



INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

**THE IMPACT OF PERIODONTAL INFLAMMATION ON
FEMALE FERTILITY**

Trabalho submetido por
Cláudia Escalda
para a obtenção do grau de Mestre em Medicina Dentária

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Trabalho orientado por
Prof. Doutor José João Mendes
e coorientado por
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setembro de 2021

*“We are all time travellers,
journeying together into the future.
But let us work together to make that
future a place we want to visit. Be
brave, be curious, be determined,
overcome the odds. It can be done.”*

*Stephen Hawking, in “Brief answers
to the big questions”*

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Abstract

Aims: To evaluate the relationship between periodontitis and systemic inflammation through C-reactive protein (CRP) serum levels and to appraise the connection between periodontitis and two female fertility conditions (Polycystic Ovary Syndrome [PCOS] and Bacterial Vaginosis [BV]).

Material and Methods: Firstly, four electronic databases (Pubmed, CENTRAL, EMBASE and Web of Science) were searched until February 2021. Observational and intervention studies were included on CRP and high-sensitivity CRP (hs-CRP) serum levels in patients with and without periodontitis and hs-CRP levels after treatment. Secondly, five databases (PubMed, Scholar, EMBASE, Web of Science and CENTRAL) were searched until May 2020. Case-control and cohort studies evaluating the association between PCOS and periodontitis were included. Lastly, National Health and Nutrition Examination Survey (NHANES) 2001-2004 dataset was analysed comprising women between 18–49 years old with or without a BV diagnosis in accordance with Nugent's method. Complete blood count and serum levels of CRP were evaluated on the presence of periodontitis and BV.

Results: In the first study, 77 case-controls and 67 intervention trials were included. Chronic and aggressive forms of periodontitis were related with higher CRP and hs-CRP levels. Intensive non-surgical periodontal treatment (NSPT) led to an instant increase of hs-CRP accompanied by a progressive decrease whereas non-intensive NSPT diminished hs-CRP levels after treatment upon 180 days. In the second study, 12 case-controls fulfilled the inclusion criteria. PCOS women present 28% more risk of having periodontitis, and periodontitis women have 46% higher risk of having PCOS. PCOS females with periodontitis had worse periodontal parameters than non-PCOS females with periodontitis. Also, in women with BV, periodontitis showed a higher inflammatory state, denoted by increased white blood cells (WBC) and lymphocyte counts.

Conclusions: Periodontitis is associated with systemic inflammation. Both PCOS and BV presented significant association with periodontitis. Future studies are mandatory to confirm such associations and underlying mechanisms.

Keywords: C-reactive protein; Polycystic Ovary Syndrome; Bacterial Vaginosis; Periodontitis.

Resumo

Objectivos: Avaliar a relação entre periodontite e inflamação sistémica através da proteína C reativa (PCR) e verificar a ligação entre a periodontite e Síndrome dos Ovários Policísticos (SOP) e a Vaginose Bacteriana (VB).

Materiais e Métodos: Primeiramente, as bases de dados (Pubmed, CENTRAL, EMBASE e Web of Science) foram avaliadas até fevereiro de 2021. Estudos observacionais sobre a PCR e PCR de alta sensibilidade (PCR-as) em pacientes com periodontite e saudáveis e estudos de intervenção com valores de PCR-as após tratamento foram incluídos. Secundariamente, cinco bases de dados (PubMed, Scholar, EMBASE, Web of Science e CENTRAL) foram avaliadas até maio de 2020. Estudos de caso-controlo e coortes associando a SOP e periodontite foram incluídos. Posteriormente, o *National Health and Nutrition Examination Survey* (NHANES) 2001-2004 foi analisado considerando mulheres entre 18-49 anos diagnosticadas ou não com VB através do método de Nugent. Foi considerada a definição de periodontite de 2012. Marcadores séricos foram comparados de acordo com a presença de periodontite e VB.

Resultados: No primeiro estudo, 77 casos-controlo e 67 estudos de intervenção foram incluídos. Periodontite crónica e agressiva foi associada a níveis mais elevados de PCR e PCR-as. Tratamento periodontal intensivo causou um aumento imediato de PCR-as seguido de um decréscimo progressivo enquanto que tratamento periodontal não intensivo diminuiu a PCR-as até 180 dias. No segundo estudo, 12 casos-controlo foram incluídos. Mulheres com SOP têm 28% maior risco de desenvolverem periodontite e mulheres com periodontite tem 46% maior risco de desenvolverem SOP. Mulheres com SOP e periodontite apresentam piores características periodontais comparativamente a mulheres sem SOP mas com periodontite. Ademais, em mulheres com VB, a periodontite estava associada a maior inflamação com aumento de leucócitos e linfócitos.

Conclusões: Periodontite está associada com inflamação sistémica. SOP e VB apresentam associações significativas com a periodontite. São necessários estudos futuros para confirmar estas associações e os mecanismos adjacentes.

Palavras-chave: Proteína C reativa; Síndrome dos Ovários Policísticos; Vaginose Bacteriana; Periodontite.

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List of Abbreviations

BMI | Body Mass Index

BoP | Bleeding on Probing

BV | Bacterial Vaginosis

CAL | Clinical Attachment Loss

CRP | C-reactive protein

DCs | Dendritic Cells

EFP/AAP | European Federation of Periodontology/ American Academy of Periodontology

FSH | Follicle-stimulating Hormone

GnRH | Gonadotropin-releasing Hormone

HA | Hyperandrogenism

HDL | High-Density Lipoprotein

hs-CRP | high sensitivity C-reactive protein

IFN | Interferon

IL | Interleukin

LH | Luteinizing Hormone

LPS | Lipopolysaccharide

MetS | Metabolic Syndrome

MMPs | Matrix Metalloproteinases

mtDNA | Mitochondrial DNA

Mw | Macrophages

NHANES | National Health and Nutrition Examination Survey

NSPT | Non-surgical Periodontal Therapy

OCPs | Osteoclast Precursors

OD | Ovulatory Dysfunction

OPG | Osteoprotegerin

OS | Oxidative Stress

PCOM | Polycystic Ovarian Morphology

PCOS | Polycystic Ovary Syndrome

PESA | Periodontal Epithelial Surface Area

PGE2 | Prostaglandin E2

PISA | Periodontal Inflamed Surface Area

PMNs | Polymorphonuclear Neutrophils

PPD | Probing Pocket Depth

RANK | Receptor Activator of Nuclear Factor κ

RANK-L | Receptor Activator of Nuclear Factor κ Ligand

ROS | Reactive Oxygen Species

STI | Sexual Transmitted Infection

Th | T helper

TNF- α | Tumour Necrosis Factor α

WBC | White Blood Cells

WHO | World Health Organization

I. INTRODUCTION

1. PERIODONTIUM AND PERIODONTAL HEALTH

In line with the World Health Organization (WHO) (WHO, 2006), the definition of health comprehends a state of complete physical, mental and social well-being and not solely the non-existence of disease. This theory can be applied to all organisms or to a specific organ/tissue. In this sense, and following the 2017 European Federation of Periodontology (EFP) / American Academy of Periodontology (AAP), periodontal health is characterized by a periodontal inflammation-free state that allows normal function and avoids mental or physical consequences due to current or past illnesses (Chapple et al., 2018). Clinically, a healthy periodontium also implies biological and inflammatory markers levels consistent with homeostasis (Lang & Bartold, 2018). Hence, gingival health on intact periodontium is marked by no bleeding on probing (BoP), erythema, oedema, symptoms and bone loss as well as physiological bone levels ranging from 1 to 3 mm below the cemento-enamel junction (Chapple et al., 2018).

2. PERIODONTAL DISEASE

Periodontal disease is a condition affecting the periodontium and comprises gingivitis and periodontitis (Gasner & Schure, 2021). On one hand, gingivitis is characterized as a gingival inflammation due to dental plaque accumulation and is reversible upon the improvement of oral hygiene habits (Gasner & Schure, 2021). On the other hand, periodontitis is a chronic multifactorial inflammatory condition that not only impacts the gingiva but also the periodontal ligament, root cementum and alveolar bone (Lindhe, 2015), and is clinically outlined by inflamed gums and bone destruction due to a dysbiotic microflora (Figure 1) (Papapanou et al., 2018). The transition from gingivitis to periodontitis occurs due to the persistence of an uncontrolled gum inflammation (Lindhe, 2015) with an irreversible destruction of the periodontium tissues (Gasner & Schure, 2021).

Hence, periodontitis patients often present red swollen gingiva characterized by gingival bleeding (Lindhe, 2015). The redness results from vasodilation and increased blood flow,

while the swelling is due to increased vascular permeability and shifted osmotic potential. Gum bleeding occurs because the vascular epithelium may become fragile in periodontitis (Armitage, 2004). However, it cannot be seen as a positive predictive factor on the activity of the disease (Tonetti et al., 2018).



Figure 1 | Main stages of periodontal disease. Schematics of healthy gingiva, gingivitis and periodontitis.

Although periodontitis is characterized by a local uncontrolled inflammatory response mediated by innate and adaptive immune systems (Ebersole et al., 2013; Hajishengallis & Chavakis, 2021), some people can be more vulnerable to the repercussions of plaque accumulation while others might be resistant and hence, never develop the disease (Kinane et al., 2017). Additionally, a few locations in the oral cavity are more prone to develop this condition than others in the same patient (Benakanakere et al., 2015).

Regarding epidemiology, periodontitis is highly prevalent affecting up to 50% of the adult population worldwide alongside other periodontal diseases (Nazir, 2017; Tonetti et al., 2017). In the American context and according to the National Health and Nutrition Examination Survey (NHANES) 2009-2012 and 2009-2014, periodontitis affects between 42.2 to 46.0% of dentate adults with more than 30 years of age (Eke et al., 2018). Furthermore, mild/moderate periodontitis is the most prevalent (34.4% to 37.1%), whereas severe periodontitis accounts for 7.8 to 8.9% (Eke et al., 2018). Very few studies have provided epidemiological data of the periodontal status in Europe (König et al., 2010). A French study estimated that clinical attachment loss (CAL) ≥ 5 mm was present in 46.68% of the population with probing pocket depth (PPD) > 5 mm reaching 10.21% (Bourgeois et al., 2007). A study in the Pomeranian region determined the prevalence of CAL ≥ 3 mm to be 89.7% with PPD ≥ 4 mm being prevalent in 69.7% of subjects (Holtfreter et al., 2009). The same study also found that 17.6% and 33.3% of the subjects had severe and moderate periodontitis, respectively (Holtfreter et al., 2009). A Norwegian

study estimated the prevalence of periodontitis to be 49.5%, specifically 9.1% for the severe form of periodontitis and 40.4% for the non-severe form (mild and moderate periodontitis combined) (Holde et al., 2017). Likewise, the prevalence estimates of severe and moderate periodontitis in an Italian study were 34.94% and 40.78%, respectively (Aimetti et al., 2015). In Portugal, a single national epidemiological investigation was undertaken, although the prevalence of periodontitis was clearly optimistic and low (10.8% in adults and 15.3% in elderly) (DGS, 2015). Recently, the prevalence of periodontitis was assessed in the southern region of the Lisbon Metropolitan Area (Botelho et al., 2019), where the prevalence of periodontitis was found to be 59.9%. Furthermore, 24.0% and 22.2% of the participants exhibited severe and moderate forms of periodontitis, respectively (Botelho et al., 2019). A possible explanation for this discrepancy may be the partial recording protocol used in the first Portuguese epidemiological study (V. Machado et al., 2018b). Also, and regarding the topic of this thesis, it was found that women who are undertaking fertility treatment presented more severe periodontitis and significantly worse clinical characteristics (periodontal epithelial surface area [PESA], PPD, CAL and number of missing teeth) compared to controls (V. Machado et al., 2020c). Specifically, 33.3% and 16.7% of women in the fertility treatment group were diagnosed with periodontitis and gingivitis, respectively (V. Machado et al., 2020c). Lower results were found in the control group which presented 22.2% of women with periodontitis and 11.1% with gingivitis (V. Machado et al., 2020c). The results of this study incite for a possible relationship between female infertility-related conditions and periodontitis and introduces the importance of verifying such relationship as well as the mechanisms upon this interplay.

2.1. Etiology and etiopathogenic mechanisms

In a healthy epithelium there is a dynamic where the host proinflammatory activities and antimicrobial reactions are balanced. In this way, the innate and adaptive immune system establish a symbiose relationship between the host and the microorganisms, keeping the gingival epithelium intact and preventing the hematogenous dissemination of periodontal organisms (Hajishengallis, 2014). This happens because a barrier between the periodontium and the dental plaque biofilm is formed due to the passage of polymorphonuclear neutrophils (PMNs) and other immune cells through the desmosomes

and gap junctions of the junctional epithelial cells (Darveau, 2010).

Periodontal disease begins when the homeostatic equilibrium ends (Hajishengallis, 2015). Overtimes, there is a shift in the normal oral microflora, diminishing commensal bacteria and/or overwhelming periodontopathogenic bacterial colonization reaching periodontal tissues (Hajishengallis, 2015). Furthermore, in a susceptible host, microbial infection by periodontal bacteria, in particular a group of specific Gram-negative anaerobic species also known as the red complex, leads to chronic inflammation (Socransky & Haffajee, 2005). These red-complex bacteria comprehends *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* and are predominantly observed in periodontal patients deep periodontal pockets (Mysak et al., 2014; Singhrao et al., 2015).

As shown in figure 2, lipopolysaccharide (LPS) of these bacteria, along with other virulence factors, induce the host macrophages and other inflammatory cells to produce a variety of pro-inflammatory cytokines (such as tumour necrosis factor [TNF]- α , interleukin [IL]-1 β and prostaglandin E2 [PGE2]) (Kwon et al., 2020). These pro-inflammatory cytokines and virulence factors, in turn enhance the creation of matrix metalloproteinases (MMPs) by macrophages, fibroblasts, junctional epithelial cells and neutrophils (Nędzi-Góra et al., 2017). These MMPs cause the destruction of collagen fibres in periodontal tissues, especially periodontal ligaments (Page et al., 1997). Furthermore, the pro-inflammatory cytokines enhance the expression of receptor activator of nuclear factor κ ligand (RANK-L) on the osteoblasts and T helper (Th) cells arising in its interaction with receptor activator of nuclear factor κ (RANK) on osteoclast precursors. Consequently, the formation of osteoclasts and their maturation leads to alveolar bone destruction (Taubman et al., 2005).

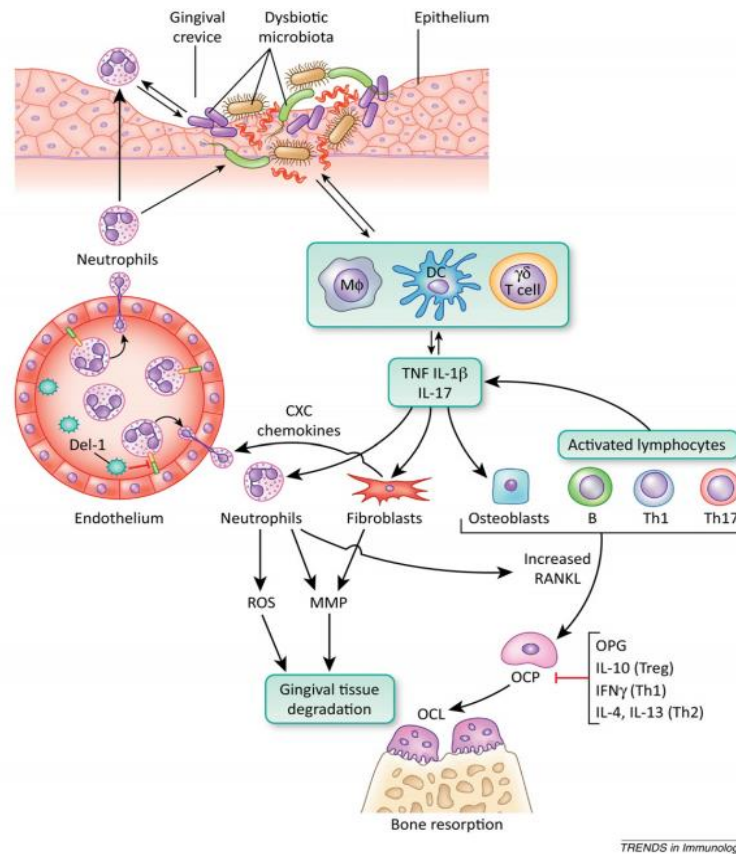


Figure 2 | Inflammatory mechanisms leading to bone loss in periodontitis. The connective tissue is invaded by dysbiotic bacteria which interacts with immune cells, such as macrophages (Mφ) and dendritic cells (DCs), leading to the production of proinflammatory mediators (such as the bone-resorptive cytokines tumour necrosis factor [TNF], interleukin [IL]-1β, and IL-17) and managing the development of T helper (Th) cells, which also play a role and enhance the inflammatory response. IL-17 acts on neutrophils, fibroblasts, and osteoblasts inducing the production of CXC chemokines, matrix metalloproteinases (MMPs) and other destructive molecules. It also takes part in osteoblast expression of receptor activator of nuclear factor κB ligand (RANKL), which drives the maturation of osteoclast precursors (OCPs). When lymphocytes are activated, they play a major role in pathological bone resorption through the same RANKL-dependent mechanism, whereas osteoprotegerin (OPG) inhibits the interaction of RANKL with its functional receptor (RANK) on OCP. The RANKL/OPG ratio is proportional to the inflammatory activity. Upon activation, neutrophils express membrane bound RANKL and can directly stimulate osteoclastogenesis. The anti-inflammatory cytokine IL-10, as well as interferon (IFN)γ and IL-4 plus IL-13 have the ability to suppress osteoclastogenesis. (Adapted from Hajishengallis, 2014 according to CCC terms and conditions [<https://www.copyright.com/>]).

2.2. Risk factors

Periodontitis is defined as a microbial-shift disease denoted by the change of microorganisms from mainly Gram-positive to mainly Gram-negative species over the passage from periodontal health to periodontal disease (Marsh, 1994). Poor hygiene habits as well as pathogenic biofilm accumulation are commonly associated with periodontal disease however, this seem insufficient to simply generate periodontitis (Hajishengallis, 2015). Nonetheless, some contributing factors such as overhanging

restorations (Jansson et al., 1994), open interproximal contacts (Koral et al., 1981), and the presence of certain anatomical factors like narrow furcation entrance (Bower, 1979) and position of the tooth (Kassab & Cohen, 2003), have been associated as they promote the accumulation of biofilm.

In addition, several environmental and lifestyle factors have since been established. Periodontitis is associated with age (Ebersole et al., 2016), educational levels (V. Machado et al., 2020a) and psychosocial stress (Botelho et al., 2018). Moreover, pregnancy, parity and motherhood can be a source of acute and chronic stress (Leung et al., 2016) promoting this condition. Also, smoking habit is a major modifiable risk factor towards the development of chronic periodontitis (V. Machado et al., 2018a). Smokers demonstrate a decreased periodontal status and more-severe tooth loss than non-smokers, as well as higher rates of progression of chronic periodontitis and tooth loss (Kumar, 2020). Notably, gingival inflammation can be less pronounced in smokers than in non-smokers in result of vasoconstriction and increased keratinization of gingival tissues (Kinane et al., 2006).

In the same fashion, systemic conditions have consistently been associated with periodontitis. This interplay occurs when the destruction of local epithelium mediated by periodontopathogenic microorganisms ends up in the release of local inflammatory mediators from the periodontal pocket into the systemic circulation (Paul et al., 2021). Bacteria can either directly or indirectly get into the bloodstream reaching distant locations (Lockhart et al., 2009) and resulting in bacteraemias (Schenkein et al., 2020) and periodontitis-related systemic inflammation (Hajishengallis & Chavakis, 2021).

Inflammation in extra-oral sites can also occur due to oro-pharyngeal or oro-digestive translocation of periodontal pathogens (Hajishengallis & Chavakis, 2021). Oro-pharyngeal translocation has been associated with aspiration pneumonia (Mammen et al., 2020), whereas oro-digestive translocation is related with intestinal dysbiosis and gut-mediated systemic inflammation (Kitamoto et al., 2020).

Although the existence of periodontal bacteria or LPSs in systemic tissues is transient, their consequences (Farrugia et al., 2020) and induction of inflammation are significant given the chronic characteristics of periodontitis and the frequency of bacteraemias which not only occur during professional dental treatments but also during tooth brushing or

mastication (Teles & Wang, 2011).

