Dentistry
ISSN: 2161-1122

Gokhale et al., Dentistry 2014, 4:10
DOI: 10.4172/2161-1122.1000270

Research Article Open Access

A Study to Evaluate the Relationship between Il-1 β and Tnf- A Levels In Gcf in Non- Smoker, Previous or Current Smoker Female Patients with Chronic Periodontitis

Shami Ajit Gokhale*, Girish Byakod, Rashmi Hegde, Sangeeta Muglikar and Ashwini Gokhale

Department of Periodontology and Implantology, M.A. Rangonwala College of Dental Sciences and Research Center, Maharashtra, India

Abstract

Background: It has been postulated that the association between periodontal disease and systemic conditions may be because of the confounding effects of smoking. Studies of this type rarely investigate the adverse pregnancy outcomes like preterm birth or low birth weight. This study evaluates relationship between periodontal disease and adverse pregnancy outcomes.

Aim: The aim of this study was to compare and evaluate the relationship between IL-1β and TNF-α levels and adverse pregnancy outcomes in females with a history of smoking and non- smokers.

Materials and methods: 40 pregnant women recruited in a hospital were examined. Data including history of smoking or passive smoking before pregnancy was collected. Patients with advanced gingivitis and periodontitis were selected. GCF samples was collected 2-4 days post operatively. Eliza kits were used for analysis of TNF- α and IL-1 β .The samples collected were immediately transferred to an eppendorf tube and stored at -80 C till the analysis was done.

Results: The results of the study suggest that increased TNF-α and IL-1 β levels signify that adverse pregnancy outcomes in women who are non- smokers and those with a history of smoking are because of the confounding effects of smoking on periodontitis.

Keywords: Periodontal disease; Periodontitis; Preterm labor; Hyper-inflammatory trait

Introduction

Periodontal diseases (PD) include a group of chronic inflammatory diseases that affect the supporting structures of the teeth involving complex inflammatory interactions with the host, leading to potential tooth loss [1].

Periodontitis is caused predominantly by gram-negative, anaerobic, and microaerophilic bacteria that colonize in the subgingival area and cause local and systemic elevations of proinflammatory prostaglandins and cytokines.

The concept that periodontal disease might influence systemic health was originally published by Miller in his "focal infection theory" in 1891 [2], suggesting that "microorganisms or their waste products obtain entrance of parts of the body adjacent to or remote from the mouth." Miller and subsequent proponents of the focal infection theory blamed oral foci of infection for a number of regional and systemic diseases, ranging from tonsillitis and middle ear infections to pneumonia, tuberculosis, syphilis, osteomyelitis, endocarditis, meningitis and septicemia [2-5].

However the recent epidemiological and microbiological–immunological studies have lent credence to the concept that periodontal disease may be a separate risk factor for cardiovascular disease, cerebrovascular disease and respiratory disease, as well as preterm delivery of low-birth-weight infants [6,7].

The term preterm labor (PTL) refers to pregnancy outcome at less than 37 weeks and after 22 weeks of gestation. Prematurity is strictly connected with preterm labor. Its obstetric criteria state infant's weight under 2500 g (LBW-low birth weight) and labor less than 37 weeks gestation [8,9]. If the two criteria meet together a term preterm low birth weight (PTLBW) is used.

Multiple factors have been associated with PTLBW. Some of these known risk factors in mothers for PTLBW include young and old maternal age, low pre-pregnancy weight, obesity, multiple gestations, anemia, gestational diabetes, genitourinary tract infections, arterial hypertension, illicit drug use, cigarette smoking, low socioeconomic status (SES), inadequate prenatal care, short stature, excessive alcohol consumption, and previous preterm delivery [10]. Further research has shown that chronic oral infections are also negatively associated with pregnancy, more specifically preterm and low birth weight deliveries [11-18].

It has been postulated that distant infections like periodontal diseases may be associated with LBW through similar mechanisms as other maternal infections [19].

Maternal periodontal infection has been proposed to influence LBW delivery through mechanisms involving inflammatory mediators or direct bacterial assault on the amnion leading to preterm labor and premature rupture of membranes [19-21].

