates discrepancies between individual studies, making it harder to compare them. An important step in any future research on this topic would be identifying a universal definition for periodontal disease. Furthermore, additional well-designed observational studies with sufficient study sizes and exposure and outcome definitions, in addition to adequate control of confounding, are needed to accurately evaluate the effect of periodontal disease on preterm birth.

CHAPTER 9: REFERENCES

1. World Health Organization. Oral health. Available at: http://www.who.int/mediacentre/factsheets/fs318/en/. Accessed: October 6, 2015.
2. Guthmiller JM, Novak KF. *Periodontal Diseases*. Washington (DC): ASM Press; 2002.
3. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005;366:1809-1820.
4. Ryalat S, Sawair F, Baqain Z, et al. Effects of oral diseases on mothers giving birth to preterm infants. *Med Princ Pract* 2011;20:556-561.
5. National Institutes of Health. Periodontal (Gum) Disease: Causes, Symptoms, and Treatments. Available at: http://www.nidcr.nih.gov/oralhealth/Topics/GumDiseases/PeriodontalGumDisease.htm. Accessed: October 6, 2015.
6. PubMed Health. Gingivitis and periodontitis: Overview. Available at: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072777/. Accessed: April 4, 2016.
7. Andonova I, Iliev V, Zivkovic N, Susic E, Bego I, Kotevska V. Can oral anaerobic bacteria cause adverse pregnancy outcomes? *Pril (Makedon Akad Nauk Umet Odd Med Nauki)* 2015;36:137-143.
8. Loesche WJ. *Microbiology of Dental Decay and Periodontal Disease*. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
9. Loesche WJ, Grossman NS. Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. *Clin Microbiol Rev* 2001;14:727-752, table of contents.
10. Kim J, Amar S. Periodontal disease and systemic conditions: a bidirectional relationship. *Odontology* 2006;94:10-21.

11. Olsen I. From the acta prize lecture 2014: the periodontal-systemic connection seen from a microbiological standpoint. *Acta Odontol Scand* 2015;20:1-6.

12. Scannapieco FA. Systemic effects of periodontal diseases. *Dent Clin North Am* 2005;49:533-550, vi.

13. Paquette D. Periodontal disease and the risk for adverse pregnancy outcomes. *Grand Rounds in Oral-Sys Med* 2006;4:14-24.

14. Dasanayake AP, Gennaro S, Hendricks-Munoz KD, Chhun N. Maternal periodontal disease, pregnancy, and neonatal outcomes. *MCN Am J Matern Child Nurs* 2008;33:45-49.

15. World Health Organization. Preterm birth. Available at: http://www.who.int/mediacentre/factsheets/fs363/en/. Accessed: October 6, 2015.

16. Behrman RE, Butler AS. *Preterm Birth: Causes, Consequences, and Prevention*. Washington, DC: National Academies Press; 2007.

17. Centers for Disease Control and Prevention. Preterm Birth. Available at: http://www.cdc.gov/reproductivehealth/maternalinfanthealth/pretermbirth.htm. Accessed: March 28, 2016.

18. Cheong JL, Doyle LW. Increasing rates of prematurity and epidemiology of late preterm birth. *J Paediatr Child Health* 2012;48:784-788.

19. Arigbede AO, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: A literature review. *J Indian Soc Periodontol* 2012;16:487-491.

20. Walia M, Saini N. Relationship between periodontal diseases and preterm birth: Recent epidemiological and biological data. *Int J Appl Basic Med Res* 2015;5:2-6.

21. Ide M, Papapanou PN. Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes--systematic review. *J Periodontol* 2013;84:181-194.

22. Martinez-Martinez RE, Moreno-Castillo DF, Loyola-Rodriguez JP, et al. Association between periodontitis, periodontopathogens and preterm birth: is it real? *Arch Gynecol Obstet* 2016;294:47-54.

23. Perunovic N, Rakic MM, Nikolic LI, et al. The Association Between Periodontal Inflammation and Labor Triggers (Elevated Cytokine Levels) in Preterm Birth: A Cross-Sectional Study. *J Periodontol* 2016;87:248-256.