Having this in consideration, periodontitis has been associated with several conditions such as cardiovascular diseases (V. Machado et al., 2020b; Mendes et al., 2021), diabetes mellitus and obesity (Winning & Linden, 2017), cancer (Hajishengallis, 2015; Michaud et al., 2017), renal diseases (Schöffner et al., 2020), rheumatoid arthritis (Hussain et al., 2020) and female fertility conditions (V. Machado et al., 2020d). The association between periodontitis and systemic conditions has been consistently studied and linked with a state of low-grade inflammation considering the systemic effect of periodontitis inducing an increase of serum C-reactive protein (CRP) and other biomarkers, which may enhance the overall inflammatory burden (Morita et al., 2016).

Moreover, genetic predispositions have been found to be involved in both the beginning and the evolution of periodontitis (Borrell & Papapanou, 2005), with an estimated value of heritability reaching 50% (Michalowicz et al., 2000). Due to the complex nature of periodontal disease as well as the biological process involved in its pathogenesis, it is estimated that periodontitis is correlated with alterations in a variety of genes (Laine et al., 2012), each contributing for the onset and progression of disease (T. C. Hart et al., 2000). Furthermore, some of the genes associated are toll-like receptors, ILs, immunoglobulin receptors and MMP which have been consistently studied in genome-wide association studies (Shaddox et al., 2020).

3. CRP AS A MARKER OF SYSTEMIC INFLAMMATION

CRP is a pentameric acute-phase reactant protein synthesized by the liver, whose level rises in the presence of inflammation (Nehring et al., 2021) due to the action of IL-6 on the gene responsible for its transcription (Kramer et al., 2019).

Two distinct inflammatory markers of CRP are currently acceptable and reported in the literature. CRP is an already well-known biomarker of inflammatory processes being characterized by its precision within the range of 10 to 1,000 mg/L, on the other hand, the high sensitivity CRP (hs-CRP) is a more precise and sensitive analyte within the range of 0.5 to 10 mg/L (Pearson et al., 2003).

Interestingly, the CRP serum levels can change promptly with the beginning and the end

of the inflammatory process (Nehring et al., 2021). There are several causes for an elevated CRP level including acute and/or chronic states, either infectious or non-infectious in aetiology. Nevertheless, exceptionally higher serum concentrations of CRP are generally associated with an infectious process (Vanderschueren et al., 2006).

It is known that the host response to a periodontal infection activates the innate and adaptive immunity (Paraskevas et al., 2008). Even though periodontitis is considered a chronic disease, the elements characteristic of an acute reaction also take part in the innate immunity and corroborate the presence of a systemic inflammation (Ebersole & Cappelli, 2003).

Considering the clinical relevance of systemic chronic inflammation and its influences on systemic health, CRP is considered an important biomarker (Paraskevas et al., 2008). Thus, it is worthy to consider periodontitis treatment to alleviate systemic inflammatory burden. Currently two different periodontal therapeutic approaches have been considered: 1) non-intensive non-surgical periodontal therapy (NSPT) consisting of standard mechanical scaling and root planning under local anaesthesia; and 2) intensive NSPT involving an intensive session of full-mouth subgingival root debridement delivered over a four-hour period under local anaesthesia (where periodontal surgery, teeth removal or local antibiotic might be delivered). In this sense, in the future it is interesting to search which treatment modality better decreases the serum levels of CRP, and which one have the highest impact on systemic inflammation.

4. FEMALE INFERTILITY-RELATED CONDITIONS

The definition of infertility is the inability to initiate a clinical pregnancy upon 12 months of regular unprotected sexual activity or due to an impairment in reproduction or after therapeutic donor insemination in women with less than 35 years or more than 35 years within 6 months of failed fertility treatments (Adamson et al., 2018; Vander Borgh & Wyns, 2018).

Infertility can also be classified as primary or secondary. Primary infertility occurs in women which have never been pregnant before and who meets the criteria for being classified as infertile. Secondary infertility applies to women unable to maintain a clinical pregnancy although have been previously diagnosed as pregnant (Vander Borgh &

Wyns, 2018). This is a problem that may constrain the growth of the population (Vander Borgh & Wyns, 2018) and have a negative impact not only on the couple (Onat & Beji, 2012) but also on its sexual satisfaction (Ramezanzadeh et al., 2006) and psychological and social health and happiness (Cousineau & Domar, 2007). Unfortunately, it is a prevalent condition worldwide being estimated to affect 186 million people and 8-12% of reproductive-aged couples, the majority belonging to developing countries (Inhorn & Patrizio, 2015). Within female infertility, secondary infertility is the most common form (Nachtigall, 2006) and occurs mostly in regions with poor maternity care and high rates of unsafe abortion which results in post-abortive and post-partum infections (Inhorn & Patrizio, 2015).

Several lifestyle factors have been associated with this condition. An important factor affecting the occurrence of pregnancy is the frequency of coitus soon after menses (Elzanaty et al., 2005). Also, dietary restriction and over exercise have been associated leading to a reduction in the frequency of ovulation, poor endometrial development and amenorrhea (Vander Borgh & Wyns, 2018). Furthermore, obesity, smoking and alcohol habits have been related with infertility as they diminish the ovulation and the probability of a spontaneous conception (Best & Bhattacharya, 2015). After conception, these also influences the occurrence of miscarriages and the predisposition for an unfavourable pregnancy outcome (Best & Bhattacharya, 2015).

In addition, several diseases affecting females are known to influence fertility and diminish the probability of conception or influence pregnancy outcomes. Some examples are premature ovarian insufficiency (Vander Borgh & Wyns, 2018), polycystic ovary syndrome (PCOS) (Dewailly, 2016), bacterial vaginosis (BV) (Jacobsson et al., 2002), endometriosis (Tanbo & Fedorcsak, 2017), uterine fibroids (Vander Borgh & Wyns, 2018), and endometrial polyps (R. J. Hart, 2016).

4.1. Polycystic Ovary Syndrome

PCOS is an endocrine, reproductive and metabolic disease, and can lead to infertility (Bozdog et al., 2016). It affects 5–15% of reproductive-aged women worldwide (Bozdog et al., 2016), although the prevalence varies among different countries (Deswal et al., 2020). It is also known that caucasian females are less likely to develop PCOS when

compared with middle east and non-white female populations (Ding et al., 2017).

4.1.1. Diagnosis

The diagnosis is based on the Rotterdam criteria established by European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine (Fauser et al., 2004). According to these criteria the patient must clinically present two out of three of the indicators: a) oligo ovulation or anovulation; b) clinical (hirsutism, acne, male pattern alopecia) or biochemical manifestations of hyperandrogenism (increased serum levels of testosterone); c) polycystic ovaries, meaning 12 or more follicles with dimensions between 2 to 9 mm in diameter or increased ovarian volume, larger than 10 cm³, observed by transvaginal ultrasound. It is important that other aetiologies of menstrual disturbance are excluded (Fauser et al., 2004).

Several phenotypes can be identified and divided in four classes with distinct characteristics, namely (1) phenotype A, marked by clinical or biochemical hyperandrogenism (HA), ovulatory dysfunction (OD), and polycystic ovarian morphology (PCOM); (2) phenotype B, denoting HA and OD, but not PCOM; (3) phenotype C, distinguished by HA and PCOM, without OD; and finally (4) phenotype D, characterized by OD and PCOM, without HA (Azziz et al., 2006).

Regarding a serological diagnosis, hs-CRP is considered the gold standard for clinical purposes (Margioris et al., 2013). Levels lower than 1 mg/L suggest no chronic low grade inflammation; levels ranging from 1 to 3 mg/L suggests clinically evident chronic low grade inflammation; levels between 3 and 10 mg/L suggests a high chronic low grade inflammation and levels above 10 mg/L suggest acute inflammation not related to chronic low grade inflammation (Margioris et al., 2013).

4.1.2. Aetiology

Throughout the years, it has been proposed that oxidative stress (OS) influences PCOS disorder as it is considered a low-grade inflammatory condition (Mancini et al., 2021). OS is defined as an alteration in the prooxidant/antioxidant ratio favouring the oxidative

state and ultimately causing damage (Sies, 2019). The mechanism of OS in PCOS relies on a multitude of genetic and environmental factors. The major phenomenon to be pointed out as main source of reactive oxygen species (ROS) arising from nutrient translation is mitochondrial dysfunction (Zhang et al., 2019). An increased production of ROS leads to damage of mitochondrial components such as mitochondrial DNA (mtDNA), proteins and lipids inducing cell apoptosis (Wallace, 2005). Another factor is diet-induced OS. When glucose is absorbed it induces an inflammatory response verified by increased ROS-related OS (González et al., 2006). It also potentiates the release of proinflammatory cytokines from circulating monocytes (Figure 3) (González et al., 2005).

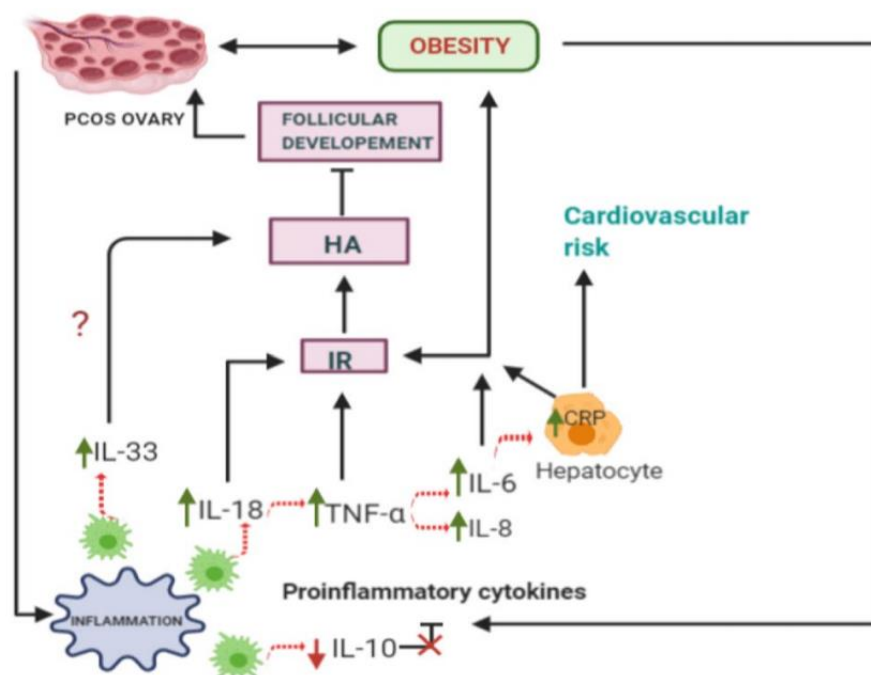


Figure 3 | Interaction between Polycystic Ovarian Syndrome (PCOS) and inflammatory markers. (Adapted from Gnanadass et al., 2021 according to CCC terms and conditions [https://www.copyright.com/]).

Hence, OS can induce and/or worsen all main features of PCOS, being these:

- Metabolic syndrome (MetS) - MetS is generally considered as a condition characterized by abdominal obesity, impaired glucose metabolism, hypertriglyceridemia, hyperinsulinemia, low HDL (high-density lipoprotein) cholesterol, hypertension and insulin resistance (Lotti et al., 2021).

Hyperinsulinemia and insulin resistance increase the secretion of androgens in women (Dumesic et al., 2015).

- Hyperandrogenism - As mentioned above and considering MetS a prominent condition of PCOS, a surplus amount of androgens is present in women with polycystic ovaries. Increased levels of androgens enhances primordial follicles and antral follicles (Gnanadass et al., 2021) and ultimately leading to numerous cysts (Abbott et al., 2005).
- Obesity - Obesity is thought to have a key role in PCOS in susceptible women (Gambineri et al., 2002). It can be responsible for insulin resistance and hyperinsulinemia, both resulting in excessive androgens levels, as mentioned above. It is also interesting to point out that this relationship is bidirectional as PCOS is marked by increased OS which in turn induces proliferation of preadipocytes and adipocyte differentiation through the increase of the size of mature adipocytes (Furukawa et al., 2004).
- Infertility - It was verified an increased difficulty for pregnancy to happen and a higher risk of miscarriage in PCOS women (Özer et al., 2016) due to enhanced ROS activity, antioxidant depletion and altered mitochondria distribution in oocytes (Zhang et al., 2019).

4.2. Bacterial Vaginosis

BV is characterized by a shift in the vaginal microbiota (Livengood, 2009), associated with a thin gray/white and malodorous discharge, a high vaginal pH (>4.5), and vaginal epithelial cells heavily coated with bacteria (Bautista et al., 2016). Concerning epidemiology, BV is a prevalent condition, accounting for 30% of the population (Livengood, 2009) and is greater among African-Americans than Caucasians (Kenyon et al., 2013).

It is associated with abortion (Haahr et al., 2019), failure in implantation (Moreno et al., 2016) and preterm birth (Yudin, 2005). However, the link between BV and woman infertility is yet to be confirmed (Haahr et al., 2019).

4.2.1. Diagnosis

Nowadays, the clinical diagnosis is based on the Amsel criteria and must meet 3 of the following 4 criteria: a) vaginal pH higher than 4.5, b) a milk-like consistency discharge characterized by being thin and homogeneous, independently of the colour and quantity, c) higher fishy odour scent of the discharge upon addition of 10% potassium hydroxide (whiff test), and d) microscopic examination of vaginal swabbing samples in saline with the presence of clue cells (Amsel et al., 1983). However, for research and clinical studies purposes, the Nugent's classification system is the gold standard (Lee et al., 2020). It consists of a Gram stain scoring system based on the density of certain bacteria, where a higher density of *Lactobacillus* morphotypes decreases the score and a greater density of *Gardnerella* and *Bacteroides* morphotypes increases the score. Further 1 or 2 points are added for the presence of *Mobiluncus* morphotypes. Hence, three different ranges are considered for BV diagnosis: 0 to 3 are normal, 4 to 6 are an intermediate state, and 7 to 10 are positive for BV (Nugent et al., 1991).

4.2.2. Aetiology

The healthy vaginal flora is represented by a hydrogen peroxide producer *Lactobacillus spp*, which is able to eliminate other bacteria unable to synthesize catalase (Livengood, 2009). They also produce organic acid using glycogen in the vaginal epithelium as the substrate contributing to the normal vaginal pH, as well as synthesize bacteriocins which are proteins that inhibit other bacterial species (Livengood, 2009). Hence, BV is marked by a depletion of hydrogen peroxide-producing lactobacilli and an increase of anaerobic species.

BV is characterized by variations in the biochemistry and microenvironment of the vagina (Swidsinski et al., 2005). The regular vaginal epithelium is protected by a thin layer of mucin whereas in BV, this layer is changed to a *Gardnerella vaginalis*-specific biofilm. Regarding biochemical changes, concentrations of β -defensin-1 and -2, secretory leukocyte protease inhibitor, IL-1 α , IL-1 β , and IL-8 are diminished; on the other hand, 70 kD heat-shock protein, lytic enzymes, nitric oxide, and endotoxin are found in higher concentrations in the vagina of BV patients. Comprehensively, these changes predispose the vagina to increased destructive and inflammatory influences due to deprivation of

normal protective mechanisms. Be that as it may, the aetiology of BV is still unknown (Bautista et al., 2016; Guédou et al., 2013; Hay, 2017).

4.2.3. Predisposing factors

Several risk factors are associated with BV. Nevertheless, since data regarding aetiology of BV is lacking (Bautista et al., 2016), it is not clear if BV is a sexual transmitted infection (STI) (Guédou et al., 2013), although, in the literature there are reports relating BV with sexual activity (Fethers et al., 2009). Aspects such as number of sexual partners and frequency of vaginal intercourse, women having sex with other women, early coitarche, recent partner change, oral and anal sex and medical records of bacterial STIs, have been demonstrated to increase the risk of BV (Guédou et al., 2013; Muzny et al., 2013). It is also important to point out the association with education level. Lower levels of schooling was shown to be a predictive factor in the development of BV (Ranjit et al., 2018). Furthermore, recent evidence also indicates an association between menstrual cycle and BV (Bautista et al., 2016).

Also, a correlation between BV and birth control methods has been established and it highly depends on which methods are used. Oral contraceptives and condom use seem to decrease the prevalence of BV (Bautista et al., 2016; Ranjit et al., 2018).

5. ASSOCIATION BETWEEN PERIODONTITIS AND FEMALE INFERTILITY-RELATED CONDITIONS

Oral health is crucial for the maintenance of regular quotidian functions. Over the years, it has been investigated the influence of periodontitis upon conception and it has been verified as a negative relationship (V. Machado et al., 2020c). Several hypotheses have been proposed to explain such association, however, the common ground relies on the low-grade systemic inflammation associated with periodontitis and its influence on the endometrial tissues (Johnson et al., 2010). Also, evidence supported that the spread of bacteria and their by-products into the bloodstream causes increased inflammatory burden, OS, and leukocyte response (Figure 4) (Porwal et al., 2014).

Also, a possible interplay between female infertility-related conditions and periodontitis is the role that steroid sex hormones have on the periodontium. Sex hormone receptors were discovered on gingival fibroblasts and epithelial cells (Vittek et al., 1982), reinforcing this relationship. Although the following findings are contradictory (Morelli et al., 2018), it was proposed that sex hormones may alter oral microbial balance contributing to periodontal alterations (D. Machado et al., 2016). It is also important to point out that oestrogen through the oestrogen- α receptor may control blood vessel tone. It is also regarded as its function on the blood vessel permeability through the release of mediators and vasoactive amines (Arnal et al., 2010). This action on blood vessels might leads to increased vascular permeability and contributes to gingival changes (Morelli et al., 2018).

Notwithstanding, the impact of female infertility-related conditions on the development of periodontitis and how periodontal infection and consequent inflammation influences female infertility-related conditions is yet not fully understood (Kassebaum et al., 2014).

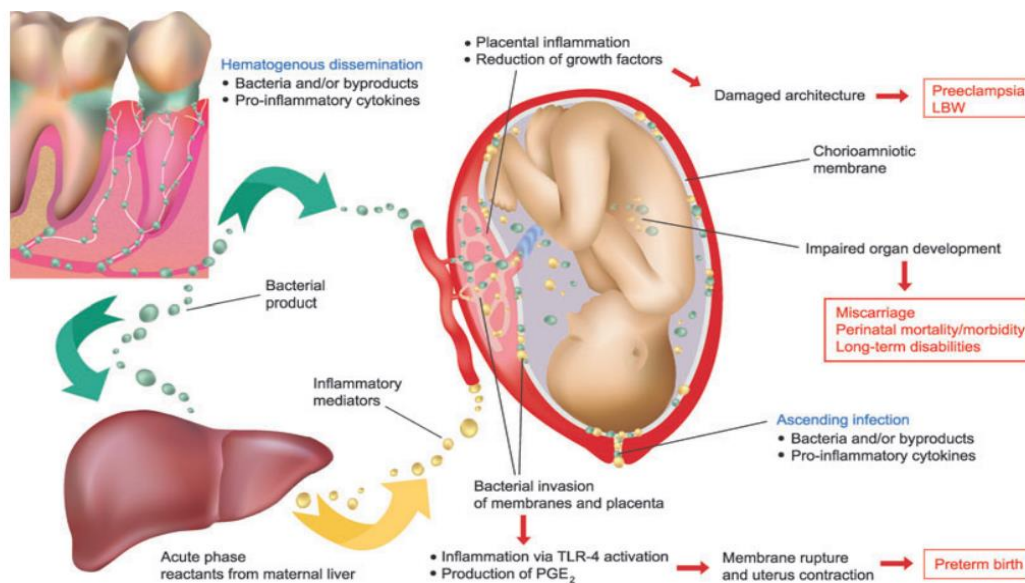


Figure 4 | Plausible biological mechanisms on the association between periodontal disease and pregnancy complications. (Adapted from Madianos et al., 2013 according to CCC terms and conditions [https://www.copyright.com/]).

5.1. Periodontitis and PCOS

Throughout the years, it has been proven a bidirectional relationship between periodontitis and PCOS based on the fact that both are characterized by chronic

subclinical inflammation (Tong et al., 2019), leading to a cascade of proinflammatory events (Figure 5) (Akcalı et al., 2017). Two theoretical mechanisms have been recently proposed (V. Machado et al., 2020d): a) PCOS is marked by hyperandrogenism, hyperinsulinemia and OS which have an impact on periodontal inflammation and destruction and b) periodontitis increases proinflammatory markers and OS levels which in turn unbalance insulin resistance and negatively influence ovarian function.