Evidence concerning the progression of periodontitis, suggests that cytokines, chemokines and inflammatory mediators involved with the

***Corresponding author:** Shami Ajit Gokhale, MDS, Department of Periodontology and Implantology, M.A. Rangonwala College of Dental Sciences and Research Center, Plot No. 8, Swojas, Shantiban Society, Sahajanand, Pune, Maharashtra, India, Tel: 9922189371; E-mail: shamiagokhale@gmail.com

Received November 05, 2014; **Accepted** November 29, 2014; **Published** December 05, 2014

Citation: Gokhale SA, Byakod G, Hegde R, Muglikar S, Gokhale A (2014) A Study to Evaluate the Relationship between Il-1β and Tnf-A Levels In Gcf in Non-Smoker, Previous or Current Smoker Female Patients with Chronic Periodontitis. Dentistry 4: 270. doi:10.4172/2161-1122.1000270

chemotaxis of leukocytes, may also contribute to a systemic promotion of PTD and LBW [22].

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that is involved in human parturition, especially in the context of infection-induced preterm birth [23].

It is involved with several gestational events. The endometrium, the epithelial tissue that forms the lining of the uterus, has been shown to produce IL-1β and TNF-α, along with many other inflammatory mediators. Current research also reports that IL-1β is present in amniotic fluid at all periods of gestation, as evidenced by a cross-sectional study conducted in 2005 by Esplin et al. [18]. An increase in IL-1β in women in active labor has also been reported [24, 25]. IL-1β and TNF-α are the cytokines significantly increased in amnion, amniotic fluid, and decidua at term and can also induce PGE2 production in amniocytes and decidual cells in vitro [26,27].

Periodontal disease shares many common risk factors with LBW, such as smoking, age, low socioeconomic level, and systemic health status [21].

Various studies have postulated that the association between periodontal disease and systemic conditions may be because of the confounding effects of smoking. Studies of this type rarely investigate the adverse pregnancy outcomes like preterm birth or low birth weight.

Hence in this study we decided to shed light on this hypothesis that the association between periodontal disease and systemic conditions like PTLBW may be because of the confounding effects of smoking by comparing the relationship between IL-1β and TNF-α concentrations and adverse pregnancy outcomes in periodontitis affected females with a history of smoking with those in non- smokers.

Aim

The aim of this study was to compare and evaluate the relationship between IL-1β and TNF-α levels and adverse pregnancy outcomes in periodontitis affected females with a history of smoking and those who were non- smokers.

Materials and Methods

The study included 50 pregnant women recruited in a private hospital in Pune (Maharashtra, India).

The patient selection included pregnant subjects undergoing normal delivery or a Caesarian. Periodontitis affected females were included in the test group and those with healthy periodontium were included in the control group.

Hypertensive patients, patients with a history of UTI, diabetic patients, patients with previous history of miscarriages or history of multiple pregnancies and other chronic diseases were excluded from the study.

Demographic data, behavioral and medical data were collected including history of smoking or passive smoking before pregnancy. The study was approved by the Institution's ethics committee and an informed consent was obtained from the patients who were willing to participate in the study after explaining them the entire study protocol.

- Patients were evaluated for their periodontal status 2-4 days post operatively and the GCF samples were collected at the same time, as the levels of biological fluid cytokines have been proved to remain high 4-5 days pre and post operatively.
- Calibrated volumetric capillaries with markings upto 5 µl were used to collect the GCF. The samples collected were immediately transferred to an Eppendorf tube and stored at -80°C till the analysis was done.
- A patient was considered to have periodontitis if there were at least six sites with PPD ≥ 4 mm and CAL ≥ 3 mm. Healthy mothers should had a PPD 4-3 mm and CAL < 3 mm
- Patients were grouped into 5 groups as follows:

o **Group 1:** Patients with periodontitis, with adverse pregnancy outcomes and with a history of smoking or passive smoking (n=6)

o **Group 2:** Patients with periodontitis, without adverse pregnancy outcomes but with a history of smoking or passive smoking (n=19)

o **Group 3:** Patients with periodontitis, with adverse pregnancy outcomes and without a history of smoking or passive smoking (n=3)

o **Group 4:** Patients with periodontitis, without adverse pregnancy outcomes and without a history of smoking or passive smoking (n=14)

o **Group 5:** (Control Group): Patients without periodontitis, without pregnancy outcomes and without any history of smoking or passive smoking. (n=10)

The GCF IL-1β and TNF-α levels in each of this category were evaluated and compared using ELISA kits. Krishgen Biosystems Human TNF-α ELISA kit was used for the evaluation of h TNF-α and Mabtech Human Il-β ELISA kit was used for the evaluation of IL-1β in GCF.