24. Muwazi L, Rwenyonyi CM, Nkamba M, et al. Periodontal conditions, low birth weight and preterm birth among postpartum mothers in two tertiary health facilities in Uganda. *BMC Oral Health* 2014;14:42.

25. Wang YL, Liou JD, Pan WL. Association between maternal periodontal disease and preterm delivery and low birth weight. *Taiwan J Obstet Gynecol* 2013;52:71-76.

26. Kumar A, Basra M, Begum N, et al. Association of maternal periodontal health with adverse pregnancy outcome. *J Obstet Gynaecol Res* 2013;39:40-45.

27. Tellapragada C, Eshwara VK, Bhat P, et al. Risk Factors for Preterm Birth and Low Birth Weight Among Pregnant Indian Women: A Hospital-based Prospective Study. *J Prev Med Public Health* 2016;49:165-175.

28. Lauren M, Minire A, Maldi X, Mirton M, Aferdita M. The impact of periodontitis in the preterm birth and body size of newborns. *Mater Sociomed* 2012;24:44-47.

29. Soucy-Giguere L, Tetu A, Gauthier S, et al. Periodontal Disease and Adverse Pregnancy Outcomes: A Prospective Study in a Low-Risk Population. *J Obstet Gynaecol Can* 2016;38:346-350.

30. Santa Cruz I, Herrera D, Martin C, Herrero A, Sanz M. Association between periodontal status and pre-term and/or low-birth weight in Spain: clinical and microbiological parameters. *J Periodontal Res* 2013;48:443-451.

31. American Academy of Periodontology. CDC: Half of American Adults Have Periodontal Disease. Available at: https://www.perio.org/consumer/cdc-study.htm. Accessed: March 28, 2016.

32. Brodeur JM, Payette M, Beniger M, Charbonneau A, Olivier M, Chabot D. Periodontal diseases among Quebec adults aged 35 to 44 years. *J Can Dent Assoc* 2001;67:34.

33. Nath SG, Raveendran R. "What is there in a name?": A literature review on chronic and aggressive periodontitis. *J Indian Soc Periodontol* 2011;15:318-322.

34. Armitage GC, Cullinan MP. Comparison of the clinical features of chronic and aggressive periodontitis. *Periodontol 2000* 2010;53:12-27.

35. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1-6.

36. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). *BMJ* 2012;345.

37. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008;371:75-84.

38. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science* 2014;345:760-765.

39. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. *Clin Microbiol Rev* 2000;13:547-558.

40. Saini R, Saini S, Saini SR. Periodontitis: A risk for delivery of premature labor and low-birth-weight infants. *J Nat Sci Biol Med* 2010;1:40-42.

41. Bansal M, Khatri M, Kumar A, Bhatia G. Relationship between maternal periodontal status and preterm low birth weight. *Rev Obstet Gynecol* 2013;6:135-140.

42. Vinturache AE, Gyamfi-Bannerman C, Hwang J, Mysorekar IU, Jacobsson B, (PREBIC) PBIC. Maternal microbiome - A pathway to preterm birth. *Semin Fetal Neonatal Med* 2016;21:94-99.

43. Offenbacher S, Jared HL, O'Reilly PG, et al. Potential pathogenic mechanisms of periodontitis associated pregnancy complications. *Ann Periodontol* 1998;3:233-250.

44. Alchalabi HA, Al Habashneh R, Jabali OA, Khader YS. Association between periodontal disease and adverse pregnancy outcomes in a cohort of pregnant women in Jordan. *Clin Exp Obstet Gynecol* 2013;40:399-402.

45. Stadelmann P, Alessandri R, Eick S, Salvi GE, Surbek S, Sculean A. The potential association between gingival crevicular fluid inflammatory mediators and adverse pregnancy outcomes: a systematic review. *Clin Oral Investig* 2013;17:1453-1463.