The first association made between periodontitis and PCOS was grounded on their association with MetS (Dursun et al., 2011). It was verified that women with PCOS possessed increased levels of periodontal inflammation and destruction when associated with glycaemic dysregulation and increased OS (Dursun et al., 2011). Also, having in consideration that MetS is one of PCOS main characteristics, it is relevant to point out that it may induce systemic inflammation and resistance to insulin (Nibali et al., 2013), reinforcing the PCOS-Periodontitis association.

Another relevant association is the fact that the chronic subclinical inflammation inherent to both diseases leads to an increase in proinflammatory markers concentrations such as CRP, TNF- α , IL-6, IL-7 and MMPs (Cekici et al., 2014) as well as potentiates OS (Akcalı et al., 2017). In this way, the subclinical inflammation associated to periodontitis and the direct invasion of periodontopathogenic bacteria may promote PCOS pathogenesis and, in a similar way, subclinical inflammation produced by PCOS may aggravate periodontal destruction (V. Machado et al., 2020d).

Nevertheless, and although the PCOS-periodontitis link might be reasonable, this relationship remains to be fully elucidated, reinforcing the importance of further studies.

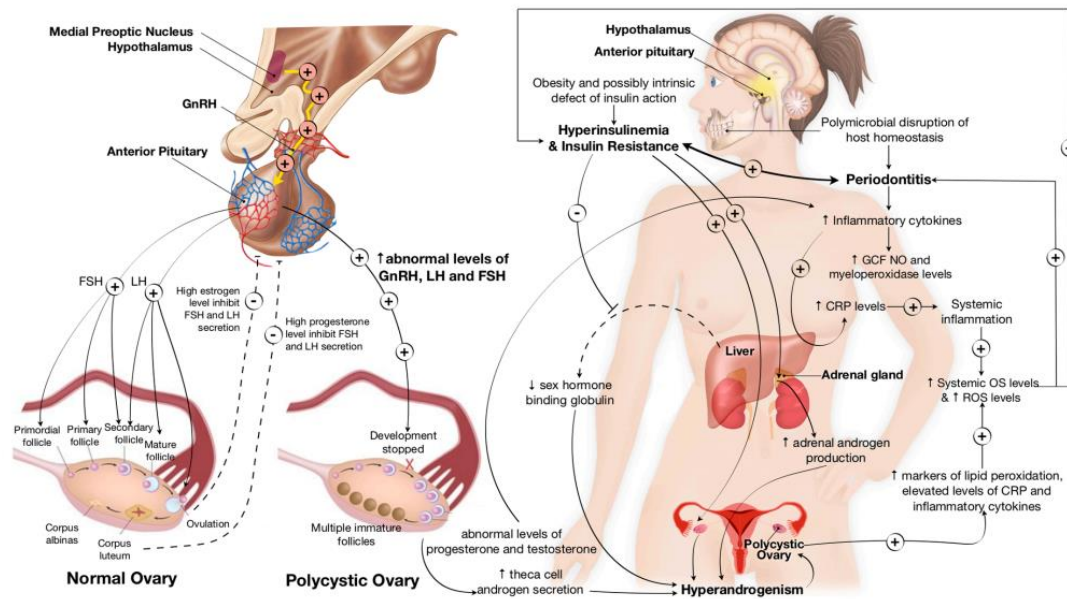


Figure 5 | PCOS pathophysiology and the role of periodontitis. Luteinizing hormone (LH) is a key hormone in hyperandrogenism in Polycystic Ovarian Syndrome (PCOS). Also, gonadotropin-releasing hormone (GnRH) is able to resist to the negative feedback of progesterone. Hence, increased levels of GnRH leads to the production of LH and restricts the production of Follicle Stimulating Hormone (FSH), enhancing androgen production and modifying the normal follicular development. PCOS is also related to resistance to insulin, altered ovarian function and adrenal steroidogenesis, promoting hyperandrogenism and female infertility. Additionally, PCOS women show increased serum levels of Interleukin (IL)-6, Tumour Necrosis Factor (TNF)- α , C-reactive protein (CRP), and white blood cell (WBC) count. In this sense, the underlying mechanisms of the PCOS-Periodontitis association seems to be systemic inflammation, oxidative stress (OS) and insulin resistance. (Adapted from V. Machado et al., 2020d according to CCC terms and conditions [<https://www.copyright.com/>]).

5.2. Periodontitis and BV

Microbial similarities in the oral and vaginal environments were described, which may indicate a common pathophysiology (Cauci, 2004). One of the main species that has been identified is *Prevotella* (Coyne et al., 2019; Larsen, 2017). These bacteria is responsible for a state of chronic inflammation due to the production of a number of inflammatory mediators such as IL which will ultimately activate neutrophils (Larsen, 2017). This inflammatory state is responsible for releasing toxic products, such as enzymes and cytokines ultimately contributing to tissue destruction (Aboodi et al., 2011). *Prevotella* also secretes peptide toxins reinforcing the spread of inflammation (Coyne et al., 2019). As a consequence, such factors will result in bone loss and tissue destruction in periodontitis (V. Machado et al., 2020d) as well as it may collapse systemic health (Larsen, 2017) creating a possible link with BV.

It is also important to mention that active smokers present a higher prevalence of BV due to the effects that smoking has on cervicovaginal immunity, characterized by higher levels of inflammatory markers on vaginal secretions (Murphy & Mitchell, 2016). Smoking is also a considerable modifiable risk for periodontitis (Palmer et al., 2005) known for compromising immune response (Buduneli & Scott, 2018; Ebersole et al., 2013).

Further, women with periodontitis and BV during pregnancy may have higher chances of preterm delivery, intrauterine and genital tract infection, as well as distant site infections such as periodontal infection (Klein & Gibbs, 2005). This may happen by two means: a) periodontopathogenic bacteria and by-products spread into the bloodstream triggering an inflammatory response when reaching the foeto-placental unit (Madianos et al., 2013); b) periodontal inflammation results in the production of inflammatory mediators extending the inflammatory response (Madianos et al., 2013).

Still, the association between BV and periodontitis needs to be better understood and additional evidence is required on the underlying mechanisms.

II. AIMS

In the Introduction section, the literature highlighted the lack of studies on the association between periodontitis, and CRP, PCOS and BV. Therefore, the aims of this thesis were divided in three articles:

- To assess the evidence regarding the impact of a periodontitis diagnosis on CRP levels in patients with no other conditions when compared to controls, and to investigate the influence of intensive and non-intensive NSPT on the hs-CRP levels after treatment (Section III).
- To research all evidence correlating PCOS and periodontitis and establish a possible bidirectional association (Section IV).
- To investigate a possible association between BV and periodontitis and to explore if this is responsible for systemic changes detected in blood samples through blood count (white, red and platelet cells) and CRP serum concentrations (Section V).

III. SERUM C-REACTIVE PROTEIN AND PERIODONTITIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Machado V., Botelho J., Escalda C., Hussain S.B., Luthra S., Mascarenhas P., Orlandi M., Mendes J.J., & D'Aiuto, F. (2021). Serum C-reactive protein and periodontitis: A systematic review and meta-analysis. *Frontiers in Immunology*, 12, 1-10. <https://doi.org/10.3389/fimmu.2021.706432>



Serum C-Reactive Protein and Periodontitis: A Systematic Review and Meta-Analysis

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Periodontitis has been associated with low-grade inflammation as assessed by C-reactive protein (CRP) levels and its treatment can decrease CRP serum levels. The aim of this systematic review was to critically appraise the evidence comparing CRP serum levels (standard and high-sensitivity [hs]) of otherwise healthy patients suffering from periodontitis when compared to controls. The impact of intensive and non-intensive nonsurgical periodontal treatment (NSPT) on hs-CRP was also investigated. Four electronic databases (Pubmed, The Cochrane Central Register of Controlled Trials [CENTRAL], EMBASE and Web of Science) were searched up to February 2021 and the review was completed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (PROSPERO No. CRD42020167454). Observational and intervention studies that: 1) evaluated CRP and hs-CRP serum levels in patients with and without periodontitis, and; 2) hs-CRP levels after NSPT were included. Following risk of bias appraisal, both qualitative and quantitative analyses were performed. Pooled estimates were rendered through ratio of means (RoM) random-effects meta-analyses. After screening 485 studies, 77 case-control studies and 67 intervention trials were included. Chronic and aggressive periodontitis diagnoses were consistently associated with higher levels of CRP and hs-CRP ($p < 0.001$). Patients with aggressive periodontitis exhibited on average more than 50% higher levels of CRP (RoM [95% confidence interval [CI]]: 1.56 [1.15; 2.12], $p = 0.0039$) than patients with chronic periodontitis. Intensive NSPT induced an immediate increase of hs-CRP followed by a progressive decrease whilst non-intensive NSPT consistently decreased hs-CRP after treatment up to 180 days ($p < 0.001$). These findings provide robust evidence that periodontitis is associated with systemic inflammation as measured by serum CRP levels. Periodontitis treatment induces a short-term acute inflammatory increase when performed in an intensive session, whilst a progressive reduction up to 6 months was demonstrated when performed in multiple visits.

Keywords: inflammation, periodontal disease, periodontitis, periodontal medicine, systematic reviews and evidence-based medicine, oral-systemic disease(s), systemic health/disease, C- reactive protein

INTRODUCTION

Periodontitis is a chronic inflammatory disease initiated by a dysbiotic dental biofilm (1) and followed by a progressive destruction of periodontal tissues (2). Bacteria and their products progressively affect the periodontium integrity, which can trigger a local inflammatory response but also a systemic response (2, 3). Raised levels of pro-inflammatory mediators have been reported in patients with periodontitis (4) who also exhibit distinct hematological changes (5) including raised levels of C-reactive protein (CRP) (6).

CRP is an acute-phase protein mainly produced by the liver in response to infection or tissue damage (7, 8). While CRP is a more traditional inflammation marker with less precision (within the range of 10 to 1,000 mg/L), high sensitivity CRP (hs-CRP) is a highly precise and accurate analyte (within the range of 0.5 to 10 mg/L) (9). The association between CRP and periodontitis received great attention in part due to the link between periodontitis and cardiovascular disease (CVD) (6). However, CRP has been established as a marker of association of periodontitis with other systemic diseases (3).

Previous systematic reviews suggested elevated CRP levels in patients with periodontitis (6, 10–12). Nevertheless, there are inconsistent evidence-based outcomes on the effect of two different periodontal therapeutic approaches (13–19). Standard non-intensive periodontal therapy (NSPT) consists of conventional mechanical scaling and root planning with polishing on a quadrant-by-quadrant with local anesthesia. Whereas intensive periodontal therapy (IPT) involves an intensive session of full-mouth subgingival root debridement delivered within a 24-hour period under local anesthesia (where periodontal surgery, teeth removal or local antibiotic might be delivered). Given the clinical relevance of chronic inflammation and future systemic health outcomes, including in patients with periodontitis, the amount of newly available evidence, an updated and robust appraisal of the data on the association of periodontitis and its treatment with CRP levels was thought to be of interest.

The overall aim of the present systematic review was to critically appraise all up-to-date evidence on the impact of diagnosis of periodontitis on serum CRP levels in otherwise healthy patients when compared to controls. We further investigated the impact of non-intensive and intensive NSPT on the variations of hs-CRP levels after treatment.

Abbreviations: CANTOS, Canakinumab Antiinflammatory Thrombosis Outcome Study; CENTRAL, The Cochrane Central Register of Controlled Trials; CI, Confidence Interval; CLIA, Chemiluminescent enzyme immunometric assay; CRP, C-Reactive Protein; CVD, cardiovascular disease; ELISA, Enzyme-Linked Immunosorbent Assay; hs-CRP, high-sensitivity CRP; IL, interleukin; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; NSPT, Nonsurgical Periodontal Therapy; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RIA, Radial immunodiffusion assay; RoB, Risk of Bias; RoM, Ratio of Means; TNF, tumor necrosis factor.

MATERIALS AND METHODS

Protocol and Registration

This systematic review protocol was previously defined and registered at the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Review (CRD42020167454). The review was conducted based on the Cochrane Handbook of Systematic Reviews of Interventions (20) and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (21) (**Supplementary Table 1**).

Focused Questions and Eligibility Criteria

We developed a protocol to answer two PI(E)CO questions:

1. “In otherwise healthy individuals, do patients with periodontitis have increased serum CRP and hs-CRP levels compared to patients without periodontitis?”
2. “Do hs-CRP levels decrease after intensive e non-intense NSPT?”

Each question had the following statements:

1. Otherwise healthy patients (Patients, P); Periodontitis (Exposure, E); Non-periodontitis (Comparison, C); CRP and hs-CRP serum levels (Outcome, O).
2. Otherwise healthy patients (Patients, P); Intensive and non-intensive NSPT (Intervention, I); Baseline serum levels of hs-CRP (Comparison, C); Posttreatment hs-CRP serum levels (Outcome, O).

Studies were eligible for inclusion based on the following criteria:

- a. Observational (cross-sectional, case-control) studies using serum CRP and hs-CRP levels of otherwise healthy participants with periodontitis and controls;
- b. Intervention studies (randomized controlled trials [RCTs] and non-randomized studies [NRSI]) reporting changes of hs-CRP levels after NSPT;
- c. Participants with periodontitis and no other comorbidities that could influence CRP serum levels, such as a history of CVD, atherosclerosis, diabetes mellitus, obesity, postmenopausal women;
- d. Studies with a clear case definition of periodontitis;
- e. Studies with clear reporting the CRP and hs-CRP method of quantification (for instance, Immunoturbidimetry, Nephelometry, Enzyme-Linked Immunosorbent Assay [ELISA], Chemiluminescent enzyme immunometric assay [CLIA], Radial immunodiffusion assay [RIA]).

Search Strategy

Identification of studies was performed through detailed search strategies developed for each database (Pubmed, The Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, Web of Science) until February 2021. Our Pubmed search strategy was based on the following algorithm: (periodontitis OR gingivitis OR periodontal health OR (periodontal diseases

[MeSH])) AND (C-reactive protein OR (C-reactive protein [MeSH]) OR CRP OR Acute Phase Protein OR high sensitivity C-reactive protein OR hs-CRP). No restrictions were applied regarding the publication year or language. Grey literature was searched through an appropriate database (<http://www.opengrey.eu/>). Hand searching in four periodontology journals (namely, Journal of Clinical Periodontology, Journal of Periodontology, Periodontology 2000, Journal of Periodontal Research) was conducted to search articles not found through other search methods.

Study Selection

Study selection was assessed independently by three investigators (VM, JB and CE), who screened the titles and/or abstracts of retrieved studies. Any disagreements were resolved by discussion with a fourth author (SBH). For measurement reproducibility purposes, inter-examiner reliability following full-text assessment was calculated *via* kappa statistics.

Data Extraction Process and Data Items

Electronic data were extracted to a predefined table, including the first author's name, study design, publication year, country where the study was conducted, project funding, mean age, number of participants per group and by gender, percentage of smokers per group, periodontal case definition, CRP and hs-CRP method and serum levels (values were expressed in mg/L). For intervention studies, hs-CRP serum levels at baseline and after intensive and non-intensive NSPT were extracted. Three independent reviewers (VM, JB and CE) completed data extraction. Corresponding authors of studies were contacted if there were missing information.

The primary aim of this systematic review was to assess CRP and hs-CRP serum levels in patients with and without periodontitis (chronic or aggressive). The secondary aims included assessment of hs-CRP serum levels after NSPT comparing to baseline data according to the type of NSPT: 1) IPT; and, 2) non-intensive periodontal treatment.

Risk of Bias (RoB) in Individual Studies

Case-control studies were appraised using the Newcastle-Ottawa Scale (NOS) by two calibrated reviewers (VM and CE). We considered studies with 7-9 stars as of low RoB, studies with 5-6 stars of moderate RoB, whilst studies with less than 5 stars were deemed of high RoB (5, 22). The Cochrane Collaboration's tool RoB2 was used to assess RCTs (23) and the RoB in Non-randomized Studies - of Interventions (ROBINS-I) tool for non-RCT studies of intervention (24) by two calibrated examiners (JB and BSH). Any disagreement was resolved by discussion.

Summary Measures and Synthesis of Results

For continuous data (serum levels of CRP and hs-CRP), mean values and standard deviations (SD) were used. The unit of measurement used in the meta-analysis was mg/L, and all studies reporting different units of measurement were converted appropriately. If median and interquartile range were reported

in the selected studies, we converted to mean and SD following Hozo's procedure (25).

Estimates were calculated in R version 3.4.1 (R Studio Team 2018) using a DerSimonian-Laird random-effects model (26). All random-effects meta-analysis and forest plots were performed using 'meta' package (27). Statistical heterogeneity was assessed through I^2 index and Cochran's Q statistic ($p < 0.1$), and the overall homogeneity was calculated through the χ^2 test (28). All tests were two-tailed, with alpha set at 0.05. Further, the weight percentage given to each study in each analysis was provided in the forest plots. We analyzed publication bias if, at least, 10 or more studies were included (28). Overall estimates were reported with 95% confidence interval (CI).

We started by conducting an *a priori* sensitivity analysis comparing Standardized Mean Difference (SMD) versus Ratio of Means (RoM) meta-analyses. In case of similar results in terms of heterogeneity and significance, RoM was used as it would allow easier and direct interpretation of the results (reported as percentage) (29, 30).

According to the type of NSPT (intensive and non-intensive) delivered, a subgroup meta-analysis was made for each day after treatment. Then values were graphically assembled in a scatterplot using 'ggplot2' package, with mean and 95% CI values in a vertical interval. Post-hoc sensitivity analyses were designed to assess the impact of high RoB in the pooled estimates, as well as the influence of different study designs (intervention and case-control).

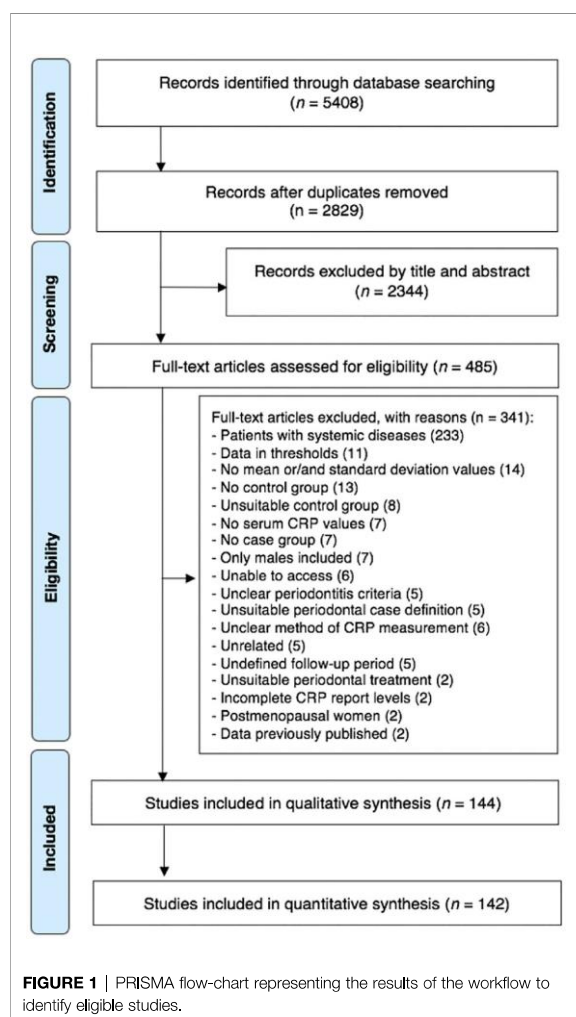
RESULTS

Study Selection

The search strategy retrieved 5,408 possibly relevant articles. After duplicate removal, 2,829 manuscripts were screened against the eligibility criteria; 2344 were excluded after titles and/or abstracts review. Among these, 485 articles were assessed for full paper review eligibility, 341 were excluded (detailed reasons for exclusion in **Supplementary Table 2**). A total of 144 studies were included for qualitative synthesis whilst 142 for quantitative analyses (**Figure 1**). Good inter-examiner reliability was confirmed at the full-text screening (kappa score = 0.92, 95% CI: 0.89; 0.94).