The analysis was carried out according to the manufacturer's instructions. The assays utilized ELISA strip plates pre-coated with a capture monoclonal antibody (mAB), to which samples were added. Captured cytokines were detected by adding a bionylated mAB followed by streptavidin-horseradish peroxidase (SA-HRP). Addition of the enzyme substrate TMB resulted in a colored substrate product with an intensity that was directly proportional to the concentration of cytokine in the sample. The concentration of the cytokine in the sample was determined by comparison to a serial dilution of recombinant cytokine standard analyzed in parallel. On arrival all the components of the kit, with the exception of the lypophilized standard were stored at 2-8°C. After reconstitution of the lypophilized cytokine standard was aliquoted and kept at -20°C. The opened kit components were used within one month. All the steps in the conduct of the assay were carried out following the instructions as mentioned in the manual provided by the manufacturers.

Statistical methods: The results were statistically analyzed using differences between mean values of each of the test groups with the mean values for the control group using independent sample t-test. Continuous variables have been reported as Mean ± SD.

All the analysis was done using a statistical program (SPSS/PC Version 11.0 for Windows, SPSS Inc. Chicago IL).

Results

The results of this study show that in the test group the total number of patients presenting with adverse pregnancy outcomes were 9 (6 out of 40 patients presented LBW, 1 with IUD and 2 with preterm and LBW).

6 patients presented with a history of smoking / passive smoking, 2 with active and 4 with passive smoking. 3 patients with adverse pregnancy outcomes presented with no history of smoking. 14 patients

who were non smokers showed no adverse pregnancy outcomes. There were 17 patients without any adverse pregnancy outcomes but with a history of smoking; 2 active and 15 passive smoking. 2 patients out of 4 who were active smokers presented adverse pregnancy outcomes.

The levels of IL-1β were more than those of TNF-α in all groups (Table 1). The levels of IL-1β and TNF-α were significantly high in Group 1 i.e. patients with adverse pregnancy outcomes and with a history of smoking or passive smoking. (TNFα = 6.82 ± 2.02 pg/ml, IL-1β = 11.3 ± 3.02 pg/ml). The values were least in group 4 i.e. patients without adverse pregnancy outcomes and without a history of smoking or passive smoking.

The concentration of IL-β and TNF-α in each test group was compared with the control group considering the values of both the cytokines in the control group as baseline values.

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for the test groups and the control group [p<0.001,t=4.46 for group 1 against group 5 (Table 2.1); p<0.001, t=6.89 for group 2 against group 5 (Table 2.2); p<0.001, t= 4.46 for group 3 against group 5 (Table 2.3); p<0.05, t=2.6 for group 4 against group 5 (Table 2.4)]. In other words the values for IL-β concentration in GCF in the test groups were significantly higher than those in the control group i.e. group 5.

Also, the results show that there is a statistical difference between the mean concentration of TNF-α in GCF for the test and control group [p<0.001,t=8.99 for group 1 against group 5 (Table 3.1); p<0.001, t=5.29 for group 2 against group 5 (Table 3.2); p<0.05, t=2.91 for group 3 against group 5 (Table 3.3); p<0.01, t= 2.84 for group 4 against group 5 (Table 3.4)]. This means that the values for TNF-α concentration in GCF in the test groups is significantly higher than that in the control group.

Group	n=	Levels of TNF-α in pg/ml	Levels of IL-1β in pg/ml
1	6	6.82 ± 2.02	11.3± 3.02
2	17	3.52 ± 1.43	7.06 ± 2.25
3	3	2.22 ± 12.1	5.31 ± 1.68
4	14	1.43 ± 0.28	3.08 ± 1.24
5	10	1.07 ± 0.34	1.82 ± 1.05

Table 1: Levels of TNF-α and IL-1β in GCF.

Group	n=	Mean(pg/ml)	Sd	P-value
1(Test)	6	11.3	3.02	df=14 t=9.22 **p<0.001**
5(Control)	10	1.82	1.05	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 1 and 5 (df=14, t=9.22, p<0.001)

Table 2.1: Comparison of IL-1β concentration in GCF between Group 1 and Group 5.