46. Corbella S, Taschieri S, Francetti L, De Siena F, Del Fabbro M. Periodontal disease as a risk factor for adverse pregnancy outcomes: a systematic review and meta-analysis of case-control studies. *Odontology* 2012;100:232-240.

47. Piscoya MD, Ximenes RA, Silva GM, Jamelli SR, Coutinho SB. Maternal periodontitis as a risk factor for prematurity. *Pediatr Int* 2012;54:68-75.

48. Siqueira FM, Cota LO, Costa JE, Haddad JP, Lana AM, Costa FO. Intrauterine growth restriction, low birth weight, and preterm birth: adverse pregnancy outcomes and their association with maternal periodontitis. *J Periodontol* 2007;78:2266-2276.

49. Bulut G, Olukman O, Calkavur S. Is there a relationship between maternal periodontitis and pre-term birth? A prospective hospital-based case-control study. *Acta Odontol Scand* 2014;72:866-873.

50. Ryu JI, Oh K, Yang H, et al. Health behaviors, periodontal conditions, and periodontal pathogens in spontaneous preterm birth: a case-control study in Korea. *J Periodontol* 2010;81:855-863.

51. Macedo JF, Ribeiro RA, Machado FC, et al. Periodontal disease and oral health-related behavior as factors associated with preterm birth: a case-control study in south-eastern Brazil. *J Periodontal Res* 2014;49:458-464.

52. Mathews TJ, MacDormanc MF. Infant Mortality Statistics from the 2010 Period Linked Birth/Infant Death Data Set. *National Vital Statistics Reports* 2013;62:1-26.

53. Mayo Clinic. Diseases and Conditions: Preeclampsia. Available at: http://www.mayoclinic.org/diseases-conditions/preeclampsia/basics/definition/con-20031644. Accessed: October 11, 2015.

54. Ide M, Linden GJ. Periodontitis, cardiovascular disease and pregnancy outcome--focal infection revisited? *Br Dent J* 2014;217:467-474.

55. Desai K, Desai P, Duseja S, Kumar S, Mahendra J, Duseja S. Significance of maternal periodontal health in preeclampsia. *J Int Soc Prev Community Dent* 2015;5:103-107.

56. Zi MY, Longo PL, Bueno-Silva B, Mayer MP. Mechanisms involved in the association between periodontitis and complications in pregnancy. *Front Public Health* 2015;2:290.

57. Taghzouti N, Xiong X, Gornitsky M, et al. Periodontal disease is not associated with preeclampsia in Canadian pregnant women. *J Periodontol* 2012;83:871-877.

58. Souza LM, Cruz SS, Gomez-Filho IS, et al. Effect of maternal periodontitis and low birth-weight - A case control study. *Acta Odontol Scand* 2016;74:73-80.

59. Schenkein HA, Koertge TE, Sabatini R, Brooks CN, Gunsolley JC. Birth weight of infants of mothers with aggressive periodontitis. *J Periodontol* 2012;83:279-286.

60. Islam MM. Increasing Incidence of Infants with Low Birth Weight in Oman. *Sultan Qaboos Univ Med J* 2015;15:e177-183.

61. American Academy of Periodontology. Types of Gum Disease. Available at: https://www.perio.org/consumer/types-gum-disease.html. Accessed: March 28, 2016.

62. Dhadse P, Gattani D, Mishra R. The link between periodontal disease and cardiovascular disease: How far we have come in last two decades ? *J Indian Soc Periodontol* 2010;14:148-154.

63. Koziel J, Mydel P, Potempa J. The link between periodontal disease and rheumatoid arthritis: an updated review. *Curr Rheumatol Rep* 2014;16:408.

64. Basha S, Swamy HS, Mohamed RN. Maternal Periodontitis as a possible risk factor for preterm birth and low birth weight - A prospective study. *Oral Health Prev Dent* 2015.

65. Govindaraju P, Venugopal S, Shivakumar MA, Sethuraman S, Ramaiah SK, Mukundan S. Maternal periodontal disease and preterm birth: A case-control study. *J Indian Soc Periodontol* 2015;19:512-515.