Characteristics of the Studies

In this systematic review, 77 case control-studies (**Supplementary 3 - References**) and 67 intervention studies (**Supplementary 4 - References**) were included. Twelve intervention studies had relevant information regarding the comparison of otherwise healthy patients with periodontitis and non-periodontitis (31–42) (**Supplementary 4 – References**). Two studies had their data reported in more than one article: thus, these two papers were grouped under a single name study as follows: Gani et al., 2009/2012 (43, 44). Overall, studies were performed in 27 countries across Asia, Europe, America and Oceania (**Supplementary Tables 5, 6**). A total of 11,242 participants in observational and 2,199 in intervention studies were included in the analysis. Among the observational studies 6,155 healthy participants and



5,087 patients with periodontitis were identified (4,319 diagnosed with chronic- and 768 with aggressive-periodontitis). Three studies reported data for combined diagnoses (chronic and aggressive) of periodontitis (45–47). Among the intervention studies, 1,389 participants received non-intensive whilst 810 received intensive NSPT.

A plethora of case definitions of periodontitis were retrieved (as shown in **Supplementary Tables 5, 6**) as well as variability in the used CRP and hs-CRP methods (i.e., immunoturbidimetry, nephelometry, ELISA, CLIA, RIA, Cardiac-specific claim, latex agglutination method and spectrophotometer) (**Supplementary Tables 5, 6**).

Seventy-one observational studies were classified as of low RoB according to the NOS for case-control studies (**Supplementary Table 7**) (28 with 9 stars, 29 with 8 stars and 14 with 7 stars). Good inter-examiner reliability was confirmed at the RoB assessment (kappa score = 0.94, 95% CI: 0.84; 1.00). Six observational studies had moderate RoB. Most articles failed

in including a representative number of cases (43.0%, $n=34$), nevertheless, most of them had adequate cases (88.6%, $n=70$) and controls (94.9%, $n=75$) definition as well as selection (72.2%, $n=57$). All studies had excellent a) comparability results (100%, $n=79$) and b) values of non-response rate (98.7%, $n=78$). All studies reported a secure record of ascertainment of exposure with equal ascertainment method for cases and controls (100.0%, $n=79$).

Forty-four non-RCT had a low RoB, two had serious RoB and two had insufficient information according to the ROBINS-I tool (**Supplementary Table 8**). Lastly, fifteen RCTs had a low RoB according to ROB2 and four had some concerns regarding RoB (**Supplementary Figure 1**).

Diagnosis of Periodontitis and CRP Levels

The *a priori* sensitivity analysis was performed to compare RoM and SMD approaches yielding similar results (**Supplementary Table 9**).

Eighteen (CRP) and fifty-nine (hs-CRP) studies were included (**Supplementary Figures 2–5**). Chronic periodontitis was associated with an increase of CRP and hs-CRP levels of 103% and 110%, respectively ($p<0.0001$) (RoM [95% CI]: 2.03 [1.59; 2.60], $p<0.0001$, $I^2 = 96.3\%$ and RoM [95% CI]: 2.10 [1.62; 2.55], $p<0.0001$, $I^2 = 97.4\%$, respectively) (**Table 1**). Sensitivity analyses showed no differences in these results based on the type of study and CRP measurement method (**Supplementary Table 10** and **Supplementary Figures 2–5**). In particular, estimates from studies with low RoB demonstrated only mild improvement in heterogeneity ($I^2 = 66.3\%$ vs $I^2 = 98.5\%$ for all studies) (**Supplementary Figure 3**). No impact of level of RoB on heterogeneity was noted for studies reporting hs-CRP levels (**Supplementary Table 11**).

A diagnosis of aggressive periodontitis was associated with an increase of CRP of 191% (RoM [95% CI]: 2.91 [1.91; 4.44], $p<0.0001$, $I^2 = 98.6\%$) and of hs-CRP of 179% (RoM [95% CI]: 2.79 [1.68; 4.65], $p<0.0001$, $I^2 = 83.6\%$) when compared to controls (**Table 1**). Sensitivity analyses showed no differences in these results based on the type of study and CRP method of measurement (**Supplementary Figures 6, 7**).

Combined diagnosis of periodontitis was associated with an increase of 80% of CRP (RoM [95% CI]: 1.80 [1.36; 2.38], $p<0.0001$, $I^2 = 99.4\%$) and of 109% of hs-CRP (RoM [95% CI]: 2.09 [1.78; 2.47], $p<0.0001$, $I^2 = 99.8\%$) when compared to healthy controls (**Supplementary Figures 8–10**). When comparing different diagnoses, aggressive periodontitis was associated with 56% higher levels of CRP than chronic periodontitis (RoM [95% CI]: 1.56 [1.15; 2.12], $p=0.0039$, $I^2 = 84.9\%$) (**Supplementary Figures 11–13**).

Publication bias was noted in studies examining CRP levels in patients with periodontitis versus controls ($p=0.041$) as well as studies comparing the specific diagnosis of chronic periodontitis versus healthy controls ($p=0.027$) (**Supplementary Figures 14–17**).

Periodontal Treatment and CRP Levels

All studies included reported hs-CRP results at baseline and after NSPT with data at 1, 7, 30, 60, 90 and 180 days of treatment delivered (non-intensive and intensive NSPT). An *a priori* sensitivity analysis confirmed the suitability of RoM meta-

TABLE 1 | CRP and hs-CRP levels according to the periodontal status.

Variable	N studies	N of participants	ROM	95% CI	p-value	I ² (%)	Tau	Egger test
Healthy periodontium vs. Chronic Periodontitis								
CRP (mg/L)	19	CP: 1411; H: 2894	2.10	1.56; 2.83	<0.0001	98.5	0.640	0.027
hs-CRP (mg/L)	59	CP: 2615; H: 2317	2.04	1.72; 2.43	<0.0001	99.8	0.399	0.083
Healthy periodontium vs. Aggressive Periodontitis								
CRP (mg/L)	7	AgP: 266; H: 243	2.91	1.91; 4.44	<0.0001	98.6	0.548	-
hs-CRP (mg/L)	6	AgP: 424; H: 270	2.79	1.68; 4.65	<0.001	83.6	0.547	-
Healthy periodontium vs. PD (Chronic- and Aggressive- Periodontitis combined)								
CRP (mg/L)	24	PD: 2062; H: 3305	2.06	1.64; 2.59	<0.0001	98.7	0.503	0.041
hs-CRP (mg/L)	65	PD: 2741; H: 2885	2.09	1.78; 2.47	<0.0001	99.8	0.401	0.064
Chronic vs. Aggressive Periodontitis								
CRP (mg/L)	5	CP: 145; AgP: 232	1.56	1.15; 2.12	0.0039	84.9	0.300	-
hs-CRP (mg/L)	5	CP: 101; AgP: 118	1.69	0.96; 2.98	0.0667	77.5	0.513	-

CRP, C-reactive protein; hs-CRP, high sensitivity CRP; ROM, Ratio of Mean.
 Bold values mean p value below 0.05.

analysis, with statistical significance and heterogeneity similar across analyses (**Supplementary Tables 12, 13**). Sensitivity analyses of studies with low RoB confirmed the same findings of the overall analyses (**Supplementary Tables 14–16**).

Non-intensive NSPT was associated with a progressive reduction of hs-CRP values up to 180 days after delivery (**Figure 2** and **Table 2**). Intensive NSPT resulted however in a sharp increase of hs-CRP one day after treatment, followed by a progressive reduction at 90 days after treatment but a new moderate increase at 180 days of follow-up (**Figure 2** and **Table 3**). Two studies reported data beyond 180 days (48, 49) with reductions of hs-CRP levels when compared those at baseline.

DISCUSSION

This systematic review confirms a strong association between periodontitis and serum levels of CRP in otherwise systemically healthy individuals. Patients with periodontitis exhibited consistently higher values of CRP compared to healthy controls

irrespective of the laboratory assessment adopted. Furthermore, different periodontal treatment modalities resulted in divergent kinetics of CRP over time with a rapid increase when intensive NSPT was performed but then a progressive reduction over time.

In contrast to previous studies (6, 10–12), this review expanded data on both observational and interventional studies linking periodontitis and systemic inflammation though CRP serum levels. So far, only one meta-analysis had shown high serum CRP levels in periodontitis patients had an average increase of 1.56 mg/L ($p < 0.001$) compared to non-periodontitis cases (6). On the basis of our results, the more aggressive forms of periodontitis would be responsible for the greater increase in CRP levels in serum. This finding would be in agreement with the latest periodontitis case definitions when referring to cases of a rapid rate of progression and higher levels of CRP levels (50). These patients (previously known as suffering from aggressive periodontitis) often present a higher inflammatory load (50–52), nevertheless this should be researched more in depth in the future.

The first ever systematic review on this topic provided inconclusive non-significant results of NSPT on serum CRP levels (10). Two subsequent reviews confirmed periodontal treatment as effective in reducing serum CRP (11, 12). Differences between the different periodontal therapy approaches is unclear and the present review provides newer evidence on the changes of CRP serum levels following two different periodontal treatment modalities (intensive and non-intensive therapy). Furthermore, evidence pointed towards a better clinical response to treatment when intensive sessions of periodontal cleaning were performed (13–19). The latter however seemed to be linked to a greater systemic inflammatory response. In this review we describe the changes over time of systemic inflammation with a different pattern of changes in serum CRP even in otherwise healthy participants. Our intent was not to test whether periodontitis treatment would reduce systemic inflammation (as this has been extensively reviewed before and tested in appropriate RCTs) (6, 10–12) but rather to ascertain the directions of changes in the systemic inflammatory response linked to two treatment modalities over time. These differences bear clinical significance because NSPT, whilst is associated with a medium-term reduction of inflammation, it is also linked to acute spikes of hs-CRP which

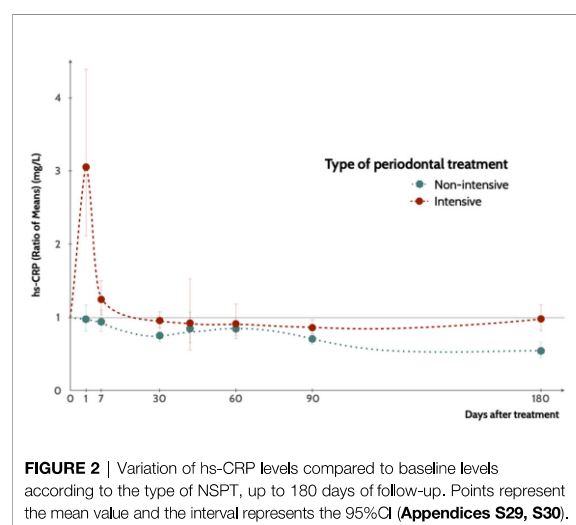


TABLE 2 | Sensitivity analysis regarding the type of meta-analytical approach for non-intensive treatment studies.

Non-intensive treatment								
Day	n	ROM	95% CI	I ² (%)	n	SMD	95% CI	I ² (%)
1	4	0.98	0.82; 1.17	0.0	4	-0.05	-0.32; 0.20	0.0
7	6	0.95	0.81; 1.1	5.7	6	-0.08	-0.29; 0.19	5.7
30	12	0.75	0.67; 0.86	83.9	12	-0.53	-0.92; -0.14	83.0
42	3	0.84	0.66; 1.08	0.0	3	-0.20	-0.52; 0.12	0.0
60	13	0.84	0.76; 0.94	77.5	13	-0.48	-0.77; -0.19	77.5
90	23	0.71	0.64; 0.78	73.0	23	-0.71	-1.01; -0.41	82.9
180	11	0.55	0.45; 0.66	88.5	11	-1.25	-1.83; -0.67	92.4

CI, Confidence Interval; n, number of included articles; SMD, Standardized Mean Difference; ROM, Ratio of Mean.

TABLE 3 | Sensitivity analysis regarding the type of meta-analytical approach for intensive treatment studies.

Intensive treatment								
Day	n	ROM	95% CI	I ² (%)	n	SMD	95% CI	I ² (%)
1	16	3.05	2.12; 4.38	95.5	16	1.86	1.25; 2.46	91.8
7	9	1.25	1.04; 1.5	73.8	9	0.39	0.08; 0.71	63.8
30	10	0.96	0.86; 1.07	4.9	10	-0.02	-0.21; 0.17	10.4
42	4	0.93	0.56; 1.52	34.7	4	-0.04	-0.32; 0.24	16.7
60	4	0.96	0.87; 1.05	0.0	4	-0.06	-0.28; 0.16	0.0
90	13	0.87	0.78; 0.97	82.8	13	-0.34	-0.61; 0.07	63.5
180	6	1.04	0.89; 1.21	64.7	6	0.02	-0.34; 0.38	35.9

CI, Confidence Interval; n, number of included articles; SMD, Standardized Mean Difference; ROM, Ratio of Mean.

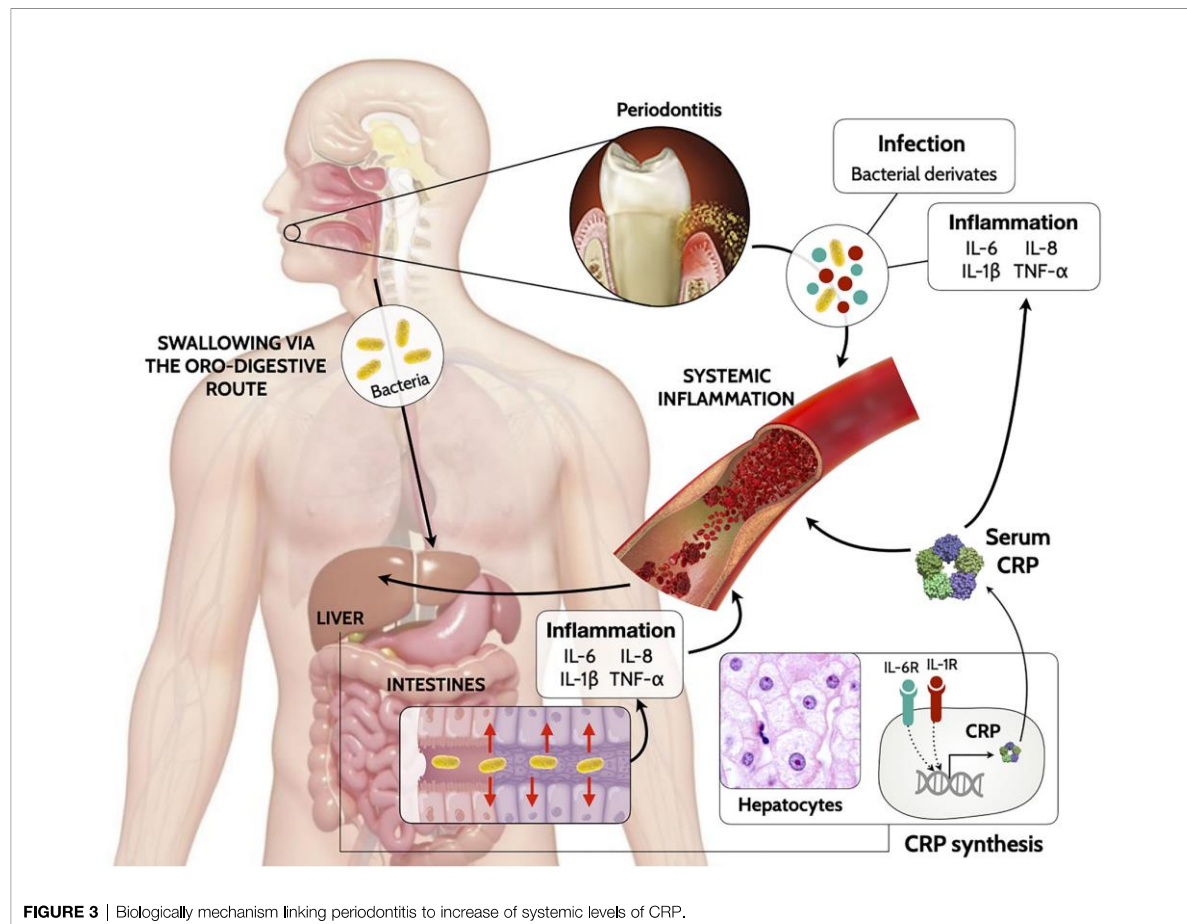
might have detrimental consequences when observed in patients who might suffer from other co-morbidities (such as, CVD or diabetes mellitus). Future studies are warranted to further expand data beyond 180 days after treatment and focus on patients reported outcomes.

Inflammation is a key biological process against injuries and infections and occurs at intervals throughout life, yet the persistence of a chronic systemic inflammatory state tracks future chronic conditions and associated complications (53). This risk prediction has been demonstrated when assessing basal levels of inflammation as assessed by CRP, particularly in the categorization of CVD risk categories (low risk <1 mg/L, medium between 1 and 3 mg/L and high >3 mg/L) (9). In the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), lower rates of myocardial infarction, stroke and death were found following Rosuvastatin regimens in otherwise healthy adults with baseline CRP level ≥ 2 mg/L and no hyperlipidemia (54). The reduction of 37% in CRP level observed in the test group stimulated further the discussion on the anti-inflammatory therapy as a valuable strategy in the prevention of CVD events. Despite CRP was excluded from the pathogenesis of inflammatory related conditions (i.e., such as atherosclerosis) by a subsequent mendelian randomization, it remains a proxy of the overall systemic inflammatory status. Additionally, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial showed the success of monoclonal antibody anti-inflammatory therapy in secondary CVD prevention confirming a benefit in terms of future CVD events in participants who maintained with CRP concentration less than 2mg/L throughout the trial (55). The increase in CRP concentration reported in patients with

periodontitis and the reduction observed following periodontal therapy do not provide a definitive explanation on the pathways by which periodontitis could affect CVD risk but supports this hypothesis. Further, it remains to be demonstrated that the reduction of CRP levels after periodontal treatment will reduce the risk for CVD events. Nevertheless, periodontitis should be considered a common trigger of systemic inflammation.

Beyond the effect of the treatment of periodontitis in reducing CRP, the clinical relevance of the observed acute increase in inflammation after an intensive NSPT is still unknown (56), particularly in patients with other comorbidities (53). Non-intensive NSPT was recently defined as a safe procedure in patients on anti-thrombotic therapy, as it does not increase the risk for CVD events (56). Exploring the clinical relevance of the acute inflammation following intensive periodontal treatment and whether it poses a risk for patients with established chronic diseases should be a matter of further research.

The exact mechanism of how periodontitis triggers CRP release is still under investigation. Gingival inflammation triggered by the dysbiotic dental biofilm is enriched with inflammatory mediators (i.e., interleukin [IL]-1 β , IL-6 and tumor necrosis factor [TNF]- α) (2). Intra-oral hematogenous dissemination of periodontal bacteria occurs through the ulcerated epithelium or the overflow of inflammatory mediators from local periodontal damaged tissues to the systemic circulation triggering a systemic inflammatory response. Alternatively, periodontal bacteria can migrate through saliva swallowing *via* the oro-pharyngeal or oro-digestive sites, and ectopically colonize the gut causing an inflammatory reaction (3). The low-grade systemic inflammation driven by local inflammatory mediators promote local secretion of CRP (by adipocytes, vascular



smooth muscle cells or gingival cells) (57) but also can generate a distant response by targeting hepatocytes (58, 59) after systemic dispersion (**Figure 3**). This state of prolonged inflammation ultimately results in alterations in the bone marrow (3) and with distinctive blood patterns (5). This is referred as trained myelopoiesis in the bone marrow, where hematopoietic stem cells undergo inflammation-adapted differentiation, leading to hyperreactive white cells (neutrophils and monocytes/macrophages) (3). As a result, when these hyperactive cells migrate through the bloodstream, they exacerbate inflammatory reactions in multiple locations and worsening on-going infectious and inflammatory diseases.

Strengths and Potential Limitations

There are some limitations worth mentioning when reading this review. Estimates produced by our analyses included both representative and unrepresentative studies (as assessed through RoB assessment), which means that these results are not applicable to all patients with periodontitis. A number of variations in periodontal case definitions as well as CRP quantification methods used in the included studies, might

have influenced the heterogeneity observed in the analyses. Further, methods to quantify CRP are becoming more precise and accurate, which may contribute to higher heterogeneity of estimates (60). Additionally, most studies did not include controls to follow and compare the hs-CRP changes over time with periodontitis. Also, although we were able to explore the possible role of smoking habits on baseline CRP levels, we could not further explore in interventional studies as this information was registered only at baseline and not updated throughout follow-ups. Similarly, we were unable to assess the impact BMI *via* meta-regressions because most studies lacked this information both at baseline and prospectively.