Group	n=	Mean(pg/ml)	Sd	P-value
2(Test)	17	7.06	3.02	df=25 t=6.89 **p<0.001**
5(Control)	10	1.82	1.05	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 2 and 5 (df=25, t=6.89, p<0.001)

Table 2.2: Comparison of IL-1β concentration in GCF between Group 2 and Group 5.

Group	n=	Mean(pg/ml)	Sd	P-value
3(Test)	3	5.31	1.68	df=11 t=4.46 **p<0.001**
5(Control)	10	1.82	1.05	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 3 and 5 (df=11, t=4.46, p<0.001)

Table 2.3: Comparison of IL-1β concentration in GCF between Group 3 and Group 5.

Group	n=	Mean(pg/ml)	Sd	P-value
4(Test)	14	3.08	1.24	df=22 t=2.60 **p<0.05**
5(Control)	10	1.82	1.05	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 4 and 5 (df=22, t=2.60, p<0.05)

Table 2.4: Comparison of IL-1β concentration in GCF between Group 4 and Group 5.

Group	n=	Mean	sd	P-value
1(Test)	6	6.82	2.02	df=14 t=8.99 **p<0.001**
5(Control)	10	1.07	0.34	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 3 and 5 (df=14, t=8.99, p<0.001)

Table 3.1: Comparison of TNF-α concentration in GCF between Group 1 and Group 5.

Group	n=	Mean	Sd	P-value
2(Test)	17	3.52	1.43	df=25 t=5.29 **p<0.001**
5(Control)	10	1.07	0.34	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 3 and 5 (df=25, t=5.29, p<0.001)

Table 3.2: Comparison of TNF-α concentration in GCF between Group 2 and Group 5.

Group	n=	Mean	Sd	P-value
3(Test)	3	2.22	1.21	df=11 t=2.91 **p<0.05**
5(Control)	10	1.07	0.34	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 3 and 5 (df=11, t=2.91, p<0.05)

Table 3.3: Comparison of TNF-α concentration in GCF between Group 3 and Group 5.

Group	n=	Mean	sd	P-value
4(Test)	14	1.43	0.28	df=22 t=2.84 **p<0.01**
5(Control)	10	1.07	0.34	

The results indicate that there is a statistically significant difference between mean concentration of IL-1β in GCF for group 3 and 5 (df=22, t=2.84, p<0.01)

Table 3.4: Comparison of TNF-α concentration in GCF between Group 4 and Group 5.

Discussion

Proponents of an association between periodontal disease and adverse pregnancy outcome have postulated theories to explain a cause-and-effect relationship. Infection and inflammation appear to be important factors in the pathogenesis of premature birth [28]. Infection and inflammation are also pathogenic processes involved in periodontitis [29]. Although periodontitis is localized to the periodontal tissues, a low-grade bacteremia or circulating inflammatory mediators such as interleukins could have a deleterious effect on distant tissues, such as the pregnant womb.

The periodontal pockets serve as a chronic reservoir of bacteria (mostly Gram-negative bacteria, such as Porphyromonas gingivalis and Prevotella intermedia) and their virulent products. The translocation of these virulence products along with proinflammatory cytokines reach the fetal-placental unit through the hematogenic pathway and can trigger premature labor.

Alternatively, an association may relate to a generalized tendency to over-produce inflammatory mediators in response to localized infection. This is known as a hyper-inflammatory trait [30].

There are several risk factors common to both periodontal disease and adverse pregnancy outcome. These include non-White ethnicity and socioeconomic factors [28,29,31]. Smoking is potentially the most important shared risk factor and is common to periodontal disease [32] and poor pregnancy outcome [33].

The role of smoking as a risk factor for periodontal disease is well documented [34-37]. A number of studies have shown that smokers, compared to nonsmokers, have deeper pockets, more alveolar bone loss and a higher frequency of tooth loss [38-43]. These associations have been confirmed in studies showing that smoking is a risk factor for increasing probing depths and loss of attachment [31,44,45].

As smoking is a risk factor common to many diseases, it may be a confounding factor that is complicating apparent associations between periodontal disease and poor pregnancy outcome [46].

The main objective of the current study was to investigate the role of smoking as a risk factor associated with periodontitis in the pregnancy outcomes in women.