66. Masoura S, Kalogiannidis I, Margioula-Siarkou C, et al. Neonatal outcomes of late preterm deliveries with pre-eclampsia. *Minerva Ginecol* 2012;64:109-115.

67. Connealy BD, Carreno CA, Kase BA, Hart LA, Blackwell SC, Sibai BM. A history of prior preeclampsia as a risk factor for preterm birth. *Am J Perinatol* 2014;31:483-488.

68. Spong CY, Mercer BM, D'Alton M, Kilpatrick S, Blackwell SC, Saade G. Timing of Indicated Late-Preterm and Early-Term Birth. *Obstet Gynecol* 2011;118:323-333.

69. Stoelhorst GM, Martens SE, Rijken M, et al. Behaviour at 2 years of age in very preterm infants (gestational age < 32 weeks). *Acta Paediatr* 2003;92:595-601.

70. Muthukumar S, Suresh R. Community periodontal index of treatment needs index: an indicator of anaerobic periodontal infection. *Indian J Dent Res* 2009;20:423-425.

71. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). *Int Dent J* 1982;32:281-291.

72. Cutress TW, Ainamo J, Sardo-Infirri J. The community periodontal index of treatment needs (CPITN) procedure for population groups and individuals. *Int Dent J* 1987;37:222-233.

73. Chandki R, Banthia P, Banthia R. Biofilms: A microbial home. *J Indian Soc Periodontol* 2011;15:111-114.

74. Markou E, Eleana B, Lazaros T, Antonios K. The influence of sex steroid hormones on gingiva of women. *Open Dent J* 2009;3:114-119.

75. Wu M, Chen SW, Jiang SY. Relationship between gingival inflammation and pregnancy. *Mediators Inflamm* 2015;2015:623427.

76. Mannem S, Chava VK. The relationship between maternal periodontitis and preterm low birth weight: A case-control study. *Contemp Clin Dent* 2011;2:88-93.

77. Shah M, Muley A, Muley P. Effect of nonsurgical periodontal therapy during gestation period on adverse pregnancy outcome: a systematic review. *J Matern Fetal Neonatal Med* 2013;26:1691-1695.

78. Michalowicz BS, Gustafsson A, Thumbigere-Math V, K B. The effects of periodontal treatment on pregnancy outcomes. *J Clin Periodontol* 2013;40:195-208.

79. Pirie M, Linden G, Irwin C. Intrapregnancy non-surgical periodontal treatment and pregnancy outcome: a randomized controlled trial. *J Periodontol* 2013;84:1391-1400.

80. Khairnar MS, Pawar BR, Marawar PP, Khairnar DM. Estimation of changes in C-reactive protein level and pregnancy outcome after nonsurgical supportive periodontal therapy in women affected with periodontitis in a rural set up of India. *Contemp Clin Dent* 2015;6.

81. Schwendicke F, Karimbux N, Allareddy V, Gluud C. Periodontal treatment for preventing adverse pregnancy outcomes: a meta- and trial sequential analysis. *PLoS One* 2015;10.

82. Rosa MI, Pires PD, Medeiros LR, Edelweiss MI, Martínez-Mesa J. Periodontal disease treatment and risk of preterm birth: a systematic review and meta-analysis. *Cad Saude Publica* 2012;28:1823-1833.

83. Joshi VM, Vandana KL. The detection of eight putative periodontal pathogens in adult and rapidly progressive periodontitis patients: an institutional study. *Indian J Dent Res* 2007;18:6-10.

84. Doyle RM, Alber DG, Jones HE, et al. Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. *Placenta* 2014;35:1099-1101.

85. Mitchell-Lewis D, Engebretson SP, Chen J, Lamster IB, Papapanou PN. Periodontal infections and pre-term birth: early findings from a cohort of young minority women in New York. *Eur J Oral Sci* 2001;109:34-39.

86. Su H, Gornitsky M, Velly AM, Yu H, Benarroch M, Schipper HM. Salivary DNA, lipid, and protein oxidation in nonsmokers with periodontal disease. *Free Radic Biol Med* 2009;46:914-921.