Our systematic review however was designed to follow a strict protocol, with a robust methodology and extensive search of all relevant evidence including all possible confounding factors. Further by using two meta-analytical approaches we are comfortable with the effect sizes across comparative groups and/or interventions (29) managing the high degree of heterogeneity (61).

In conclusion, periodontitis is associated inflammation as assessed by raised CRP levels and this is greater in those patients with more aggressive gingival inflammation. Further,

periodontal treatment whether performed in an intensive or conventional fashion causes changes in CRP including a sharp increase over the first week and then a progressive reduction up to 6 months. These findings are relevant when discussing the potential implications of periodontitis on systemic health and especially in those individuals who suffer from other comorbidities where systemic inflammation is recognized as a key driver not just of their onset but also disease-progression like diabetes and cardiovascular diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

VM, JB, FD'A, conception and design of the work. VM, JB, SL, SH, data collection. VM, JB, PM, data analysis and

interpretation. VM, JB, and CE, drafting the article. VM, JB, CE, SL, SH, PM, MO, JJM, FD'A, critical revision of the article and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2021.706432/full#supplementary-material>

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IV. IS THERE A BIDIRECTIONAL ASSOCIATION BETWEEN POLYCYSTIC OVARIAN SYNDROME AND PERIODONTITIS? A SYSTEMATIC REVIEW AND META-ANALYSIS

Machado, V., Escalda, C., Proença, L., Mendes, J.J., & Botelho, J. (2020). Is there a bidirectional association between polycystic ovarian syndrome and periodontitis? A systematic review and meta-analysis. *Journal of Clinical Medicine*, 9(6), 1-16. <https://doi.org/10.3390/jcm9061961>

Review

Is There a Bidirectional Association between Polycystic Ovarian Syndrome and Periodontitis? A Systematic Review and Meta-Analysis

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Abstract: Polycystic ovary syndrome (PCOS) has reproductive and metabolic properties that may be linked to periodontitis (PD). This study aimed to update and render a robust critical assessment on all evidence linking PCOS and PD, and appraising a hypothetical bidirectional association. Five databases (PubMed, Scholar, EMBASE, Web of Science and CENTRAL) were searched up to May 2020. Case-control and cohort studies on the association of PCOS and PD were included. The risk of bias of observational studies was assessed through the Newcastle-Ottawa Scale (NOS). Random effects meta-analyses of standardized mean difference (SMD) and risk ratio (RR) were performed. We followed Strength of Recommendation Taxonomy (SORT) to appraise the strength and quality of the evidence. Twelve case-controls fulfilled the inclusion criteria (876 with PCOS and 48170 healthy controls), all scored as having a low risk of bias. Meta-analysis revealed that PCOS females have 28% more risk towards PD, and PD females have 46% more risk to have PCOS. PCOS females with PD had higher gum bleeding, periodontal pocket depth and clinical attachment loss than non-PCOS females with PD. Populations with undefined periodontal status contribute to underestimated results. On the basis of the available evidence, it is possible to assume a bidirectional link between PCOS and PD. That is, PCOS increases by 28% the risk of having PD and in the same fashion, PD increases by 46% the risk of having PCOS. Furthermore, women with PCOS were associated with worsening clinical characteristics and inflammation of PD. These findings suggest that PCOS and PD may be linked. Hence, further prospective and clinical trial studies with nonsurgical periodontal therapy are necessary to clarify the existence of an increased risk of PCOS in women with PD and vice-versa.

Keywords: polycystic ovary syndrome; periodontitis; periodontal disease; systematic review; meta-analysis

1. Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine, reproductive and metabolic condition, with a worldwide prevalence ranging 5–15% [1]. PCOS is characterized by polycystic ovaries, hyperandrogenism with impaired gonadotropin secretory activity, hyperinsulinemia, hypothalamic–pituitary–ovarian (HPO) axis changes, and ovulatory and menstrual dysfunction [2–8]. Additionally, PCOS is also accompanied by psychological alterations, such as anxiety, depression and poor quality of life [9]. The pathogenesis of PCOS remains to be fully understood due to its

multifactorial profile. Nevertheless, chronic infections have gained relevance, for instance, because of the associated high levels of oxidative stress (OS), inflammatory molecules, adhesion molecules, as well as blood lymphocytes and monocytes. In particular, the proinflammatory state has been one of the most investigated for the PCOS link with periodontitis.

Periodontitis (PD) is a chronic multifactorial inflammatory condition caused by a dysbiotic plaque and causes the destruction of the periodontium, the tissues that surround teeth [10,11]. Periodontitis is a pandemic noncommunicable disease and its symptoms start with gum inflammation (gingivitis) to an exacerbated and uncontrolled inflammatory response from the innate and adaptive immune system [12,13]. The destruction of the periodontium allows for bacteria and bacterial products to gain access to the systemic circulation through the ulcerated epithelium and destruction of the periodontium.

Over the last decade, cross-sectional evidence has suggested that PCOS patients may be more prone to develop periodontitis [14–22] and with higher local levels of OS [14,17]. Also, a randomized clinical trial showed that nonsurgical periodontal therapy together with myo-inositol therapy contributed to a higher decrease of systemic inflammatory levels compared with myo-inositol solely in women with PCOS and periodontitis [23]. Furthermore, three systematic reviews have supported a possible association between PCOS and PD, however none of them were able to synthesize estimates on this association nor the impact on clinical characteristics [9,24,25]. However, there is inconclusive evidence regarding the risk of PCOS patients to develop periodontitis and vice-versa, in a potential bidirectional relationship, and the potential role of PCOS associated inflammation on periodontal tissue health (worsen or better the gingival condition).

Given the recent increase of newly available studies, the primary aim of the present review was to update and render a robust critical assessment on all evidence linking PCOS and periodontitis, and appraising a hypothetical bidirectional association, following a meticulous systematic protocol.

2. Materials and Methods

2.1. Protocol

This systematic review was defined *a priori* and was performed following the Cochrane Handbook of Systematic Reviews of Interventions [25] and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26].

2.2. Focused Question and Eligibility Criteria

We set the following broad research question so as not to introduce bias between PCOS and Periodontitis. The following focused question was addressed: “Is there a bidirectional association between PCOS and Periodontitis?”, with the following specific questions:

1. “Does PCOS have an effect on the healthy periodontium?”
2. “Does PCOS influence periodontal clinical characteristics of Periodontitis?”
3. “Does Periodontitis influence clinical characteristics of PCOS?”

We developed a protocol to answer the following PECO question:

1. Female patients without PD (Patients-P); PCOS (Exposure-I); No PCOS (Comparison-C); periodontal probing depth (PPD), clinical attachment loss (CAL), bleeding on probing (BoP) levels (Outcome-O)
2. Female patients with PD (Patients-P); PCOS (Exposure-I); No PCOS (Comparison-C); PPD, CAL, BoP levels (Outcome-O)
3. Female patients with PD (Patients-P); PCOS (Exposure-I); No PCOS (Comparison-C); endocrine outcomes (Ferriman–Gallwey score, free androgen index, dehydroepiandrosterone sulfate (DHEAS), free testosterone and total testosterone levels); glycemic (fasting blood insulin, fasting blood glucose, homeostatic model assessment (HOMA-IR)); and physical (waist-to-hip ratio (WHR)) outcomes - O

To address the first PECO research question, studies reporting on the presence of PPD, CAL and BoP evaluation in PCOS female patients and healthy controls with non-PD diagnosis, were included. Studies comparing these two groups of patients without clinical and radiophonic diagnostic confirmation in the control group were excluded.

For the second and third PECO questions, studies reporting the presence of PPD, CAL and BoP values of PCOS patients and healthy controls were included. Studies that have included PCOS patients and healthy controls without previously knowing their periodontal status following periodontal clinical assessment were excluded due to the high risk of reporting bias.

2.3. Search Strategy

We searched in Pubmed, Medical Literature Analysis and Retrieval System Online (MEDLINE), CENTRAL (The Cochrane Central Register of Controlled Trials), EMBASE, Web of Science from the earliest data available until March 2020, and last updated in May 2020. We merged keywords and subject headings in accordance with the thesaurus of each database and applied exploded subject headings. Our Pubmed search strategy was based on the following algorithm: (chronic periodontitis OR periodontitis, chronic OR adult periodontitis OR periodontitis, adult OR periodontal disease OR alveolar bone loss OR attachment loss, periodontal OR periodontal pocket) and (polycystic ovary syndrome or PCOS). Gray literature was searched through the OpenGrey portal [27]. Additional relevant literature was included after a manual search of the reference lists of the final included articles. If there were multiple publications for the same study, data from the largest sample were used. The study type was restricted to human studies. We included both randomized clinical trials (RCTs) and non-RCTs (case-control, cohort studies and cross-sectional) that reported on (A) PCOS female patients with and without associated PD and/or (B) patients with PD with and without a concomitant PCOS diagnosis. The authors were contacted when necessary additional data clarification was required.

2.4. Study Processes

Two independent investigators (V.M. and J.B.) screened the titles and abstracts of the retrieved studies. We resolved disagreement through adjudication or by a third reviewer (J.J.M.). The final selection of studies was independently performed by three authors who reviewed the full text of the selected papers based on the inclusion criteria mentioned above. Any disagreements were resolved by discussion.

A predefined table was used to extract necessary data from each eligible study, including the first author's name, study design, publication year, the country of origin, inclusion/exclusion criteria, number of female participants, mean age in year, periodontal case definition, and PCOS diagnosis based on validated classification criteria. Clinical periodontal measures included: PPD, CAL, BoP, missing teeth. All data were independently extracted by two reviewers with a consensus in all aspects. Concerning additional data clarifications, we attempted to contact the corresponding authors twice, with an interval time of one week, without success.

2.5. Risk of Bias (RoB) in Individual Studies

Methodological quality was appraised using the Newcastle-Ottawa (NOS) Scale by one calibrated author. In this last tool, case-control and cohort studies are scored across three categories: studies with 7–9 stars of low RoB, studies with 5–6 stars of moderate RoB, whilst studies with less than 5 stars were deemed as being of high RoB. If any doubt occurred, they were resolved by discussion with a second author.

2.6. Statistical Analysis

All statistical analyses were performed in R version 3.4.1 (R Studio Team 2018) using a DerSimonian-Laird random-effects model [28]. All random-effects meta-analysis and forest plots were performed using ‘meta’ package [29,30]. Risk ratio (RR), 95% confidence intervals (CI) and the weight for each included study were calculated for the risk of PCOS towards PD and vice-versa. For continuous data, mean values and standard deviations (SD) were used and analyzed with standard mean differences (SMD) and 95% CI. We used mm as the unit of measurement in the SMD meta-analysis. In the case of median and interquartile range reported, we converted to mean and SD following Hozo procedure [31]. The data was reported regardless of the individual case definition. Subgroup meta-analysis estimates were pooled according to samples with PD, without PD and with undefined periodontal status (that is, studies where periodontal diagnosis was not performed, but measures of PPD and CAL were recorded). Statistical heterogeneity was inspected through I^2 index and Cochrane’s Q statistic ($p < 0.1$), and the overall homogeneity was calculated through the χ^2 test [25]. All tests were two-tailed, with alpha set at 0.05. Further, the weight percentage given to each study in each analysis was provided in the forest plots. Publication bias was planned if, at least, 10 or more studies were included [25]. The overall estimates were reported with 95% CI.

2.7. Strength of Recommendation

We used the SORT (Strength of Recommendation Taxonomy) to judge the strength and quality of the evidence [32]. We discussed the outcomes of the present systematic review, clinical recommendations, and future necessary research.

3. Results

3.1. Characteristics of Included Studies

The search strategy identified a total of 239 possibly relevant articles. After duplicates removal, 54 papers were judged against the eligibility criteria, and 185 were excluded after titles and/or abstracts review. Out of these 19 articles which were subjected to full paper review eligibility, 7 articles were excluded as they did not address the research questions (Supplementary Table S1). As a result, a final number of 12 case-control studies [14–19,21,22,33–36] met all of the inclusion and exclusion criteria and were included for qualitative synthesis (Figure 1). Interexaminer reliability at the full-text screening was considered very substantial (kappa score = 1.00).

The characteristics of the included studies are shown in Table 1. We identified 12 case-control studies from five different countries, across Europe and Asia. These studies were published between 2011 and 2020. The sample sizes ranged from 40 [35] to 48.820 participants [18] per study. A total of 49.965 participants were included in this review, comprising 768 PCOS female individuals without PD, 613 PCOS female individuals with PD, 24.152 female patients with PD and 24.445 healthy controls.

Table 1. Characteristics of the included studies

Authors (Year)	Country	Funding	Number of Subjects	PCOS	PCOS–PD	PD	H	Mean Age ± SD (PCOS/PCOS–PD/PD/H)	Exclusion criteria	PD Diagnostic Criteria	PCOS Criteria
Portwal et al. (2014)	India	None	126	85	0	0	41	23.50 ± 2.67/NA/NA/22.9 ± 4.7	Thyroid dysfunction, hyperprolactinemia, and androgen-secreting tumors to avoid misdiagnosis of PCOS; chronic inflammatory disease; smoking and alcohol habits; Systemic ATB within 3 months; periodontal treatment within 6 months; and AgP diagnosis	Page and Eke 2012	Rotterdam Criteria
Akcali et al. 2014	Turkey	IADR	125	80	0	0	45	25.6 ± 5.2/NA/NA/26.1 ± 4.7	Hyperandrogenism, DM, hyperprolactinemia, congenital adrenal hyperplasia, thyroid disorders, Cushing syndrome, HTA, hepatic or renal dysfunction; BMI > 30 kg/m ² ; CVD; medications (e.g., oral contraceptive agents, steroid hormones, insulin-sensitizing drugs and ATB or anti-inflammatory); periodontal status within the last 6 months	Armitage 1999	Rotterdam Criteria
Hameed et al. 2017	India	None	80	20	20	20	20	NA	Smoking habits; pregnant women; periodontal therapy at the previous 3 months; anti-inflammatory or ATB therapy during the last 3 months; contraceptives or hormonal drugs or medications for PCOS; systemic diseases (e.g., DM, HTA, CVD) which could affect periodontal health	AAP 1999	Rotterdam Criteria
Saglam et al. 2017	India	None	88	22	22	22	22	27.6 ± 4.0/28.6 ± 4.5/28.2 ± 4.3/27.8 ± 3.9	Cushing syndrome, non-classic congenital adrenal hyperplasia, hyperprolactinemia, thyroid	Page and Eke 2012	Rotterdam Criteria

dysfunction, and androgen-secreting tumors											
Tong et al. 2019	Taiwan	None	48820	304	441	23969	24106	NA	Endocrine disorders (e.g. Cushing syndrome, non-classic congenital adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction and androgen-secreting tumors)	ICD-9-CM code: 523.4	ICD-9-CM code: 256.4X
Saljoughi et al. 2020	Iran	Arak University of Medical Sciences	110	25	30	23	32	45.3 ± 3.0/45.2 ± 3.2/45.3 ± 3.1/45.5 ± 3.3	Interfering drugs (e.g., ATB, oral contraceptives, antihypertensive, and DM drugs); infection in the last 6 months; systemic diseases (e.g., thyroid disorders, hyperprolactinemia, DM, HTA, malignancies, osteoporosis); obesity and overweight; smoking and alcohol habits, and pregnant women	Armitage 1999	Rotterdam Criteria

AgP—aggressive periodontitis; ATB—antibiotics; CVD—Diagnosis of cardiovascular diseases; DM—diabetes mellitus; H—periodontal healthy patients; HTA—hypertension; PCOS—polycystic ovarian syndrome; PD—patients with periodontitis; PCOS-PD—Patients diagnosed with polycystic ovarian syndrome and periodontitis; SD—Standard deviation.

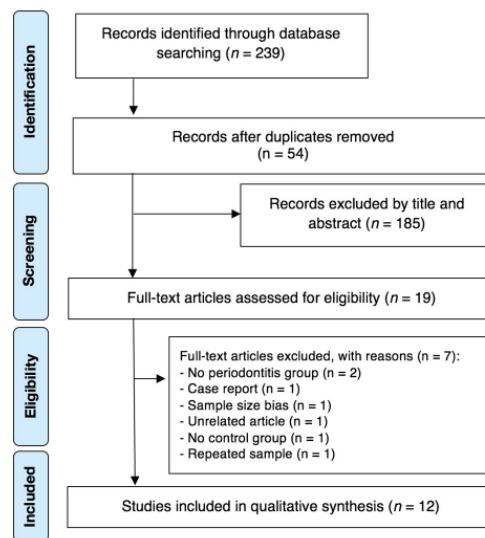


Figure 1. Article selection flow chart for the systematic review according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

3.2. Methodological Quality

The RoB for observational studies, with Newcastle-Ottawa scale, varied across the studies, ranging from 7 to 9 stars (Supplementary Table S2). After appraisal, four articles presented the highest score (9/9) [15,19,21,22]. Furthermore, six articles scored 8/9 [16,17,33–36]. Almost half of the articles failed in including a representative amount of cases (41.7%, $n = 5$), nevertheless, most of them made a good definition of the cases (91.7%, $n = 11$) as well as a good selection (91.7%, $n = 11$) and definition of the controls (75.0%, $n = 9$). All studies had excellent comparability results, secure record of ascertainment of exposure, equal ascertainment method for cases and controls and the same nonresponse rate for both cases and controls (100.0%, $n = 12$).

3.3. Methodological Quality

3.3.1. Bidirectional Association between Polycystic Ovarian Syndrome and Periodontitis

To investigate the likelihood of PCOS female individuals to have PD, three studies were included [15,18,21], in a total of 49,046 participants. The overall results reveal that PCOS individuals have on average 28% more risk to develop periodontitis (RR [95% CI]: 1.28 [1.06–1.55], $p < 0.0001$, $I^2 = 43.0\%$) with moderate heterogeneity, revealing some variation among the included studies (Figure 2).

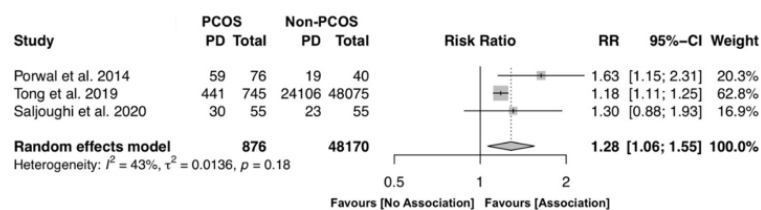


Figure 2. Forest plot of risk ratio of polycystic ovary syndrome (PCOS) female individuals to have periodontitis (PD) (p -value < 0.0001). Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. The size of the squares is proportionate to the study

sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.

To investigate the likelihood of female individuals with periodontitis to have PCOS, the same three studies were included [15,18,21]. The overall results reveal that females with periodontitis have, on average, 46% more risk to be diagnosed with PCOS (RR [95% CI]: 1.46 [1.29–1.66], $p < 0.0001$, $I^2 = 0.0\%$), with complete homogeneity among the included studies (Figure 3).

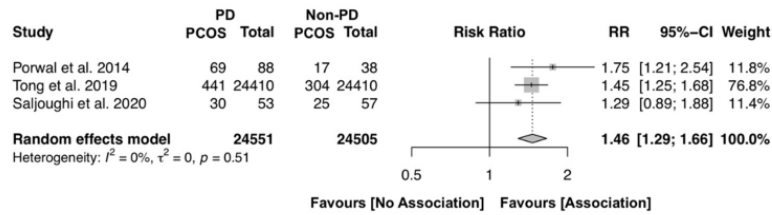


Figure 3. Forest plot of risk ratio of PD female individuals to have PCOS (p -value < 0.0001). Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. The size of the squares is proportionate to the study sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.

3.3.2. PCOS Effect on the Gingival Inflammation

For the BoP analysis, ten studies were included to investigate the association of PCOS on gingival bleeding [14–17,19,21,22,33,34,36]. Five studies compared BoP levels of PD patients with and without PCOS [16,17,21,33,34], four studies compared BoP levels of non-PD patients with and without PCOS [17,21,34,36] and four studies compared BoP between PCOS and controls [14,15,19,22] (Figure 4). Overall, this analysis had 594 and 490 individuals with PCOS and without PCOS, respectively. A significant MD was found (MD [95% CI]: 2.21 [1.32–3.11] $p < 0.01$), and the heterogeneity among studies was high (98% and 94%, respectively). Specifically, PD patients with PCOS presented more gingival inflammation than PD females without PCOS (MD [95% CI]: 1.56 [1.07–2.05] $p = 0.75$), with complete homogeneity ($I^2 = 0.0\%$).