In this study IL-1β and TNF-α were detected in all GCF samples from periodontally healthy and periodontitis sites, which is in agreement with the findings of some authors [47]. However, few investigators have reported that IL-1β was not found in healthy sites, but only in periodontally diseased sites [48,49].

The data of the current study suggests that patients with periodontitis whether in presence or absence of smoking showed significantly higher levels of IL-1β and TNF-α than the control group (without periodontitis, without adverse pregnancy outcomes and non-smokers).

The present study has shown a positive association between smoking and periodontitis as the levels of IL-1β and TNF-α are higher in patients with a history of smoking than those without any history of active or passive smoking. This result is similar to that reported in a number of cross-sectional and longitudinal studies that have investigated the effect of smoking on periodontal status, either as the main exposure factor of interest or as a co-variable. Studies support an association between chronic periodontitis and premature birth.

There may be several mechanisms by which smoking affects the periodontal condition. For example, smoking diminishes the host response in terms of a decrease in Neutrophil function). Furthermore, it has been suggested that smoking may favour infection with periodontal pathogens [50-58].

Various studies associated smoking strongly with periodontitis and also with adverse pregnancy outcomes. Studies also suggest a correlation between periodontitis and adverse pregnancy outcomes. In the current study we have attempted to show that in periodontitis affected pregnant patients with a history of smoking, smoking proves to be an aggravating factor for adverse pregnancy outcomes.

Authors have suggested that smokers have a reduced capacity for an inflammatory response to microbial changes. These studies reported greater colonization by periodontal pathogens in the bacterial plaque of smokers. The mean TNF-α and IL- 1β levels in GCF of mothers with adverse with adverse pregnancy outcomes was significantly higher than the other groups. The levels were higher in-group2, which is suggestive of an increased risk of these patients to adverse pregnancy outcomes.

Group 3 which includes subjects without history of smoking but with adverse pregnancy outcomes also shows significantly high levels of the cytokines which could be attributed to other factors like socioeconomic status, oral hygiene, obesity, stress, and specific hormonal alterations or maternal infections during pregnancy which the patient is not aware of.

Group 4, which includes patients without any history of smoking and no adverse pregnancy outcomes, shows relatively lesser levels of both the cytokines.

Group 5, (control) which includes patients without periodontitis, non-smokers and no adverse pregnancy outcomes, shows the least levels of cytokines.

Thus above results are suggestive of a positive correlation between smoking and adverse pregnancy outcomes in subjects with periodontitis.

The limitation of our study was the lesser availability of the number of subjects who were smokers especially in group 3 which involved patients with periodontitis, with adverse pregnancy outcomes and without a history of smoking or passive smoking. The reason to this could be attributed to the fact that very few females are habitual to smoking, more so during pregnancy even in the urban population of the area where the study was conducted.

In future, we propose to conduct a study with a larger sample size such that the values for comparison can establish a strong correlation between the parameters that have been considered in the current study.

Conclusion

Thus the increased TNF-α and IL-1 β levels signify that adverse pregnancy outcomes which could be seen in women who had a history of smoking, are because of the confounding effects of smoking on periodontitis.

References

1. Silva TA, Garlet GP, Fukada SY, Silva JS, Cunha FQ (2007) Chemokines in oral inflammatory diseases: apical periodontitis and periodontal disease. J Dent Res 86: 306-319.
2. Miller WD (1891) The human mouth as a focus of infection. Dental Cosmos 33: 689-713.
3. Hunter W (1900) Oral Sepsis as a Cause of Disease. Br Med J 2: 215-216.
4. Billings F (1912) Chronic focal infections and their etiologic relations to arthritis and nephritis. Arch Intern Med 9: 484-498.
5. Mayo OH (1922) Focal infection of dental origin. Dental Cosmos 64: 1206-1208.
6. DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM (1993) Dental disease and risk of coronary heart disease and mortality. BMJ 306: 688-691.
7. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, et al. (1996) Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol 67: 1103-1113.
8. Breborowicz GH, Szymanekiewicz M, Anholcer A, Gadzinowski JA, Wczesniac (1999) Two Problem Polozniczy I Neonatologiczny. Medipress Ginekol. Poloz 5: 3-12.
9. Klimek M, Lauterbach R, Tomaszczyk B, Rzepecka-Weglarz B, Klimek M, et al. (1994) Neonatal evaluation of obstetrical criteria of prematurity. Ginekol Pol 65: 250-253.
10. da Silva TR (2012) Nonbiological maternal risk factor for low birth weight on Latin America: a systematic review of literature with meta-analysis. Einstein (Sao Paulo) 10: 380-385.
11. Offenbacher S, Jared HL, O'Reilly PG, Wells SR, Salvi GE, et al. (1998) Potential pathogenic mechanisms of periodontitis associated pregnancy complications. Ann Periodontol 3: 233-250.
12. Armitage GC (2001) Periodontal disease and pregnancy: discussion, conclusions, and recommendations. Ann Periodontol 6: 189-192.
13. Bobetsis YA, Barros SP, Offenbacher S (2006) Exploring the relationship