87. Offenbacher S, Katz V, Fertik G, et al. Periodontal infection as a possible risk factor for preterm low birth weight. *J Periodontol* 1996;67:1103-1113.

88. Wiebe CB, Putnins EE. The periodontal disease classification system of the American Academy of Periodontology--an update. *J Can Dent Assoc* 2000;66:594-597.

89. Eke PI, Dye B, Wei L, Thornton-Evans G, Genco R. Prevalence of Periodontitis in Adults in the United States: 2009 and 2010. *J Dent Res* 2012:1-7.

90. National Health Service. Gum disease. Available at: http://www.nhs.uk/conditions/Gum-disease/Pages/Introduction.aspx. Accessed: October 6, 2015.

91. . Heterogeneity in Meta-Analysis. StatsDirect Ltd. http://www.statsdirect.com/help/Default.htm - meta_analysis/heterogeneity.htm. England: StatsDirect Ltd. 2013.

92. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;327:557-560.

93. Nikolakopoulou A, Mavridis D, Salanti G. Demystifying fixed and random effects meta-analysis. *Evid Based Ment Health* 2014;17:53-57.

94. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: a systematic review. *BJOG* 2006;113:135-143.

95. Caughey AB, Robinson JN, Norwitz ER. Contemporary diagnosis and management of preterm premature rupture of membranes. *Rev Obstet Gynecol* 2008;1:11-22.

96. Al Riyami N, Al-Ruheili I, Al-Shezaw F, Al-Khabori M. Extreme preterm premature rupture of membranes: risk factors and feto maternal outcomes. *Oman Med J* 2013;28:108-111.

97. Andriankaja OM, Genco RJ, Dorn J, et al. The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. *J Periodontol* 2006;77:1067-1073.

98. Beltran-Aguilar ED, Eke PI, Thornton-Evans G, Petersen PE. Recording and surveillance systems for periodontal diseases. *Periodontol 2000* 2012;60:40-53.

99. Bassani DG, da Silva CM, Oppermann RV. Validity of the "Community Periodontal Index of Treatment Needs" (CPITN) for population periodontitis screening. *Cad Saude Publica* 2006;22:277-283.

100. Goepfert AR, Jeffcoat MK, Andrews WW, et al. Periodontal disease and upper genital tract inflammation in early spontaneous preterm birth. *Obstet Gynecol* 2004;104:777-783.

101. Jarjoura K, Devine PC, Perez-Delboy A, Herrera-Abreu M, D'Alton M, Papapanou PN. Markers of periodontal infection and preterm birth. *Am J Obstet Gynecol* 2005;192:513-519.

102. Davenport ES, Williams CE, Sterne JA, Murad S, Sivapathasundram V, Curtis MA. Maternal periodontal disease and preterm low birthweight: case-control study. *J Dent Res* 2002;81:313-318.

103. Noack B, Klingenberg J, Weigelt J, Hoffmann T. Periodontal status and preterm low birth weight: a case control study. *J Periodontal Res* 2005;40:339-345.

104. Offenbacher S, Lieff S, Boggess KA, et al. Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. *Ann Periodontol* 2001;6:164-174.

105. Offenbacher S, Boggess KA, Murtha AP, et al. Progressive periodontal disease and risk of very preterm delivery. *Obstet Gynecol* 2006;107:29-36.

106. Martinez de Tejada B, Gayet-Ageron A, Combescure C, Irion O, Baehni P. Association between early preterm birth and periodontitis according to USA and European consensus definitions. *J Matern Fetal Neonatal Med* 2012;25:2160-2166.

107. Baskaradoss JK, Geevarghese A, Kutty VR. Maternal periodontal status and preterm delivery: a hospital based case-control study. *J Periodontal Res* 2011;46:542-549.

108. Vogt M, Sallum AW, Cecatti JG, Morais SS. Periodontal disease and some adverse perinatal outcomes in a cohort of low risk pregnant women. *Reprod Health* 2010;7:29.