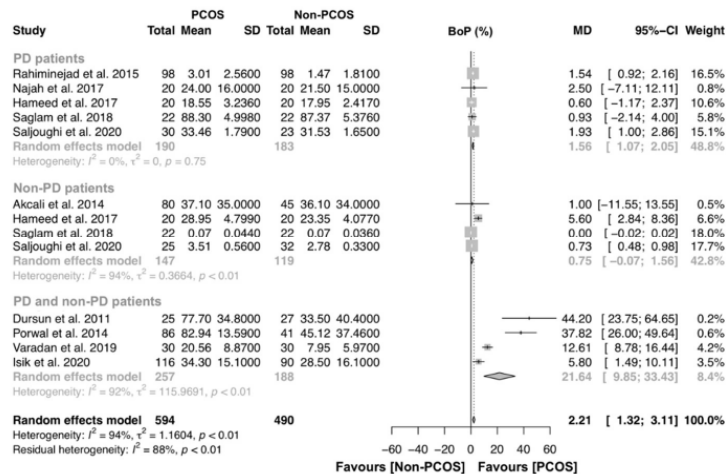


Figure 4. Forest plot of studies evaluating bleeding on probing (BoP) levels in non-PD patients with and without PCOS (p -value < 0.0001). Mean effect size estimates have been calculated with 95%

confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.

3.3.3. PCOS effect on Periodontal Structure Loss

For the assessment of PPD, nine studies were included to investigate the association of PCOS on PPD [14–17,19,21,22,33,34,36]. Four studies compared PPD levels of PD patients with and without PCOS [17,21,33,34], four studies compared PPD levels of non-PD patients with and without PCOS [17,21,35,36] and three studies compared PPD between PCOS and controls [14,15,22] (Figure 5). Overall, this analysis had 507 and 354 individuals with PCOS and without PCOS, respectively. A significant MD was found (MD [95% CI]: 0.61 [0.28–0.94] $p < 0.01$), and the heterogeneity among studies was high ($I^2 = 96\%$). Subgroup analysis was performed, and women with PCOS had greater PPD levels than women without PCOS (MD [95% CI]: 0.98 [0.15–1.81] $p < 0.01$).

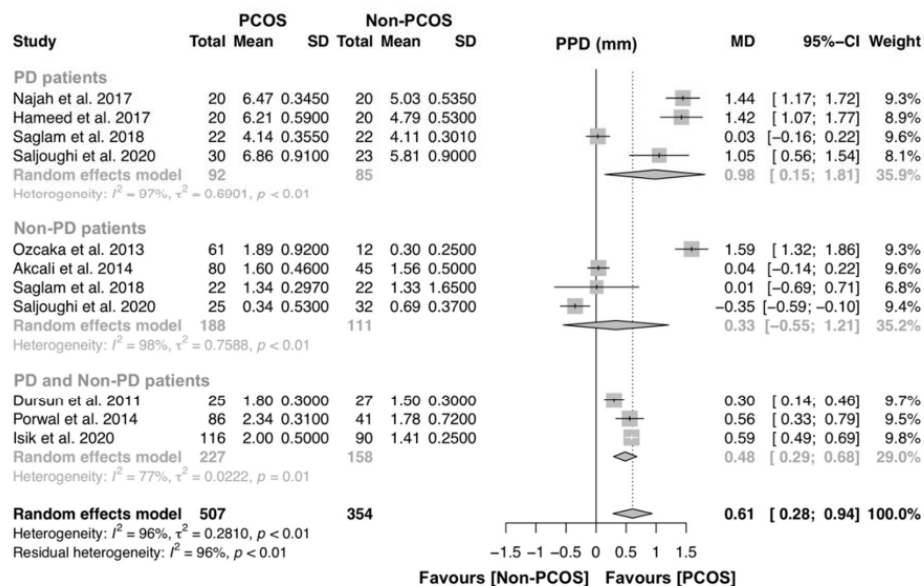


Figure 5. Forest plot of studies evaluating periodontal probing depth (PPD) levels in PD patients with and without PCOS (p -value = 0.0003). Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.

For the assessment of CAL, eight studies were included to investigate the association of PCOS on CAL [14–17,21,22,33,34]. Five studies compared CAL levels of PD patients with and without PCOS [16,17,21,33,34], two studies compared CAL levels of non-PD patients with and without PCOS [17,21] and three studies compared CAL between PCOS and controls [14,15,22] (Figure 5). Overall, this analysis had 464 and 395 individuals with PCOS and without PCOS, respectively. A significant MD was found (MD [95% CI]: 0.33 [0.13–0.53] $p < 0.01$), and the heterogeneity among studies was high ($I^2 = 92\%$). Subgroup analysis was performed and women with PCOS had greater CAL levels than women without PCOS (MD [95% CI]: 0.51 [0.12–0.89] $p < 0.01$) (Figure 6).

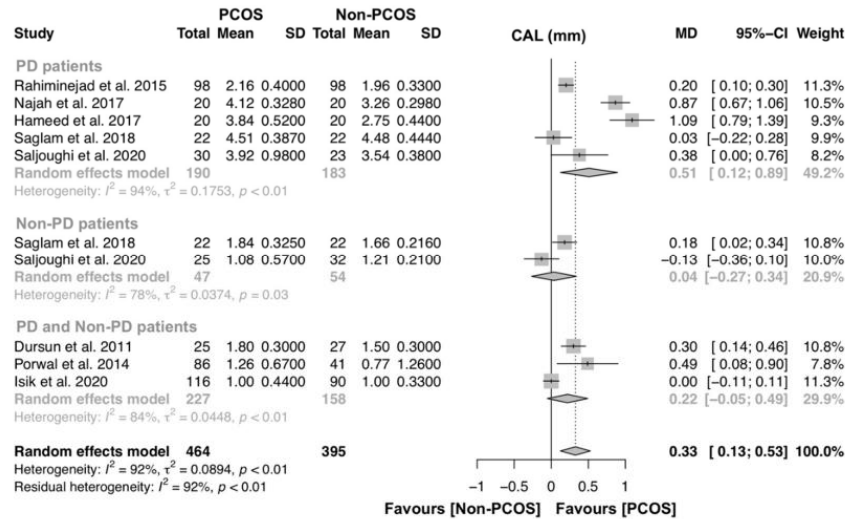


Figure 6. Forest plot of studies evaluating clinical attachment loss (CAL) levels in PD patients with and without PCOS (p-value = 0.0014). Mean effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Diamond and the vertical dotted line represent the overall pooled estimate.

3.3.4. Additional Analyses

Considering the minimum number of 10 studies, publication bias analyses was not possible.

According to the SORT recommendation, the evidence from observational studies revealed that PCOS and PD are strongly associated, based on consistent findings of at least two good-quality meta-analyses that obtained significant results and complete homogeneity (SORT A) [32].

4. Discussion

4.1. Summary of Main Findings and Quality of The Evidence

This systematic review provides an association of PCOS and PD, with an overall SORT A recommendation. Despite the number of reports, our systematic review is the first to investigate the likelihood of a bidirectional link between PCOS and PD. These results emphasize the importance of the association between PD and the clinical status of PCOS women as a common shared low-grade systemic inflammation status.

Overall, the results of this systematic review support a bidirectional link between PCOS and PD. That is, PCOS increases by 28% the risk of having PD and in the same fashion, PD increases by 46% the risk of having PCOS. It is important to emphasize that these results had very interesting degrees of heterogeneity, however, they need to be carefully interpreted due to the low number of articles available. This bidirectional association demands further studies because we were only able to infer how the presence of PCOS links with some periodontal characteristics (such as gingival inflammation and periodontal structure loss), rather than ascribe how the variation of the PD condition influences PCOS clinical characteristics.

Further, PCOS females with PD had higher gingival inflammation and periodontal structure loss than non-PCOS females with PD. While the results are in line with a previous systematic review [25], our outcomes regarding BoP on PD females (PCOS vs. non-PCOS) present complete homogeneity.

Also, for the remaining measures (PPD and CAL), our results showed higher heterogeneity levels, which might be explained by the increased the number of studies in each meta-analysis.

Comprehensively, the link between PD and PCOS is based on the chronic subclinical inflamed status of both conditions [18,37]. In other words, a persistent subclinical inflammation triggers the synthesis of a panel of proinflammatory markers (such as C-Reactive Protein (CRP), tumor necrosis factor- α , interleukin (IL)-6, IL-17, and matrix metalloproteinase-9) [36,38,39], and potentiates an oxidative stress environment (through local oxidant status-like myeloperoxidase and nitric oxide) [14,17,36]. Unfortunately, this systematic review was not able to provide pooled estimates for these inflammatory markers within the PCOS–PD axis due to the lack of studies. In this sense, further investigations on inflammation are of the utmost importance because they may explain the biological mechanisms connecting these two entities. Additionally, PD management has been implicated in insulin levels control with periodontal treatment allowing the alleviation of high glycemic levels [40], and therefore uncontrolled periodontitis may indirectly impact on PCOS clinical status.

4.2. Strengths and Potential Limitations

This systematic review followed a rigorous protocol, with up-to-date international reporting guidelines, a thorough literature search and all included articles were of low risk of bias. There are, however, a number of limitations to mention. The results are derived from observational studies denting the inference of causality. In addition, the case definition of PD among the included studies was poorly uniform and may explain the elevated heterogeneity levels. Even so, the diagnosis of PCOS was mostly based on the Rotterdam criteria 2003 [41], which may have minimized the degree of heterogeneity, except for the study Tong et al. [18] that used the ICD-9-CM system. Another limitation is that the included articles are not coherent on the blood analysis and physical assessments of PCOS so we can only have an objective insight of the periodontal parameters, plus, the articles vary considerably on the number of subjects included, which may be a cause of heterogeneity. It is also important to mention that some articles did not exclude patients with gingivitis from the periodontally healthy groups which may lead to differences in the results.

Importantly, a novelty of our study is that our approach grouped whether studies considered the periodontal status of the included patients, differently from the systematic review by Farook et al. [25]. In other words, we intended to differentiate studies that had made periodontal diagnosis a priori from those that included PCOS and control patients regardless of the periodontal status. Our results confirm that the pooled estimates from studies with undefined periodontal status are more likely to produce lower differences and more heterogeneous results. Interestingly, no study used the most recent classification on PD [42] and is a limiting factor since it is highly advisable that studies use updated classifications. Furthermore, the new PD case definition presents a potential improved ability to transmit the entire periodontal condition [43,44]. Therefore, future studies on PCOS and PD should diagnose the periodontal status of the included participants and follow the later PD diagnosis definition.

4.3. Interpretation and Clinical Implications

The cause of PCOS remains unknown [7], however is characterized as a syndrome related to the interplay of genetic and environmental factors [45]. From this standpoint, metabolic abnormalities associated with insulin resistance have become increasingly relevant [45–47]. Metabolic syndrome (MetS) is defined by a clump of hyperglycemia, obesity, dyslipidemia, and hypertension [45], and several clinical studies have reported marked prevalence among PCOS patients [48–54].

Compensatory hyperinsulinemia caused by MetS nettles excessive action of insulin, which is a major extraovarian factor in the steroidogenic dysregulation in PCOS [53,55–57]. Comprehensively, excessive insulin overwhelms theca cell steroidogenesis [58–60], inducing abnormal production of ovarian androgens and causes subsequent ovarian dysfunction [45,61,62]. PD and MetS have been consistently associated in the past two decades [63,64]. Furthermore, the relationship between PD and poor glycemic control is well-established in a bidirectional fashion based on the impact of chronic low-grade systemic inflammation [10,65–79]. Similar to MetS, this long standing inflamed status is

closely involved with abnormal hyperglycemia levels, poor glycemic control, and insulin resistance [68,80]. Our results support this hypothesis, showing a bidirectional association between PCOS and PD, however we were not able to provide further analysis to establish mechanistic relationships between these conditions, and therefore more research is warranted to clarify this link.

The complexity of cytokine networks and oxidative stress in periodontal pathogenesis is becoming increasingly evident. In addition, insulin resistance is central in the development of PCOS due to stimulation of androgens in theca cells and consequently remaining a mechanism of PCOS. The sum of all periodontal factors in women with PCOS can lead to exacerbation of its clinical characteristics, (hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphologic features). Further, PD treatment can be a coadjuvant in PCOS therapy, since one randomized clinical trial showed that periodontal treatment together with a myo-inositol regimen greatly reduced the systemic inflammatory burden [23]. In other words, periodontal care may aid the clinical management of PCOS and that constitutes a paradigm shift in the multidisciplinary approach of this condition.

4.4. Research Implications

This systematic review provides evidence for a possible bidirectional association between PCOS and PD. These results shed light on the need for more studies further investigating the underlying mechanisms between these two conditions. Future investigations should incorporate serum and physical analysis of the main biomarkers of PCOS (namely for hyperandrogenemia, testosterone and insulin resistance) in their design. Also, studies should divide participants according to the PCOS and PD status in longer prospective study designs, to ensure more evidence on the impact between these diseases.

In addition, new clinical trials assessing the impact of nonsurgical periodontal treatment (NSPT) on PCOS patients are encouraged to clarify if treating PD might alleviate PCOS clinical course (biomarkers for reproductive and metabolic health) and impact its risk factors (e.g., insulin resistance).

The development and implementation of a core set of research guidelines on PCOS and PD are highly recommended to ensure that results are collected and reported in a similar and consistent manner to allow for future definite conclusions on this association.

5. Conclusions

Within the limitations of this review, our results show that there is a higher risk of individuals with PCOS being diagnosed with PD and vice-versa. PCOS may worsen the periodontal condition as higher levels of gingival inflammation, PPD and CAL were found. The opposite cannot be said as the PCOS variables are not fully elucidative. Obstetrician-gynecologists and dentists should collaborate and improve the clinical outcomes of their PCOS patients.

Supplementary Materials: The following are available online at www.mdpi.com/2077-0383/9/6/1961/s1, Table S1: List of excluded articles, Table S2: Case-control studies bias assessment using the Newcastle-Ottawa Scale (NOS).

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**V. ASSOCIATION OF BACTERIAL VAGINOSIS WITH PERIODONTITIS IN
A CROSS-SECTIONAL AMERICAN NATIONWIDE SURVEY**

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OPEN Association of bacterial vaginosis with periodontitis in a cross-sectional American nationwide survey

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To explore the association between bacterial vaginosis (BV) and periodontitis (PD) and to determine whether PD and BV might be linked with systemic serum alterations. We used the National Health and Nutrition Examination Survey 2001–2004, with women aged 18–49 years old and diagnosed with or without BV according to Nugent's method. PD was defined according to the 2012 case definition. We compared serum counts according to the presence of PD and the presence of BV. Multivariable regression was used to explore and identify relevant variables towards the presence of BV. 961 women fulfilled the inclusion criteria. In women with BV, PD was associated with higher inflammation, characterized by increased white blood cells ($p = 0.006$) and lymphocyte ($p = 0.009$) counts. Predictive models presented a statistically significant association between PD and BV [Odds Ratio (OR) = 1.69, 95% Confidence Interval (CI): 1.09–2.61 for periodontitis; OR = 2.37, 95% CI: 1.30–4.29 for severe PD]. Fully adjusted models for age, smoking, body mass index, diabetes mellitus and number of systemic conditions reinforced this association [OR = 1.71, 95% CI: 1.06–2.76 for PD; OR = 2.21, 95% CI: 1.15–4.25 for severe PD]. An association between BV and PD is conceivable. PD was associated with higher systemic markers of inflammation in women with BV. Our data is novel and could serve as a foundation to guide future studies in the confirmation of this association and the underlying mechanisms.

Bacterial vaginosis (BV) is a common vaginal condition with unknown aetiology which represents a relevant shift in the vaginal microbiota. BV is characterized by a thin gray/white and malodorous “fishy” discharge, with high vaginal pH (> 4.5), and vaginal epithelial cells heavily coated with bacteria^{1–4}. The abnormal vaginal discharge is the result of an imbalanced vaginal microbiota with increase of anaerobic microorganisms (e.g., *Gardnerella vaginalis*, *Prevotella intermedia*, *Peptostreptococcus*, and *Bacterioides spp*)^{1,2,5}. Also, BV is associated with reproductive and obstetric sequelae, increasing women's risk of pelvic inflammatory disease, spontaneous abortion, preterm delivery, low birth weight, and postpartum endometritis^{2,5–9}. Susceptibility to BV is dependent on the host immune response and is multifactorial^{1,2,10–12}. Genetics, environmental and physiologic stressors, hormonal variations based on the reproductive status and use of hormonal contraceptives are relevant factors towards BV^{1,10}.

Periodontitis (PD) is a chronic multifactorial inflammatory condition characterized by inflamed gums and bone destruction caused by a dysbiotic plaque^{13–15}. It is preceded by gum inflammation (gingivitis) and an uncontrolled inflammatory response from the innate and adaptive immune system¹⁶. PD has also been associated with several chronic and systemic diseases^{17–23}. A recent review discussed the available evidence and possible mechanisms between female infertility related conditions as a modifiable risk factor towards PD^{24,25}. Particularly, women with BV were associated with a significant diversification of salivary microbiota and higher counts of *Prevotella intermedia* (a PD-related bacteria) in the supragingival microbiota compared with women without BV²⁶. However, the mechanism through which the oral microbiota suffers dysbiosis in the presence of BV is not determined²⁶.

Data from the Longitudinal Study of Vaginal Flora reported a significant association between PD and BV²⁷, and women with BV were also found to have higher risk of gingivitis²⁸. Comprehensively, bacterial communities

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	Non-BV (n = 510)	BV (n = 451)	p value ^a
Age, mean (SD) (years)	32.4 (10.1)	32.0 (10.3)	0.556
Race/ethnicity, n (%)			
Mexican American	122 (51.1)	117 (49.0)	0.000
Non-Hispanics White	258 (63.1)	151 (36.9)	
Non-Hispanic Black	148 (63.0)	87 (37.0)	
Other Hispanic	26 (51.0)	25 (49.0)	
Other race	10 (50.0)	10 (50.0)	
Education			
< High school	126 (43.8)	162 (56.3)	0.000
High school	129 (54.0)	110 (46.0)	
> High school	255 (58.8)	179 (41.2)	
Active smokers, n (%)	95 (9.9)	115 (12.0)	0.007
BMI, mean (SD) (kg/m ²)	27.2 (7.2)	28.2 (8.3)	0.017
Marital status			
Single	163 (47.8)	178 (52.2)	0.002
Married/living with a partner	299 (58.7)	210 (41.3)	
Divorced/separated/widowed	48 (47.5)	53 (52.5)	
Medical conditions, n (%)			
Diabetes mellitus	17 (1.8)	19 (2.0)	0.325
Other medical conditions	0.5 (0.8)	0.6 (0.9)	0.528
Periodontal health, n (%)	472 (49.1)	397 (41.3)	0.013
Periodontitis staging, n (%)			
Mild (stage I)	21 (51.2)	20 (48.8)	
Moderate (stage II)	15 (39.5)	23 (60.5)	
Severe (stage III)	2 (15.4)	11 (84.6)	
PISA	11.8 (26.4)	13.9 (27.8)	0.012
PESA	93.5 (232.0)	87.0 (260.4)	0.543

Table 1. Participants characteristics. Significant correlations are identified in bold ($p < 0.05$). BV, patients with bacterial vaginosis; n, number; SD, standard deviations; Non-BV, patients without bacterial vaginosis; PESA, periodontal epithelial surface area; PISA, periodontal inflamed surface area; <, higher than; >, less than. Values are given as mean (standard error) or % (standard error). ^aChi-square test for categorical variables, Mann–Whitney test for continuous variables, $p < 0.05$.

from the vagina and the periodontium have higher counts of *Fusobacteria nucleatum* and *Prevotella intermedia*^{27,29–31}. Also, the presence of these bacteria may trigger infections in primed women^{27,32}, and may have unpleasant consequences during pregnancy, such as preterm birth and low birth weight^{2,5–9}. Further, some studies also support the notion of haematogenous spread or oral-genital direct transfer^{27,29,33}, however, the relationship between these two pathologies remains poorly understood.