between periodontal disease and pregnancy complications. J Am Dent Assoc 137 Suppl: 7S-13S.

14. Buduneli N, Baylas H, Buduneli E, TÃrkoÄŸlu O, KÃse T, et al. (2005) Periodontal infections and pre-term low birth weight: a case-control study. J Clin Periodontol 32: 174-181.
15. Institute of Medicine (2006) Preterm birth: Causes, consequences, and prevention.
16. López NJ, Smith PC, Gutierrez J (2002) Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res 81: 58-63.
17. Offenbacher S, Boggess KA, Murtha AP, Jared HL, Lieff S, et al. (2006) Progressive periodontal disease and risk of very preterm delivery. Obstet Gynecol 107: 29-36.
18. Esplin MS, Romero R, Chaiworapongsa T, Kim YM, Edwin S, et al. (2005) Monocyte chemotactic protein-1 is increased in the amniotic fluid of women who deliver preterm in the presence or absence of intra-amniotic infection. J Matern Fetal Neonatal Med 17: 365-373.
19. Page RC (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 26: 230-242.
20. Gibbs RS (2001) The relationship between infections and adverse pregnancy outcomes: an overview. Ann Periodontol 6: 153-163.
21. Williams CE, Davenport ES, Sterne JA, Sivapathasundaram V, Fearne JM, et al. (2000) Mechanisms of risk in preterm low-birthweight infants. Periodontol 23: 142-150.
22. Gamonal J, Bascones A, Jorge O, Silva A (2000) Chemokine RANTES in gingival crevicular fluid of adult patients with periodontitis. J Clin Periodontol 27: 675-681.
23. Lappas M (2014) Caspase-1 activation is increased with human labour in foetal membranes and myometrium and mediates infection-induced interleukin-1Î² secretion. Am J Reprod Immunol 71: 189-201.
24. Buonocore G, De Filippo M, Gioia D, Picciolini E, Luzzi E, et al. (1995) Maternal and neonatal plasma cytokine levels in relation to mode of delivery. Biol Neonate 68: 104-110.
25. Elliott CL, Loudon JAZ, Brown N, Slater DM, Bennett PR, et al. (2001), "IL-1ß and IL-8 in human fetal membranes: changes with gestational age, labor, and culture conditions," American Journal of Reproductive Immunology 46: 260-267.
26. Keelan JA, Sato TA, Hansen WR (1999) "Interleukin-4 differentially regulates prostaglandin production in amnion-derived WISH cells stimulated with pro-inflammatory cytokines and epidermal growth factor," Prostaglandins Leukotrienes and Essential Fatty Acids 60: 255-262.
27. Pollard JK, Thai D, Mitchell MD (1994) "Mechanism of cytokine stimulation of prostaglandin biosynthesis in human decidua," Journal of the Society for Gynecologic Investigation 1: 31-36.
28. Goldenberg RL, Hauth JC, Andrews WW (2000) Intrauterine infection and preterm delivery. N Engl J Med 342: 1500-1507.
29. Page RC, Kornman KS (1997) The pathogenesis of human periodontitis: an introduction. Periodontol 14: 9-11.
30. Offenbacher S, Lieff S, Beck JD (1998) Periodontitis-associated pregnancy complications. Prenatal and Neonatal Medicine 3: 82-85.
31. Ismail AI, Morrison EC, Burt BA, Caffesse RG, Kavanagh MT (1990) Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Disease Study, 1959-87. J Dent Res 69: 430-435.
32. Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, et al. (1993) Evidence for cigarette smoking as a major risk factor for periodontitis. J Periodontol 64: 16-23.
33. Wilcox MA, Smith SJ, Johnson IR, Maynard PV, Chilvers CE (1995) The effect of social deprivation on birthweight, excluding physiological and pathological effects. Br J Obstet Gynaecol 102: 918-924.
34. Bergström J, Eliasson S (1987) Noxious effect of cigarette smoking on periodontal health. J Periodontal Res 22: 513-517.
35. Bergström J (1989) Cigarette smoking as risk factor in chronic periodontal disease. Community Dent Oral Epidemiol 17: 245-247.