109. Guimaraes AN, Silva-Mato A, Miranda Cota LO, Siqueira FM, Costa FO. Maternal periodontal disease and preterm or extreme preterm birth: an ordinal logistic regression analysis. *J Periodontol* 2010;81:350-358.

110. Rakoto-Alson S, Tenenbaum H, Davideau JL. Periodontal diseases, preterm births, and low birth weight: findings from a homogeneous cohort of women in Madagascar. *J Periodontol* 2010;81:205-213.

111. Nabet C, Lelong N, Colombier ML, et al. Maternal periodontitis and the causes of preterm birth: the case-control Epipap study. *J Clin Periodontol* 2010;37:37-45.

112. Srinivas SK, Sammel MD, Stamilio DM, et al. Periodontal disease and adverse pregnancy outcomes: is there an association? *Am J Obstet Gynecol* 2009;200:497 e491-498.

113. Lohsoonthorn V, Kungsadalpipob K, Chanchareonsook P, et al. Is maternal periodontal disease a risk factor for preterm delivery? *Am J Epidemiol* 2009;169:731-739.

114. Kushtagi P, Kaur G, Kukkamalla MA, Thomas B. Periodontal infection in women with low birth weight neonates. *Int J Gynaecol Obstet* 2008;101:296-298.

115. Toygar HU, Seydaoglu G, Kurklu S, Guzeldemir E, Arpak N. Periodontal health and adverse pregnancy outcome in 3,576 Turkish women. *J Periodontol* 2007;78:2081-2094.

116. Le HT, Jareinpituk S, Kaewkungwal J, Pitiphat W. Increased risk of preterm birth among non- smoking, non- alcohol drinking women with maternal periodontitis. *Southeast Asian J Trop Med Public Health* 2007;38:586-593.

117. Mumghamba EG, Manji KP. Maternal oral health status and preterm low birth weight at Muhimbili National Hospital, Tanzania: a case-control study. *BMC Oral Health* 2007;7:8.

118. Santos-Pereira SA, Giraldo PC, Saba-Chujfi E, et al. Chronic periodontitis and pre-term labour in Brazilian pregnant women: an association to be analysed. *J Clin Periodontol* 2007;34:208-213.

119. Alves RT, Ribeiro RA. Relationship between maternal periodontal disease and birth of preterm low weight babies. *Braz Oral Res* 2006;20:318-323.

120. Bosnjak A, Relja T, Vucicevic-Boras V, Plasaj H, Plancak D. Pre-term delivery and periodontal disease: a case-control study from Croatia. *J Clin Periodontol* 2006;33:710-716.

121. Lunardelli AN, Peres MA. Is there an association between periodontal disease, prematurity and low birth weight? A population-based study. *J Clin Periodontol* 2005;32:938-946.

122. Rajapakse PS, Nagarathne M, Chandrasekra KB, Dasanayake AP. Periodontal disease and prematurity among non-smoking Sri Lankan women. *J Dent Res* 2005;84:274-277.

123. Corbella S, Taschieri S, Del Fabbro M, Francetti L, Weinstein R, Ferrazzi E. Adverse pregnancy outcomes and periodontitis: A systematic review and meta-analysis exploring potential association. *Quintessence Int* 2016;47:193-204.

124. Vettore MV, Lamarca Gde A, Leao AT, Thomaz FB, Sheiham A, Leal Mdo C. Periodontal infection and adverse pregnancy outcomes: a systematic review of epidemiological studies. *Cad Saude Publica* 2006;22:2041-2053.

125. Khader YS, Ta'ani Q. Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis. *J Periodontol* 2005;76:161-165.

126. Mobeen N, Jehan I, Banday N, et al. Periodontal disease and adverse birth outcomes: a study from Pakistan. *Am J Obstet Gynecol* 2008;198:514 e511-518.

127. Sheiham A, Striffler DF. A comparison of four epidemiological methods of assessing periodontal disese. 2. Test of periodontal indices. *J Periodontal Res* 1970;5:155-161.