For this reason, we aimed to investigate the likelihood of an association between BV and PD in a representative American cohort of adult women. Secondly, we explored whether this association might lead to systemic alterations measured via blood samples, through complete blood count (white, red and platelet lineages) and C-reactive protein (CRP) levels.

Results

Baseline characteristics. From a total of 21,161 participants in National Health and Nutrition Examination Survey (NHANES) 2001–2002 and 2003–2004, we excluded 10,301 (48.7%) males, 7395 women under 18 years or over 50 years old (34.9%), 301 pregnant women (1.4%) and 38 women who did not perform the pregnancy test or had an invalid result (0.2%). Overall, from 3126 included women, a final sample of 961 completed both BV and periodontal clinical examinations. Table 1 displays the characteristics of the included women participants.

The prevalence of PD in the overall sample was 9.6% (54% in patients with BV and 38% in non-BV women), although women with BV had higher prevalence ($p = 0.013$). Women with BV had a higher percentage of active smokers (12.0%), higher levels of body mass index (BMI) (28.2 kg/m²) and presenting with higher education levels (56.3%) when compared to participants with no clinical signs of BV. Indeed, the number of medical conditions recorded was similar in both groups. Mean $\pm$ Standard deviation (SD) of periodontal inflamed surface area (PISA) and periodontal epithelial surface area (PESA) were 11.8 ± 26.4 and 93.5 ± 232.0 in patients without BV versus 13.9 ± 27.8 and 87.0 ± 260.4 for those with BV, respectively ($p = 0.012$ for PISA and $p = 0.543$ for PESA).

Variables, mean (SD)	BV (n = 510)			Non-BV (n = 451)			Global (n = 961)		
	Non-PD (n = 472)	PD (n = 38)	p value ^a	Non-PD (n = 397)	PD (n = 54)	p value ^a	Non-PD (n = 869)	PD (n = 92)	p value ^a
WBC count (10 ⁹ /L)	7.2 (2.6)	8.2 (2.7)	0.006	7.0 (2.5)	7.1 (2.6)	0.857	7.1 (2.5)	7.8 (2.7)	0.019
Monocyte (%)	7.0 (2.6)	6.8 (2.3)	0.578	7.0 (2.3)	6.2 (2.6)	0.013	7.0 (2.5)	6.6 (2.5)	0.050
Monocyte (10 ⁹ /L)	0.5 (0.2)	0.5 (0.2)	0.387	0.5 (0.2)	0.5 (0.2)	0.192	0.5 (0.2)	0.5 (0.2)	0.993
Segmented neutrophils (%)	55.8 (15.5)	55.7 (15.2)	0.896	57.0 (13.7)	57.0 (17.2)	0.469	56.4 (14.6)	56.2 (16.0)	0.660
Segmented neutrophils (10 ⁹ /L)	4.2 (1.8)	4.4 (2.1)	0.086	4.2 (1.8)	4.4 (2.1)	0.646	4.3 (1.9)	4.6 (2.2)	0.094
Eosinophils (%)	2.4 (2.5)	2.3 (1.8)	0.810	2.3 (2.1)	1.7 (1.1)	0.058	2.4 (2.3)	2.1 (1.6)	0.178
Eosinophils (10 ⁹ /L)	0.2 (0.2)	0.1 (0.1)	0.735	0.2 (0.2)	0.1 (0.1)	0.266	0.2 (0.2)	0.2 (0.1)	0.662
Basophils (%)	0.6 (0.5)	0.6 (0.4)	0.988	0.6 (0.4)	0.5 (0.5)	0.080	0.7 (0.4)	0.3 (0.3)	0.281
Basophils (10 ⁹ /L)	0.0 (0.1)	0.0 (0.1)	0.075	0.0 (0.1)	0.0 (0.1)	0.339	0.0 (0.1)	0.0 (0.1)	0.361
Lymphocyte (%)	29.6 (10.6)	30.6 (10.7)	0.474	29.4 (9.2)	28.8 (10.9)	0.506	29.5 (9.9)	29.9 (10.7)	0.850
Lymphocyte (10 ⁹ /L)	2.1 (0.8)	2.5 (0.8)	0.009	2.1 (0.7)	2.1 (0.7)	0.914	2.1 (0.8)	2.3 (0.8)	0.021
RBC count (million cells/ μ L)	4.3 (1.0)	4.4 (0.7)	0.211	4.3 (0.9)	4.3 (1.2)	0.398	4.3 (1.0)	4.4 (0.9)	0.613
Hemoglobin (g/dL)	12.6 (3.1)	13.0 (2.4)	0.395	12.9 (2.8)	12.8 (3.6)	0.501	12.8 (2.9)	12.9 (2.9)	0.890
Hematocrit (%)	37.3 (8.9)	38.7 (6.8)	0.301	38.0 (8.2)	38.2 (10.5)	0.642	37.7 (8.5)	38.4 (8.4)	0.668
MCV (fL)	83.6 (19.4)	85.5 (14.4)	0.488	85.0 (17.7)	83.0 (22.0)	0.240	84.3 (18.5)	84.5 (17.7)	0.791
MCH (pg)	28.3 (6.7)	28.8 (5.2)	0.585	28.9 (6.2)	28.0 (7.6)	0.179	28.6 (6.4)	28.5 (6.2)	0.617
MCHC (g/ dL)	32.3 (7.1)	33.0 (4.8)	0.416	32.7 (6.5)	31.6 (8.1)	0.100	32.5 (6.8)	32.5 (6.3)	0.657
RDW (%)	12.3 (3.0)	12.8 (2.4)	0.144	12.2 (2.7)	12.4 (3.8)	0.575	12.2 (2.8)	12.6 (3.0)	0.420
Platelet count (10 ⁹ /L)	278.9 (91.4)	297.4 (82.7)	0.183	281.6 (90.7)	292.4 (106.9)	0.672	280.3 (90.9)	295.4 (92.6)	0.197
MPV (fL)	8.0 (1.9)	8.3 (1.6)	0.134	8.0 (1.8)	7.6 (2.1)	0.075	8.0 (1.9)	8.0 (1.8)	0.903
CRP (mg/dL)	0.4 (1.1)	0.7 (1.0)	0.045	0.4 (1.0)	0.4 (0.6)	0.939	0.4 (1.0)	0.6 (0.9)	0.093

Table 2. Hematologic counts according to bacterial vaginosis and periodontal state. Significant correlations are identified in bold ($p < 0.05$). BV, patients with bacterial vaginosis; CRP, C-reactive protein; Non-BV, patients without bacterial vaginosis; P(−), no periodontitis, P(+), periodontitis; WBC, white blood cells; RBC, red blood cells; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RCD, red cell distribution; MPV, mean platelet volume. ^aMann–Whitney test for continuous variables, $p < 0.05$.

Hematologic counts. The complete blood count was used to compare the blood counts of the PD group with the periodontally healthy women, according to the BV diagnosis. Globally, women with PD presented higher counts of white blood cells (WBC) ($p = 0.019$) and lymphocytes ($p = 0.021$), and lower counts of monocyte ($p = 0.050$). Specifically in women without BV, PD was associated with lower counts of monocytes ($p = 0.013$). Women diagnosed with both BV and PD presented increased counts of WBC ($p = 0.006$), lymphocytes ($p = 0.009$) and CRP ($p = 0.045$) (Table 2).

Then, we graphically explored the behaviour of CRP levels of women with and without BV according to the levels of PISA (Fig. 1). In BV women, CRP levels tend to increase with the increase of PISA levels. Up to 4.4 levels of PISA, women with BV presented lower levels of CRP than non-BV counterparts.

Predictive models of periodontitis on bacterial vaginosis patients. In the crude model (model 1), the association of PD with BV was statistically significant [odds ratio (OR) = 1.69, 95% confidence interval (CI): 1.09–2.61 for PD; OR = 2.37, 95% CI: 1.30–4.29 for severe PD] (Table 3). Also, the fully adjusted models with age, smoking habits, BMI, diabetes mellitus and number of systemic conditions confirmed a significant association (OR = 1.71, 95% CI: 1.06–2.76 for PD; OR = 2.21, 95% CI: 1.15–4.25 for severe PD) (Table 3).

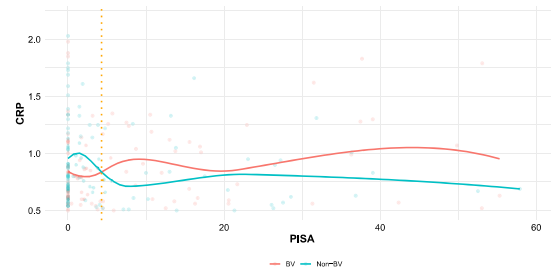


Figure 1. Comparison of C-reactive protein (CRP) (ng/mL) according to Periodontal Inflamed Surface Area (PISA) for women with and without BV.

	Periodontitis OR (95% CI)	Severe periodontitis OR (95% CI)
Model 1	1.69 (1.09–2.61)*	2.37 (1.30–4.29)**
Model 2	1.76 (1.13–2.74)*	2.52 (1.37–4.63)**
Model 3	1.66 (1.04–2.65)*	2.29 (1.21–4.33)*
Model 4	1.67 (1.04–2.69)*	2.14 (1.12–4.09)*
Model 5	1.68 (1.04–2.70)*	2.16 (1.13–4.12)*
Model 6	1.71 (1.06–2.76)*	2.21 (1.15–4.25)*

Table 3. Odds ratios (OR) and correspondent 95% confidence intervals (CI) towards bacterial vaginosis, according to the periodontal status, calculated within binary logistic regression analyses for different adjustment levels. Model 1, unadjusted model; Model 2, includes adjustment for age; Model 3, includes adjustment for age and smoking habits; Model 4, includes adjustment for age, smoking habits and BMI; Model 5, includes adjustment for age, smoking habits, BMI and diabetes mellitus; Model 6, includes adjustment for age, smoking habits, BMI, diabetes mellitus and number of systemic diseases (excluding diabetes). Statistically significant: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Discussion

We aimed to investigate whether BV and PD were associated in a representative nationwide sample of women Americans. Our results support our initial hypothesis of a possible link between BV and PD. Women with BV had higher prevalence of PD, levels of periodontal inflammation, percentage of active smokers and average BMI. Also, women with BV and PD presented significantly more systemic inflammation than those with healthy periodontium.

Thus so far, this study is the first to explore the likelihood of a systemic association via inflammatory hallmarks between BV and PD. Our findings emphasize that when both BV and PD co-exist, a significant systemic inflammatory load is detected (measured as CRP) and may be dependent on the periodontal inflammation (measured as PISA). Further, BV patients with PD present higher counts of white and lymphocyte cells than BV patients without PD.

Considering the leukocyte data in this study, our results are consistent with the available evidence, where an increase in WBC is expected from an indirect impact of PD^{34,35}. Still, the counts of neutrophils were not a significant parameter in this analysis, despite their strong involvement in the pathogenesis of periodontal disease as they contribute to tissue destruction by releasing toxic products, such as enzymes and cytokines^{36–38}. BV is commonly defined by the absence of neutrophils in vaginal swabs observed by flow cytometry^{4,39,40}, and therefore an impact on circulating neutrophil counts might be unlikely to exist. However, since the periodontal examination was partially employed, this may have influenced these contradictory results.

The reported prevalence of BV in this American population is consistent with the report by Coudray and Madhivanan⁴¹ which shows a prevalence of 29.2%. Others, Javed et al.⁴² and Peebles et al.⁴³ both found BV to be more prevalent in Non-Hispanic Black women with 51.4% and 33.2%, respectively. However, our results demonstrate higher rates in Non-Hispanic White women. Also, our result contrasts with the prevalence of BV in NHANES 2001–2004⁴⁴. A possible explanation for those differences may be due to the data mining when evaluating patients with both BV and PD examinations, that resulted in the exclusion of a substantial percentage of the overall sample (44.4%).

Our results are in accordance with previous literature stating there is a higher number of women who were diagnosed with BV and had smoking habits. In this sense, smoking habits has a significant effect on innate immunity, and it has been shown to have varying effects on markers of cervicovaginal immunity, namely with higher interleukin (IL)-10 levels in cervicovaginal secretions^{10,45–47}. It is also a major modifiable risk factor for PD^{48–50} and it is hypothesized that smoking may lead to an increase in the prevalence of periodontal pathogens as well as delays the neutrophils recruitment into periodontal tissues, and thus compromise the immune response^{16,51–56}.

Further, our results also show that women with BV had higher BMI compared with women without BV, and this result is in contrast with Loken et al.⁵⁷ result, however this can be explained due to the distinct types of populations and environmental factors.

These results might contribute to increase awareness of obstetricians/gynecologists for the higher risk of women with BV towards the presence of PD and the elevated inflamed burden that these patients may be exposed to.

Further, the coexistence of BV and PD in pregnant women may have severe consequences. Several lines of evidence indicate that infectious processes are associated with preterm delivery, namely overt or subclinical intrauterine infection, lower genital tract infection and distant infections, such as periodontal infection⁵⁸. On the one hand, periodontal bacteria and/or their pathogenic subproducts may spread into the bloodstream and reach the foeto-placental unit triggering an inflammatory response⁵⁹. On the other hand, inflammatory cytokines and mediators (such as CRP) due to periodontal inflammation also play a key role, perpetuating this inflammatory response⁵⁹. Lastly, high levels of maternal CRP in early pregnancy are associated with preterm birth^{60–62}, and PD might be seen as a contributing element for this unpleasant outcome.

Undoubtedly, BV may cause genital mucosal inflammation³, though evidence on local cervicovaginal cytokine levels in BV is still debatable^{3,63–65}. Nevertheless, the consequences of maintained elevated systemic CRP levels in women with BV are unknown given the lack of clinical evidence. On the other hand, PD is a recognized factor towards persistent inflammatory states on a plethora of other chronic diseases, for instance in diabetes, cardiovascular diseases or rheumatoid arthritis^{17,66–68}. Further, the treatment of gum disease is effective in alleviating this inflammatory burden^{69–71}. Therefore, future studies are warranted to unveil whether treating PD in women with BV might mitigate the inflammatory load observed in this study and the added value of doing so.

The present study is an epidemiological representative study of a segment of the American population composed of women in fertile age. A big strength of this study is the size of the data set used, comprising two NHANES waves. BV was assessed using Nugent's score which is widely regarded as the gold standard for the diagnosis of BV in research and clinical studies^{72–75}, and we employed the most up-to-date periodontal classification⁷⁶. Also, these results raise awareness on the interplay between oral health and women genital tract health and stress the fact that there is a need for more research to conclude a possible relationship between both diseases as well as to understand their linking mechanisms.

There are, however, some limitations to mention. Due to its novelty, we are not able to compare our results. Also, NHANES waves stated in this paper used a partial index to assess periodontal status which diminishes the sensitivity and specificity^{77,78}, and this may limit the validity of the established association due to a possible underestimation of PD. Hence future studies shall implement full-mouth periodontal charts to contribute to more consistent results. Furthermore, this is a cohort study that precluded the inference of causality or temporal relationship between the analyzed variables.

Conclusions. Women with BV have higher risk of presenting PD, and therefore an association between BV and PD is possible. Also, women with BV and PD present significantly higher systemic markers of inflammation than those without PD. Future studies are warranted to ascertain further this association as well the mechanisms upon this interplay.

Materials and methods

Study design and participants. We conducted a secondary analysis on data from the NHANES, a stratified multistage national representative survey with civilian noninstitutionalized population in fifty states of the United States of America (USA) and the District of Columbia. All specific details about the sampling, design, medical records and periodontal data-collections are available at www.cdc.gov/nchs/nhanes.htm. Both NHANES 2001–2002 and 2003–2004 were reviewed and approved by the Centers for Disease Control (CDC) and Prevention National Center for Health Statistics Research (NCHS) Ethics Review Board, and all included participants provided written informed consent.

For the purpose of this study, NHANES 2001–2002 and NHANES 2003–2004 data was retrieved with the following inclusion criteria: women between the ages of 18 and 49 years; non-pregnant; who received periodontal examination and the assessment to BV. This secondary study was performed following the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines⁷⁹.

Bacterial vaginosis assessment. Self-vaginal swabs were collected and then the swab was onto a glass slide to air dry^{80,81}. The smears were processed at Magee-Women's Hospital (Pittsburgh, Pennsylvania), and the BV score was calculated according to Nugent's method⁸². In this sense, scores between 0 and 3 were considered normal vaginal microbiota, scores of 7 or higher were considered positive for BV, whereas scores between 4 and 6 were considered intermediate. For analysis, the outcome was defined as BV confirmed (positive) or not (negative or intermediate).

Periodontal examination. Half-mouth periodontal examination was conducted on randomly assigned quadrants, one upper and one lower, by calibrated examiners as described elsewhere^{83,84}. Probing pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) measurements were performed at three sites per tooth (mesio-buccal, mid-buccal and disto-buccal). The diagnosis and staging of PD was carried out according to the CDC and Prevention-American Academy of Periodontology consensus for epidemiologic studies recommendation⁸⁵. As such, mild PD was defined as ≥ 2 interproximal sites with CAL ≥ 3 mm and ≥ 2 interproximal sites with PPD ≥ 4 mm (not on the same tooth) or 1 site with PPD ≥ 5 mm. Moderate PD was defined as ≥ 2 interproximal sites with CAL ≥ 4 mm (not on the same tooth) or ≥ 2 interproximal sites with PPD ≥ 5 mm,

also not on the same tooth. Severe PD was defined as the presence of ≥ 2 interproximal sites with CAL ≥ 6 mm (not on the same tooth) and ≥ 1 interproximal site with PPD ≥ 5 mm. The sum of mild, moderate and severe PD corresponded to the overall number of PD.

Also, the PISA and the PESA for every tooth in all subjects were estimated in Microsoft Excel spreadsheet. PISA is the area of bleeding of the pocket epithelium (mm^2) and PESA is the surface area of the root covered with pocket epithelium (mm^2)^{86,87}.

Covariates. Self-reported sociodemographic characteristics regarding age, race or ethnicity (i.e., Mexican American, Non-Hispanics White, Non-Hispanics Black, other Hispanics and Other race) and highest education level (i.e., less than high school, complete high school or similar, higher than high school). Further, in this analysis we collected data about current smokers (i.e. who had smoked ≥ 100 cigarettes during their lifetime and were still smoking). BMI was calculated as weight in kilograms divided by height in meters squared. Marital status was defined as single, married/living with a partner, divorced/separated/widowed. Diabetes mellitus was categorized as yes or no according to the self-reported questionnaire. Medical conditions were assessed as the sum of binary variables (to note the presence of asthma, congestive heart failure, coronary heart disease, angina, stroke, heart attack, emphysema, overweight, bronchitis, liver, thyroid and cancer) as previously done in Leira et al.⁸⁸.

Blood levels data included WBC count ($10^9/\text{L}$), monocyte percentage (%), monocyte ($10^9/\text{L}$), segmented neutrophils percentage (%), segmented neutrophils ($10^9/\text{L}$), percentage of eosinophils (%), eosinophils ($10^9/\text{L}$), percentage of basophils (%), basophils ($10^9/\text{L}$), lymphocyte percentage (%), lymphocyte ($10^9/\text{L}$), red blood cell (RBC) count (million cells/uL), hemoglobin (g/dL), hematocrit (%), mean cell volume (MCV) (fL), mean cell hemoglobin (MCH) (pg), mean cell hemoglobin concentration (MCHC) (g/dL), percentage of red cell distribution (RCD) width (%), percentage of platelet count (%), mean platelet volume (MPV) (fL), CRP (mg/dL). Overall, the markers expected to present association were WBC, Segmented neutrophils percentage, RBC, Hemoglobin, Hematocrit, MCV, MCH and MCHC according to Botelho⁸⁹.

We used PISA as a proxy for periodontal (local) inflammation and serum levels of CRP as a proxy for systemic inflammation, respectively^{88,90}.