36. Bergström J, Preber H (1994) Tobacco use as a risk factor. J Periodontol 65: 545-550.
37. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, et al. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J Periodontol 65: 260-267.
38. Arno A, Schei O, Lovdal A, Waerhaug J (1959) Alveolar bone loss as a function of tobacco consumption. Acta Odontologica Scandinavica 17: 3-10.
39. Solomon HA, Priore RL, Bross ID (1968) Cigarette smoking and periodontal disease. J Am Dent Assoc 77: 1081-1084.
40. Sheiham A (1971) Periodontal disease and oral cleanliness in tobacco smokers. J Periodontol 42: 259-263.
41. Feldman RS, Bravacos JS, Rose CL (1983) Association between smoking different tobacco products and periodontal disease indexes. J Periodontol 54: 481-487.
42. Ismail AI, Burt BA, Eklund SA (1983) Epidemiologic patterns of smoking and periodontal disease in the United States. J Am Dent Assoc 106: 617-621.
43. Axelsson P, Paulander J, Lindhe J (1998) Relationship between smoking and dental status in 35-, 50-, 65-, and 75-year-old individuals. J Clin Periodontol 25: 297-305.
44. Beck JD, Offenbacher S (2002) Relationships among clinical measures of periodontal disease and their associations with systemic markers. Ann Periodontol 7: 79-89.
45. Locker D, Leake JL (1993) Risk indicators and risk markers for periodontal disease experience in older adults living independently in Ontario, Canada. J Dent Res 72: 9-17.
46. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA (2002) Periodontitis-systemic disease associations in the presence of smoking--causal or coincidental? Periodontol 30: 51-60.
47. Rawlinson A, Dalati MH, Rahman S, Walsh TF, Fairclough AL (2000) Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. J Clin Periodontol 27: 738-743.
48. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, et al. (1990) Measurement of interleukin-1 alpha and -1 beta in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. J Periodontal Res 25: 156-163.
49. Wilton JMA, Brampton JLM, Griffiths GS, Curtis MA, Life JS, et al. (1992) Interleukin-1ß levels in gingival crevicular fluid from adults with previous evidence of destructive periodontitis. A cross sectional study. Journal of Clinical Periodontology 19: 35-57.
50. Thomas W, Holt PG, Keast D (1973) Effect of cigarette smoking on primary and secondary humoral responses of mice. Nature 243: 240-241.
51. Noble RC, Penny BB (1975) Comparison of leukocyte count and function in smoking and nonsmoking young men. Infect Immun 12: 550-555.
52. Kenney EB, Kraal JH, Saxe SR, Jones J (1977) The effect of cigarette smoke on human oral polymorphonuclear leukocytes. J Periodontal Res 12: 227-234.
53. Kraal JH, Chancellor MB, Bridges RB, Hawke JE (1977) Variation in the gingival polymorphonuclear leukocyte migration rate in dogs induced by chemotactic autologous serum and migration inhibitor from tobacco smoke. Journal of Periodontal Research 12: 242-249.
54. Kraal JH, Kenney EB (1979) The response of polymorphonuclear leukocytes to chemotactic stimulation for smokers and non-smokers. J Periodontal Res 14: 383-389.
55. Gerrard JW, Heiner DC, Ko CG, Mink J, Meyers A, et al. (1980) Immunoglobulin levels in smokers and non-smokers. Ann Allergy 44: 261-262.
56. Hughes DA, Haslam PL, Townsend PJ, Turner-Warwick M (1985) Numerical and functional alterations in circulatory lymphocytes in cigarette smokers. Clin Exp Immunol 61: 459-466.
57. Holt PG (1987) Immune and inflammatory function in cigarette smokers. Thorax 42: 241-249.
58. Johnson GK (1999) Position paper: tobacco use and the periodontal patient. Research, Science and Therapy Committee of the American Academy of Periodontology. J Periodontol 70: 1419-1427.