Data management, test methods and analysis. Data from the NHANES 2001–2002 and 2003–2004 were uploaded through SAS Universal Viewer for Windows and transformed to SPSS version 25.0 for Macintosh (Armonk, New York, IBM Corp.). All periodontal database was transferred to a Microsoft Excel with an appropriate algorithm to assess the periodontal case definition. Descriptive measures are described as mean $\pm$ SD for continuous variables, and number of cases (n) and percentage (%) for categorical variables. Mean values explicit comparison was performed by t-Student test when data assumptions for the application of this test were met (normality and homoscedasticity). When those assumptions were not verified, Mann–Whitney was used. For categorical variables we compared baseline variables according to BV patients diagnosed using Chi-square test. Additionally, in order to investigate the trend in CRP levels according to PISA values in patients with and without BV, we explore a graph using scatterplots from ggplot2 package for R (version 4.0), and the tendency was computed and fitted via 'geom_smooth'. A multivariate stepwise adjusted logistic regression method was used to model the influence of the investigated factors towards the presence of PD in patients with BV. Logistic regression analyses were performed, accounting for PD staging, for all women and as a function of positive diagnosis of BV. Logistic regression analyses calculated the OR and the 95% CI, for different adjustment levels. Model variables were selected among clinical and demographic characteristics. Following the initial crude model (model 1), five progressively adjusted models were calculated (model 2: age; model 3: age and smoking habits; model 4: age, smoking habits and BMI; model 5: age, smoking habits, BMI and diabetes mellitus; model 6: age, smoking habits, BMI, diabetes mellitus and number of systemic diseases excluding diabetes mellitus). A significance level of 5% was set in all inferential analyses.

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Author contributions

C.E., J.B., J.J.M. and V.M. conceived and planned the investigation. J.B. and V.M. performed the data curation and formal analysis. C.E., J.B. and V.M. were responsible for the methodology and project administration; J.J.M. and V.M. supervised and validated the manuscript. C.E., J.B., J.J.M. and V.M. written the original draft; J.B. and V.M. reviewed the manuscript. C.E., J.B., J.J.M. and V.M. have approved the submitted version.

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VI. GENERAL DISCUSSION

The main goal of the present thesis was to evaluate the relationship between periodontitis and female infertility-related conditions. To achieve this purpose, firstly, it was conducted a systematic review to assess the evidence-based regarding the CRP serum concentrations in otherwise healthy patients with and without periodontitis and the estimation of the impact of NSPT on CRP levels up to 180 days. Secondly, all evidence linking PCOS and periodontitis was assessed. Thirdly, using a population-based epidemiological survey of females with and without BV and periodontitis, it was intended to investigate a possible association between BV and periodontitis and its systemic repercussions, by a blood count analysis (white, red and platelet lineages) and CRP serum quantifications.

Comprehensively, this systematic review exhibits a marked correlation between periodontitis and CRP serum levels in otherwise healthy patients. In other words, periodontally compromised patients demonstrated consistently higher values of CRP compared to healthy controls. Specifically, patients with chronic periodontitis showed increased levels of CRP and hs-CRP of 103% and 110%, respectively. Also, patients with an aggressive periodontitis diagnosis revealed increases of 191% and 179% of CRP and hs-CRP, respectively. In this sense, it appears that the more aggressive form of periodontitis, the greater CRP serum levels. These findings are in accordance with the most recent case definitions of periodontitis (Ramich et al., 2018; Tonetti et al., 2018), which determines that a fast rate of progression had higher CRP serum levels (Tonetti et al., 2018). Furthermore, upon different periodontal treatment modalities, distinct responses on the CRP levels occur. Non-intensive NSPT was shown to be associated with a gradual reduction of hs-CRP values up to 180 days after delivery. On the other hand, intensive NSPT demonstrated a sharp increase of hs-CRP just one day after treatment, accompanied by a progressive reduction 90 days after treatment and another moderate increase upon 180 days. These results agree with past reviews showing that periodontitis therapy can reduce systemic inflammation (Demmer et al., 2013; Freitas et al., 2012).

Inflammation is a key biological process occurring consistently throughout life which protects us against injuries and infections. Yet the permanent state of chronic systemic inflammation leads to consequent chronic conditions and related systemic complications (Furman et al., 2019). Although the increased CRP serum levels of patients with

periodontitis and the consequent reduction noticed following periodontal therapy do not provide a final clarification on the mechanisms by which periodontitis impacts systemic health, periodontitis should be regarded as a common trigger of systemic inflammation. The exact mechanism of how periodontitis triggers CRP release is still unclear. On one hand, the low-grade systemic inflammation induced by local inflammatory mediators promotes local secretion of CRP (Lu & Jin, 2010). On the other hand, these events can also generate a distant response by targeting hepatocytes after systemic dispersion (Ebersole & Cappelli, 2003).

Regarding systemic inflammation, it is known that the bidirectional correlation between PCOS and periodontitis is based on a low-grade systemic inflammatory status inherent to both diseases. In general, the obtained results support this association. Women with PCOS presented an increased risk of 28% of having periodontitis. In the same manner, women with periodontitis showed an enhanced risk of 46% of having PCOS. Specifically, and being in accordance with previous literature (Farook et al., 2019), it was found that PCOS female patients with periodontitis present higher gingival inflammation and periodontal structure loss than non-PCOS females with periodontitis.

As mentioned above, the connection between periodontitis and PCOS lies on the chronic subclinical inflammatory status present in both conditions (Tong et al., 2019). This means that a continued state of inflammation leads to the production of a range of proinflammatory markers (CRP, TNF- α , IL-6, IL-17, and MMPs) (Akcalı et al., 2017; Cekici et al., 2014) potentiating an OS environment (Akcalı et al., 2017). Furthermore, both periodontitis and PCOS share an association with MetS which is defined by the presence of hyperglycaemia, obesity, dyslipidaemia, and hypertension (Rosenfield & Ehrmann, 2016). On one hand, MetS leads to compensatory hyperinsulinemia and consequently to an excessive action of insulin, which is an extraovarian factor in the steroidogenic dysregulation in PCOS (Tosi et al., 2015), ultimately overwhelming theca cell steroidogenesis (Munir et al., 2004), leading to an altered production of ovarian androgens and ending up in ovarian dysfunction (Wu et al., 2016). On the other hand, the connection between periodontitis and a deficient control of glycaemia is well characterized in a bidirectional manner based on the action of chronic low-grade systemic inflammation (Kocher et al., 2018; Papapanou et al., 2018).

The implication of OS and cytokine networks in periodontal disease is being increasingly

recognized (Cekici et al., 2014). Also, insulin resistance is a key factor for PCOS development which is related with the stimulation of androgens in theca cells, being regarded as a mechanism of PCOS (Gnanadass et al., 2021).

Despite the data regarding PCOS and periodontitis, the association between BV and periodontitis remains poorly understood. Some studies hold up the hypothesis of haematogenous spread or oral-genital direct transfer of bacteria (Zabor et al., 2010). It is also known that both in the vagina and the periodontium there are higher counts of *Fusobacterium nucleatum* and *Prevotella intermedia* (Zabor et al., 2010) and that these bacteria usually induces infections (Ren & Du, 2017; Zabor et al., 2010) and have unpleasant outcomes during pregnancy culminating in preterm birth and low birth weight (D. Machado et al., 2016). While periodontal bacteria and/or their pathogenic byproducts have the ability to reach the bloodstream and the foeto-placental unit, ultimately precipitating an inflammatory response (Madianos et al., 2013), the inflammatory cytokines and mediators (such as CRP) due to periodontal inflammation can also take part (Madianos et al., 2013). Also, it is a fact that BV may cause inflammation of the genital mucosa (Mitchell & Marrazzo, 2014), nevertheless the evidence on cervicovaginal cytokine levels in BV is arguable (Jespers et al., 2017) and the repercussions of higher CRP levels in this condition are undetermined due to lack of scientific evidence.

In view of the leukocyte data, the presented results are in accordance with the existing evidence (Cafferata et al., 2018) where a higher white blood cells (WBC) count is expected from an indirect impact of periodontitis. However, and despite the involvement of neutrophils in the pathogenesis of periodontitis and the destruction of tissues (due to the release of toxic products, such as enzymes and cytokines) (Aboodi et al., 2011), their counts were not a meaningful marker in the results analysis. The reason for this might be the fact that BV is commonly not characterized by the presence of neutrophils in vaginal swabs observed by flow cytometry (Roselletti et al., 2020), hence an effect on these cell counts might be improbable to exist.

Regarding CRP serum levels, these were graphically assessed in women with and without BV based on the levels of Periodontal Inflamed Surface Area (PISA). An increase in CRP levels was found in BV women, concomitantly with an elevation in the PISA levels.

Considering the results of the BV prevalence in the studied American population, it was

found that 29.2% of women had a positive diagnosis which is in accordance with Coudray and Madhivanan (2020). Also, regarding participants' characteristics, the results show higher rates of BV in non-hispanic white women, contradicting other studies which found the prevalence to be higher in non-hispanic black women (Javed et al., 2019; Peebles et al., 2019).

Additionally, the results demonstrate the existence of a higher number of women who smoked and were diagnosed with BV. Smoking highly impacts innate immunity and specifically it has been demonstrated to differently affect markers of cervicovaginal immunity (Murphy & Mitchell, 2016). It is also considered a major modifiable risk factor towards periodontitis (Palmer et al., 2005), not only because it can cause an increase in the levels of periodontal bacteria, but also because it hinders the neutrophils uptake into periodontal tissues, compromising the immune response (Buduneli & Scott, 2018; Ebersole et al., 2013).

Further and being in contrast with existing literature (Lokken et al., 2019), it was found that women with BV presented higher body mass index (BMI) when compared with a control group of women without BV. Although the relationship between obesity and infertility demands further clarification, a review by Best and Bhattacharya (2015) concluded that infertility was progressively worse with increasing BMI and that obesity directly impacts the oocytes and leads to ovulatory problems, poor embryo development and poor endometrial receptivity. Also, CRP serum levels were shown to have a positive correlation between BMI and obesity (Maiorino et al., 2018; Pavela et al., 2018) meaning that obesity is also considered a chronic low-grade inflammatory condition (Pavela et al., 2018; Sudhakar et al., 2018) and reinforcing its association with BV.

Finally, and as shown by previous studies (D'Aiuto et al., 2018; Deepti et al., 2017; Orlandi et al., 2020), it is remarkable to notice that in both PCOS and BV, the treatment of periodontitis is effective in lessening the inflammatory load. Hence, it is worthy to explore if treating periodontitis in women with PCOS and BV can alleviate the inflammatory burden, constituting a shift in the paradigm of the current multidisciplinary approach of these conditions.

VII. CONCLUSIONS

Addressing the first question of the first study - “In otherwise healthy individuals, do patients with periodontitis have increased serum CRP and hs-CRP levels compared to patients without periodontitis?” – it was found that chronic periodontitis was marked by an increase of 103% in CRP levels and of 110% in hs-CRP levels. Furthermore, aggressive periodontitis was correlated with an increase of CRP of 191% and of hs-CRP of 179%. Combined periodontitis (chronic and aggressive) diagnosis was related with an increase value of 80% of CRP and of 109% of hs-CRP, respectively, when compared to healthy controls. Considering different diagnoses, the aggressive form of periodontitis demonstrated 56% higher levels of CRP than the chronic one.

Regarding the second question of the first study - “Do hs-CRP levels decrease after intensive e non-intense NSPT?” – it was found that non-intensive NSPT led to a gradual reduction of hs-CRP values upon 180 days. However, intensive NSPT ended up in a high increase of hs-CRP one day following treatment, accompanied by a continuous reduction at 90 days after treatment and another moderate increase at 180 days of follow-up.

When acknowledging the first question of the secondary established aim - “Does PCOS have an effect on the healthy periodontium?” – the results show that PCOS females have on average 28% more risk in developing periodontitis. In the same way, women with periodontitis present, on average, 46% higher risk to be diagnosed with PCOS.

Secondly, addressing the second question of the secondary established aim - “Does PCOS influence periodontal clinical characteristics of periodontitis?” - periodontitis patients with PCOS demonstrated more gingival inflammation and greater PPD and CAL levels than periodontitis females without PCOS.

However, not all questions initially established were answered in the present investigation project. It was not possible to infer the mechanism by which the periodontal condition influences the clinical characteristics of PCOS. Hence, the third question of the secondary established aim - “Does periodontitis influence clinical characteristics of PCOS?” – needs further investigation to be answered in the future.

Regarding the third aim of this thesis, included in the third research article – “Do patients with both BV and periodontitis present altered blood and standard biochemical surrogates

when compared to healthy controls?” – it was found that, in women presenting BV, periodontitis was linked to higher inflammation, distinguished by increased WBC and lymphocyte counts.

VIII. FUTURE PERSPECTIVES

Periodontitis is a multifactorial low-grade systemic inflammatory condition which is widely prevalent in the world (Nazir, 2017; Tonetti et al., 2017). It is related with several systemic conditions, some of them already well studied, however, its relationship with female infertility remains poorly understood (Kassebaum et al., 2014). Considering the existing literature on this subject, and in order to disentangle the mechanisms upon this interplay, some considerations on the future approaches must be taken into account.

In the future, a standard definition and CRP quantification method should be implemented in order to diminish the heterogeneity observed in the analysis. Also, healthy controls must be included to observe and compare the hs-CRP variations over time with the population presenting periodontitis. Besides, future studies ought to expand data beyond 180 days after treatment and focus on patients reported outcomes so one can better understand the efficacy and effectiveness of the treatment.

Although the findings demonstrate that NSPT diminishes inflammation as shown by decreased CRP levels, more evidence is required to bring out whether NSPT in women with either PCOS or BV might alleviate the inflammatory load and attenuate the clinical course of these conditions as well as their risk factors.

Furthermore, studies should consider the main biomarkers of PCOS in serum and physical analysis, as well as divide the patients according to the PCOS and periodontitis status in order to clarify the impact between these diseases. Also, the use of a core set of research guidelines on PCOS and periodontitis are of utmost importance to guarantee that results are gathered and documented in a homogenous, and consistent manner to empower definite interpretations on this interconnection in future studies. It is also important to reinforce the implementation of full-mouth periodontal records in a way that they contribute to more consistent results.

Although previous literature has reported a possible interaction between PCOS and periodontitis (Farook et al., 2019; Márquez-Arrico et al., 2020; Tong et al., 2019) and between BV and periodontitis (Zabor et al., 2010), there is not enough documentation on the risk of these female fertility conditions on the development of periodontitis and vice-versa.

These results are important when addressing the potential repercussion of periodontitis on systemic health and, particularly in individuals suffering from other associated diseases impacted by systemic inflammation. In general, these findings highlight the fact that more studies are warranted to further investigate the association between PCOS and periodontitis and between BV and periodontitis as well as the mechanisms upon this interplay. Therefore, and based on the scientific importance of these associations, the next step ought to be the search for causality between these female fertility conditions and periodontitis, as well as their impact in periodontitis development and the overall systemic interaction between them.

IX. REFERENCES

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X. APPENDICES



Cláudia Escalda <claudia.escalda4@gmail.com>

Autorização para estrutura da tese

Cláudia Escalda <claudia.escalda4@gmail.com> 27 de julho de 2021 às 09:13
 Para: galcoforado@egasmoniz.edu.pt, Cristina Manso <mansocristina@gmail.com>, Ana Azul <anazul2@gmail.com>, imvcr.ireneventura@gmail.com, Paulo Mauricio <pmauricio@egasmoniz.edu.pt>
 Cc: Jose joao baltazar mendes <cdmi.mendes@gmail.com>, vanessa machado <vanessamachado558@gmail.com>

Exmos.

Magnífico Reitor do IUEM, Professor Doutor Gil Alcoforado

Vice-Reitora do IUEM, Prof.ª Doutora Cristina Manso,

Coordenadora do MIMD Prof. Doutora Ana Mano Azul

Presidente da Comissão Científica do MIMD Prof. Doutora Irene Ventura

Regente da UC Orientação Tutorial de Projecto Final, Prof. Doutor Paulo Maurício

No seguimento da minha tese de mestrado, com o tema "O impacto da inflamação periodontal na fertilidade feminina", orientada pelo Prof. Doutor José João Mendes e co-orientada pela Prof. Doutora Vanessa Machado, vimos questionar-vos relativamente às Normas para Apresentação das Dissertações, Trabalhos de Projecto e Monografias Integrantes do Relatório de Estágio (Anexo I do R.EM.DI.04).

Face aos resultados meritórios deste projecto, este grupo de trabalho viu ser premiado o esforço com a publicação de três artigos científicos:

- Serum C-reactive protein and periodontitis: a systematic review and meta-analysis. Front. Immunol. 2021, doi: 10.3389/fimmu.2021.706432
- Is There a Bidirectional Association between Polycystic Ovarian Syndrome and Periodontitis? A Systematic Review and Meta-Analysis. J. Clin. Med. 2020, 9, 1961; doi:10.3390/jcm9061961
- Association of bacterial vaginosis with periodontitis in a cross-sectional American nationwide survey. Scientific Reports. 2021, 11: 630; doi: <https://doi.org/10.1038/s41598-020-79496-4>

É importante sublinhar a importância deste feito já que se trata da primeira vez em que um aluno publica três artigos científicos, no âmbito do projecto de tese de Mestrado em Medicina Dentária, previamente à entrega e apresentação da Dissertação na Egas Moniz. Consequentemente, urge a necessidade de esclarecer alguns pontos relativamente ao formato de apresentação da dissertação.

- Como disposto no ponto 5.m do Anexo I R.EM.DI.04, "Corpo do trabalho dividido nos capítulos considerados relevantes (ex.: Introdução, Desenvolvimento do tema e Conclusões; ou Introdução, Materiais e Métodos, Resultados e Discussão, e Conclusões);".
- Pela interpretação deste ponto, parece-nos existir latitude para a apresentação de cada um dos artigos publicados como um capítulo individual.
- Parece-nos existir latitude para não existir um capítulo de Materiais e Métodos isolada, já que a metodologia é diferente para cada artigo publicado e os mesmos contemplam a metodologia já descrita.
- Esclarecemos que a publicação do artigo integral está prevista e aprovada pelas revistas onde foram publicados, e comprometemo-nos a anexar as mesmas autorizações na zona de Anexos da dissertação.

Assim, sendo a organização prevista para esta dissertação a seguinte:

1. Introduction (inclui Aims)
2. Serum C-reactive protein and periodontitis: a systematic review and meta-analysis
3. Is There a Bidirectional Association between Polycystic Ovarian Syndrome and Periodontitis? A Systematic Review and Meta-Analysis
4. Association of bacterial vaginosis with periodontitis in a cross-sectional American nationwide survey
5. Conclusions (inclui Brief Discussion of the results e Future Perspectives),

Gostaríamos de questionar a adequabilidade e permissão para proceder com a mesma.

Na esperança de que estejam todos bem, ficamos a aguardar com entusiasmo o vosso parecer.

Os melhores cumprimentos,

Cláudia Escalda (112717)



Cláudia Escalda <claudia.escalda4@gmail.com>

Autorização para estrutura da tese

Cristina Manso <mansocristina@gmail.com>

28 de julho de 2021 às 11:04

Para: Cláudia Escalda <claudia.escalda4@gmail.com>

Cc: Gil Alcoforado <galcoforado@egasmoniz.edu.pt>, Ana Azul <anazul2@gmail.com>, "Prof. Irene Ventura (MD)" <imvcr.ireneventura@gmail.com>, Paulo Mauricio <pmauricio@egasmoniz.edu.pt>, jose joao baltazar mendes <cdmi.mendes@gmail.com>, vanessa machado <vanessamachado558@gmail.com>

Estimada aluna,

Em nome do Senhor Reitor, tenho a transmitir que a Reitoria do IUEM congratula-a pelo trabalho de mérito publicado, bem como a toda a equipa de investigação que a acompanha, agradecendo o empenho de todos.

Neste sentido, e também ouvido o Regente de Orientação Tutorial, a Presidente da Comissão Científica e a Coordenadora de ciclo de estudo, não vimos nenhum impedimento à forma como pretende organizar e adequar o seu trabalho escrito, permitindo a sua execução.

Cumprimentos

Ana Cristina Manso

DDS, PhD

Professora Associada no IUEM

Vice-Reitora do IUEM

Instituto Universitário Egas Moniz – IUEM

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Author/Editor	DEUTSCHE GESELLSCHAFT FÜR GYNAKOLOGIE UND GEBURT	Start Page	631
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Article Title	Adverse pregnancy outcomes (APOs) and periodontal disease: pathogenic mechanisms.	Start Page	S170
Author/Editor	BRITISH SOCIETY OF PERIODONTOLOGY.	End Page	S180
Date	01/01/1974	Issue	SUPPL. 14
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